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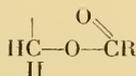
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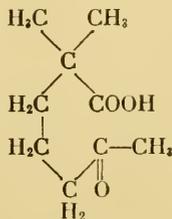
THE LIPIDS, Volume I

by Harry J. Deuel, Jr.

PAGE	LINE	CHANGE
24	21	"ox" to "hog"
	Reference 67	"90, 555-573 (1932)" to "99, 555-573 (1953)." Note also correct initials of Snider are "R. H."
88	14	Should read: "(b) <i>Triethenoid Acids</i> . Linolenic acid, elaeostearic acid, and octadeca-6,9,12-trienoic acid are the"
	17	"two" to "three"
90	Fig. 18	One-half of diagram should rotate 180°
168	Formula given as 1,2-benzylideneglycerol	"1,2-benzylideneglycerol" to "1,2-triphenylmethyl-glycerol"
171	Second equation	"1-Acylacrolein" to "Acyl ester of allyl alcohol"
203	Last column head	"Elaeostearic" to "Erucic"
313	Triglyceride formula	Last line of formula to:



356	Reference <i>f</i>	"Dureiul" to "Dureuil"
406	Reference 2	"Roper" to "Raper"
428	Equation	"Choline chloride" to "Choline"
500	17	"Rennkamp" to "co-workers"
	Reference 466	"F. Rennkamp" to "E. Schumann"
515	Geronic acid formula	Should read:



602	Azafrinone formula	Replace entire side-chain in ionone ring by "-R"
801	24	"milligrams" to "grams"
803	16	Insert ref. 68 at end of line
	25	Delete ref. 65
804	6	"Lange. ^{79f} " to "Lange ^{79f} and by Harris and co-workers. ^{79g} "
	Bottom of page	Introduce new footnote: ^{79g} P. L. Harris, M. L. Quaife, and W. J. Swanson, <i>J. Nutrition</i> , 40, 367-381 (1950).

(See over)

PAGE	LINE	CHANGE
866		"DeJust" to "Dejust"
867		"Dureiul" to "Dureuil"
868		"Elsden, G. D." to "Elsdon"
883		Insert "Raper, H. S., 406" between Rao and Rapoport
884		Delete "Roper, H. S., 406"
886		Add "500" to Schumann, E., entry
887		"Snider, R. A." to "Snider, R. H."
933	Elaeostearic acid, content in vegetable fats	"201" to "202"
934	Erucic acid, content in fats	"201" to "203"
970		Transpose up last two lines of column 1 to precede Sphingomyelin
977		Insert: "Triphenylmethylglycerol, structure, 168" between Tripentadecylin and Tristearin
980		"recurrence" to "occurrence" under "Vitamins E"

D 2/8

THE LIPIDS

Their Chemistry and Biochemistry

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University of Southern California, Los Angeles*

Volume I: CHEMISTRY

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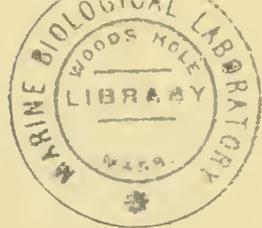
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PREFACE

No single treatise in recent years has attempted to correlate the available information as to the various components included under the classification of lipids. Such a work is obviously desirable, since all the constituents so listed are associated to some extent in plant and animal tissues. Inasmuch as the present work is intentionally slanted at the biological interpretations, a consideration of all the lipid-like components present in the animal body is imperative.

The present volume is the first of two dealing with lipids. As is evident from the Table of Contents, Volume I includes only the chemical approach to the subject. Volume II will be concerned exclusively with the biochemical and nutritional implications.

In this day of specialization, when many texts are compilations of sections prepared by masters in the several fields discussed, one may question the advisability of the preparation by a single author of a monograph on a wide field such as the chemistry of the lipids. An author attempting such a task can obviously not be an authority in all fields. Therefore, a book of this nature must necessarily be less involved and more understandable to readers who, also, are not specialists in the individual fields. The style should be more uniform and the book as a whole more cohesive when prepared by a single writer.

In this work, plant and animal species have been identified, insofar as is possible, by their systematic as well as by their common names. Since some confusion does exist in classifications, and in the spelling of the systematic names, an attempt has been made to achieve as much uniformity as possible.

The primary authority consulted for plant names has been the publication of the American Joint Committee on Horticultural Nomenclature (H. P. Kelsey and W. A. Dayton) on *Standardized Plant Names*, McFarland Co., Harrisburg, 1942. If the desired information was not available in this compilation, the *Standard Cyclo-pedia of Horticulture* by L. H. Bailey, Volumes I-III, Macmillan, New York, 1947, and *Hortus Secund*, by L. H. Bailey and E. Z. Bailey, Macmillan, New York, 1947, were consulted. Other useful sources of information included the following: J. D. Hooker and B. D. Jackson, *Index Kewensis Plantarum Phanerogamarum*, Parts I-IV, Clarendon Press, Oxford, 1893-1895, and the supplementary volumes, I-X, compiled by A. W. Hill and E. J. Salisbury, Clarendon Press, Oxford, 1906-1940; L. H. Bailey, *Manual of Cultivated Plants*, Macmillan, New York, 1944; G. S. West, *Algae*, Volume I, *Fresh-Water Algae*, Cambridge Botanical Handbooks, Cambridge University Press, 1916; and two works by G. M. Smith, *Fresh-Water Algae of the*

United States, McGraw-Hill, New York and London, 1933; and *Marine Algae of the Monterey Peninsula, California*, Stanford University Press, 1943.

No single reference volume was found to be as comprehensive for animal names as the *Standardized Plant Names* and the *Standard Cyclopedia of Horticulture* are for plants, although the Cambridge Natural History Series, from Volume I, *Protozoa and Echinoderms*, to Volume X, *Mammals*, Macmillan, New York, 1923-1936, was found to be very useful. For the names of fishes, the best information was obtained from: *A List of Common and Scientific Names of the Better-Known Fishes of the United States and Canada*, Transactions of American Fisheries Society, 75, Special Publication No. 1, 355-397, Ann Arbor, Michigan, 1948; the monograph by D. S. Jordan and B. W. Evermann entitled *A Checklist of the Fishes and Fish-like Vertebrates of North and Middle America*, Government Printing Office, Washington, D. C., 1896; and the book, in four parts, by the same authors, *The Fishes of North and Middle America; A Descriptive Catalogue of the Species of Fish-like Vertebrates Found in the Waters of North America, North of the Isthmus of Panama*, Bulletin of the United States National Museum, No. 47, Government Printing Office, Washington, D. C., 1896-1900.

Microorganisms were checked in D. H. Bergey's *Manual of Determinative Bacteriology*, sixth edition, Williams and Wilkins, Baltimore, 1948, and in W. A. N. Dorland's *Medical Dictionary*, twentieth edition, W. B. Saunders, Philadelphia, 1944.

Although much of the information given here has been obtained from recent monographs and reviews, the original references cited in the reviews have been consulted whenever possible. When the original article was not available, the source of the secondary citation has likewise been appended to the original reference.

Acknowledgment should be made to Professor L. Zechmeister of the California Institute of Technology, whose brilliant scientific and editorial career has been a continued source of stimulation to the present author over recent years.

The author is aware of the extremely valuable assistance of Mrs. Margaret Ritter, who has served as an editorial assistant throughout the preparation of the manuscript, and who has been able to locate and bring to the attention of the author much of the material cited. He would also like to acknowledge the excellent work on the figures and formulas carried out by Mrs. Mildred Greenberg and Mr. Howard Skypeck. Finally, mention should be made of the skill and accuracy of Mrs. Marie Visser in the preparation of the manuscript for the publisher.

Pasadena, California
June 4, 1951

HARRY J. DEUEL, JR.

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CHAPTER I

GENERAL CHARACTERISTICS AND CLASSIFICATION OF LIPIDS

1. Introduction

Fats have been recognized as a separate category of foodstuffs since prehistoric times. According to Markley,¹ the use of fats as foods is probably instinctive; the application of fats, and also of waxes, as illuminants, in cosmetics, in medicinals, and as lubricants, dates back to before our earliest records of civilized man.

The understanding of the chemical nature of one class of lipids, namely the fats, also predates our knowledge of the other foodstuffs. Although proteins were recognized as a definite class of substances from the time Mulder² coined the term *protein* in 1839, the determination of their structural relationship to the amino acids had to await the classical work of Kössel and especially of Emil Fischer, late in the nineteenth and early in the twentieth century. The chemical nature of the carbohydrate molecule was also obscure until the classical research of Emil Fischer and his co-workers. In contrast to these findings, Chevreul,³ as early as 1823, was able to establish the fact that the common animal and vegetable fats are combinations of the alcohol, glycerol, with the higher fatty acids. In fact Schule had obtained glycerol in 1779 by the saponification of olive oil with litharge, but he did not recognize how this fact was related to the structure of fat.

In spite of the advantage which fat gained from its early discovery and the recognition of its structure, few chemists specialized in the study of fats during the nineteenth century. Methods of analysis and of purification which were applicable to such water-soluble compounds as carbohydrates and proteins, and especially to their hydrolysis products, could not be applied to any great extent to the fats. Fats did not crystallize, and hence they could not readily be purified. Moreover, in nature they occurred as mixtures in which the individual components had a simple structure when

¹ K. S. Markley, *Fatty Acids*, Interscience, New York, 1947.

² G. J. Mulder, *J. prakt. Chem.*, 16, 129-152 (1839).

³ M. E. Chevreul, *Recherches chimique sur les corps gras d'origine animale*, Levrault, Paris, 1823. Cited by J. W. Lawrie, *Glycerol and the Glycols*, Reinhold, New York, 1928, pp. 17-18.

compared with protein, but which could not be separated into their several components by the most ingenious devices then available. Even as recently as 1917, Bull⁴ noted that only approximately 150 articles on lipids had appeared in the literature, as estimated from the references to them in *Chemical Abstracts*. However, with the widespread upsurge in research which has followed World War II, somewhat over 783 references are included in the 1947 volume of *Chemical Abstracts*. New methods have now become available for their separation and purification, such as molecular distillation. This procedure has been especially valuable for the preparation of several components of the non-saponifiable residue in especially pure condition and in sufficient yields to facilitate further study. The application of x-ray methods for the investigation of molecular structure has been particularly useful. Much of the more recent work on the qualitative identification and quantitative estimation of particular fats is traced to the discovery of the specific absorption curves which these compounds possess, in the infrared as well as in the ultraviolet region of the spectrum.

Another stimulus which has focused interest on the fat field has been the discovery of the pronounced physiological activity of a variety of substances which may be regarded as impurities of fat. These interesting products can be separated and concentrated in the non-saponifiable extract. They presumably occur in natural fats, because the triglycerides are excellent solvents for them, while they are completely insoluble in water. In this group of substances the most interesting, undoubtedly, are the fat-soluble vitamins, A, D, and E. However, equal importance should be centered on the carotenoids, which may or may not serve as provitamins A. Similarly, the sterols are important not only as possible precursors of vitamin D, but also as components of all plant and animal cells. Because of their widespread distribution and the importance of hormones and of other compounds with the steroid nucleus, studies in this field have been especially active.

2. Definitions

The term *lipid*⁵ has gained much favor during the last few years to define fats and fat-like substances. Most biochemists now consider it synonymous with the term *lipoids* (used by the English and Germans) or *lipins*, although the latter term was formerly applied only to such complex lipids as phospholipids and galactolipids.⁶

The lipids include all those substances which are insoluble in water but soluble in the so-called fat solvents (diethyl ether, petroleum ether, chloro-

⁴ H. B. Bull, *The Biochemistry of the Lipids*, Wiley, New York, 1937.

⁵ In current American literature, *lipid* is frequently spelled *lipide*. However, in conformity to the Hilditch usage, *lipid* will be the form used throughout this monograph.

⁶ I. Smedley-MacLean, *Ann. Rev. Biochem.*, 1, 135-150 (1932).

form, hot alcohol, benzene, carbon tetrachloride, acetone, etc.) which are related either actually or potentially to fatty acid esters, and which at the same time are utilizable by the animal organism. The latter qualification is essential to exclude mineral oil derivatives. This definition should not be interpreted too rigorously, or it would exclude certain compounds long considered to be members of this group. Thus, lecithin is to some extent water-soluble; lecithin is insoluble in acetone, cephalin in alcohol, while the sphingomyelins and the cerebroside are insoluble in such a widely accepted fat solvent as diethyl ether.

The terms *fats* and *oils* are generally considered to refer to substances which have a similar chemical structure, and which have the same metabolism in the animal body. Fats are those substances which are solids at ordinary temperatures, while oils are liquids under similar circumstances. Thus, what may be classed as a fat in one locality may be considered as an oil in a warmer climate.

3. Classification

The most convenient classification and the one which has been most widely employed in this country is the one originally suggested by Bloor.⁷ This is as follows:

(1) Simple Lipids

a. Neutral Fats. These are glycerol esters (glycerides) of the fatty acids (chiefly palmitic, stearic, oleic, and linoleic).

b. Waxes. These are esters of monatomic alcohols higher than glycerol. Most of these are substances with high melting points which are hydrolyzable with difficulty. These may be divided into several subclasses as follows:

(a) *True Waxes.* These consist of products of both animal and plant origin in which the esters are composed of palmitate, stearate, oleate, or other higher fatty acid esters of cetyl ($\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\text{OH}$), octadecyl or steryl ($\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{OH}$), or other higher straight-chain alcohols.

(b) *Cholesterol Esters.* These are fatty acid esters of the sterol, cholesterol. Lanolin, which contains a mixture of cholesterol palmitate, stearate, and oleate, is a member of this group.

(c) *Vitamin A Esters.* Vitamin A occurs naturally as the palmitic or stearic acid ester. In contradistinction to the waxes, these compounds are readily hydrolyzable. The colored fats which are mono- or di-esters of the carotenols, also, should be included in this category.

(d) *Vitamin D Esters.* Since vitamin D occurs naturally as an ester, it also should be included as a part of the group of waxes.

⁷ W. R. Bloor, *Chem. Revs.*, 2, 243-300 (1925-1926).

(2) *Compound or Conjugate Lipids*

This group is distinguished by the presence in the molecule of products other than fatty acids and alcohol. In some cases no alcohol is present and the fatty acids are combined in a peptide-like linkage rather than as esters.

a. Phospholipids or Phosphatides. The former term is most generally employed in the United States while the latter one is favored in English and German literature. This sub-group is characterized by the fact that it contains a phosphoric acid molecule as an integral part of the structure.

(a) *Lecithin.* This consists of glycerol, two fatty acid residues (one saturated and one unsaturated), phosphoric acid, and choline ($\text{HOCH}_2\text{-CH}_2\text{N}(\text{CH}_3)_3\cdot\text{OH}$).

(b) *Cephalin.* There are at least three, and possibly more, types of compounds which belong in this group. These are the following:

a'. Phosphatidyl Ethanolamine: This is the classical *cephalin* which hydrolyzes to glycerol, two fatty acids (one saturated and one unsaturated), phosphoric acid, and ethanolamine. The latter nitrogenous base appears in the molecule in place of the choline in lecithin.

b'. Phosphatidyl Serine: This is a product recently separated from brain cephalin by Folch⁸ which hydrolyzes to glycerol, two fatty acids, phosphoric acid, and serine. The latter amino acid is attached to H_3PO_4 through the hydroxyl group.

c'. Phosphatidyl Inositol or Lipositol: This is a phosphatide recently isolated from brain^{9,10} and from soybean oil,¹¹ and shown to contain inositol, fatty acids, galactose, tartaric acid, phosphoric acid, and ethanolamine in an undefined combination.¹²

(c) *Sphingomyelin.* This contains a nitrogenous base, sphingosine ($\text{CH}_3(\text{CH}_2)_{12}\text{CH:CHCHOHCHOHCH}_2\text{NH}_2$), a single fatty acid, phosphoric acid, and choline, but no glycerol.

(d) *Phosphatidic Acids.* These are compounds which have been prepared from plant sources and which are similar to lecithin minus the choline. Components isolated on hydrolysis are fatty acids (saturated and unsaturated), glycerol, phosphoric acid, and calcium.¹³

b. Cerebrosides. These compounds contain a carbohydrate as well as a fat molecule, but no phosphoric acid or glycerol.

(a) *Galactolipids.* These compounds, found chiefly in the brain, hydrolyze to a fatty acid (usually containing 24 carbon atoms), sphingosine, and galactose.

⁸ J. Folch, *J. Biol. Chem.*, 139, 973-974 (1941).

⁹ J. Folch and D. W. Woolley, *J. Biol. Chem.*, 142, 963-964 (1942).

¹⁰ J. Folch, *J. Biol. Chem.*, 146, 35-44 (1942).

¹¹ E. Klenk and R. Sakai, *Z. physiol. Chem.*, 258, 33-38 (1939).

¹² D. W. Woolley, *J. Biol. Chem.*, 147, 581-591 (1943).

¹³ A. C. Chibnall and H. J. Channon, *Biochem. J.*, 23, 176-184 (1929).

(b) *Glucolipids*. These substances, first isolated from the spleen in Gaucher's disease, were shown to contain glucose in place of galactose,¹⁴ but the other components, *viz.*, sphingosine and a lignoceric acid derivative, were similar to those found as galactolipids.

c. *Sulfolipids*. These contain a sulfate radicle.

(3) *Derived Lipids*

This class includes derivatives of the first two classes of lipids obtained by hydrolysis which still retain the lipid characteristic of solubility in ether, etc., and insolubility in water. This class contains the important components of the so-called non-saponifiable extract or non-saponifiable fraction (NSF).

a. *Fatty Acids*. This group includes all fatty acids obtained from natural products which are soluble in fat solvents and which are insoluble in water. The fatty acids starting with caprylic acid ($\text{CH}_3(\text{CH}_2)_6\text{COOH}$) and the higher ones belong in this category. Such fatty acid derivatives as soaps are not considered to be members of this group.

b. *Alcohols*. The alcohols of higher molecular weight which are obtained by hydrolysis of waxes and which are insoluble in water belong in this category. Glycerol is water-soluble and hence is not included. These alcohols may be classified as follows:

(a) *Straight-Chain Alcohols*. The common representatives are cetyl (or palmityl), $\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\text{OH}$; octadecyl (or steryl), $\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{OH}$; ceryl, $\text{CH}_3(\text{CH}_2)_{24}\text{CH}_2\text{OH}$; and myricyl (or melissyl), $\text{CH}_3(\text{CH}_2)_{28}\text{CH}_2\text{OH}$.

(b) *Sterols*. These all contain the steroid nucleus (cyclopentanophenanthrene). The commonest are cholesterol, which is obtained from animal fats and the phytosterols, sitosterol, and stigmasterol, present in the vegetable oils of the higher plants or *phanerogams*. Ergosterol, which is a sterol characterizing a lower order of plants (*cryptogams*), is also a member of this group.

(c) *Alcohols Containing the β -Ionone Ring*. These include vitamin A itself and such carotenols as cryptoxanthin, lutein, and zeaxanthin.

c. *Hydrocarbons*. This group includes products which contain no alcohol groups and which cannot be saponified. Several classes of hydrocarbons can be distinguished, as follows:

(a) *Aliphatic Hydrocarbons*. This group includes iso-octadecane ($\text{C}_{18}\text{H}_{38}$), which is liquid and is found as a component of liver fats. There are also a number of solid hydrocarbons which are largely found in beeswax and plant waxes, such as pentacosane ($\text{CH}_3(\text{CH}_2)_{23}\text{CH}_3$) and homologues up to hentriacontane ($\text{C}_{31}\text{H}_{64}$).

¹⁴ N. Halliday, H. J. Deuel, Jr., L. J. Tragerman, and W. E. Ward, *J. Biol. Chem.*, **132**, 171-180 (1940).

(b) *Carotenoids*. Most important are the three carotene isomers α , β , and γ , all of which have an identical empirical formula, $C_{40}H_{56}$. Lycopene also falls in this category, but the ionone ring is ruptured in the latter compound.

(c) *Squalene* ($C_{30}H_{50}$). This hydrocarbon related to the carotenoids is found in shark-liver oil.

d. Vitamins D. These compounds are found in the non-saponifiable extract, but differ from the sterols principally because the steroid nucleus is ruptured between carbons 9 and 10. These are present in fish-liver oils as esters (before saponification) or may be synthesized in other oils which contain 7-dehydrocholesterol or ergosterol when these are subjected to irradiation with ultraviolet light.

e. Vitamins E. The chroman derivatives, α , β , γ , and δ tocopherols, are compounds found in natural oils (chiefly vegetable) of varying vitamin E potency. These occur in high concentration in corn, cottonseed, and soybean oil.

f. Vitamins K. These vitamins are derivatives of 1,4-naphthoquinone and contain long hydrocarbon side chains. Their occurrence in fats and oils is extremely limited.

CHAPTER II

THE CHEMISTRY OF FATTY ACIDS AND GLYCEROL

I. Introduction

In his monumental treatise on the chemical constitution of various fats, Hilditch¹ has discussed the properties of some 600 different fats, which he has arranged according to their biological relationship. The fats considered include 420 samples from various plant species, 80 from land animals, and about 100 of aquatic origin. In only a few instances have these included more than one fat from different parts of the same animal or plant.

However, the number of natural fats which differ sufficiently in properties and composition to be considered as entities must be many times those listed by Hilditch. Each plant and animal has at least one lipid which is characteristic of its particular species; in fact, in the case of the higher animals a considerable variety of fats occur in the different tissues and organs of the same animal. It is a well-known fact that the composition of subcutaneous fat varies considerably from that of mesenteric fat, while perirenal, omental, genital, intermuscular, and liver fat each possesses its own characteristic composition and properties. Moreover, the lipids present in the heart, brain, spinal cord, and in fact in every separate tissue, can also be classified as individual fats.

The number of individual fats is further augmented by species variations. Samples of cottonseed or soybean oil from diverse varieties of the plants have a somewhat different make-up typical of that species. In the case of animals, it is well known that milk fat differs in each kind of animal, and that it may be identified by its characteristic fatty acid content. Even in two species as closely related as the Holstein and the Guernsey cow, the milk fat may appear to be quite different, since it has a low carotene content in the first case and a relatively high one in the second instance. Such variations make it advisable to consider that butter fat is not a single fat of uniform composition but rather that a number of different butters actually exist which may have wide alterations in composition.

¹ T. P. Hilditch, *The Chemical Constitution of Natural Fats*, Wiley, New York, 2nd ed., 1947.

The composition of fat from a single tissue in the same species of animal may again show wide variations. One may go so far as to say that in the same animal the fat may change with alterations in diet. One need only be reminded of the variation of fat laid down by hogs on a normal diet as contrasted with "soft pork" produced when the food contains large amounts of soybean oil.² Similar variations in the composition of egg-yolk fat have been produced by changes in the dietary fat.³

From these considerations, it should be self-evident that the fats are too numerous and subject to too great variations in composition to allow for a strict classification. Identification by the terminology currently employed will, of course, continue to be of value for many commercial applications. It can hardly be expected to serve for the characterization of fats for research study or even for critical industrial uses. Here, the composition of any product must be identified by the proportion of the various triglycerides, the quantity and type of unsaturated linkages, the amount of mono- or diglycerides, and by the specific composition of the non-saponifiable fraction. The present chapter will include a discussion of the structure and properties of the fatty acids and glycerol which make up the triglycerides. A similar treatment of the components of the non-saponifiable residue will follow in the later chapters.

The subject of fatty acids has been exhaustively reviewed in the two excellent recent monographs of Markley⁴ and of Ralston.⁵ The most complete treatment of glycerol is given by Lawrie⁶ in a monograph of the American Chemical Society.

2. Classification and Structure of Fatty Acids

The most important conditions which regulate the nature of fat are the kind and proportion of the various fatty acids present in the triglyceride molecule. Since fats and oils consist almost entirely of triglycerides, *i.e.*, fatty acid esters of the trihydric alcohol, glycerol, the fatty acid component accounts for 90% of the molecule of most natural fats. Although small amounts of di- or monoglycerides may exist in some fats, these constituents are present in too small an amount to alter the 90% proportion of the fatty acids.

The fatty acids present in animal and vegetable fats are limited to a comparatively small number of representatives. One important generaliza-

² N. R. Ellis and H. S. Isbell, *J. Biol. Chem.*, 69, 239-248 (1926).

³ R. W. Riemenschneider, N. R. Ellis, and H. W. Titus, *J. Biol. Chem.*, 126, 255-263 (1938).

⁴ K. S. Markley, *Fatty Acids*, Interscience, New York, 1947. This contains an excellent discussion on nomenclature and a complete listing and discussion of the individual fatty acids.

⁵ A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948.

⁶ J. W. Lawrie, *Glycerol and the Glycols*, Reinhold, New York, 1928, pp. 1-147.

tion which can be made is that the natural fats and oils of animal as well as of vegetable origin, almost without exception, are composed only of fatty acids having an even number of carbon atoms. Furthermore, they consist almost exclusively of straight-chain acids rather than of forked-chain components; only in a few isolated cases are any ring compounds found to occur as part of the fatty acid molecule. Although fats with an odd number of carbon atoms can be synthesized in the laboratory and would appear to be metabolized in the animal body, they are almost never deposited in the tissues as such. Some exceptions have, however, been noted (see page 237).

The fatty acids most frequently occurring as components of natural fats and oils can be classified into several series. The first of these is referred to as the saturated fatty acid series or simply as the fatty acid series. These have no unsaturated linkages and cannot be altered by hydrogenation or halogenation. The second group is characterized by the presence of one double bond, and is called the oleic acid series (or monoethenoid acids). A third group is the linoleic acid or diethenoid acid series, which is characterized by two unsaturated linkages. The triethenoid and tetraethenoid acids may be classified under the general group of polyethenoid acids. The acids having more than one double bond make up some of the most important of the fatty acids, since they cannot be synthesized by the higher animals, and some of them are required by the animal; hence they are of considerable importance from a nutritional standpoint.

(1) Nomenclature

Most of the fatty acids were known before their structural relations were established and before the adoption by the Geneva Congress^{7,8} in 1892, of the system of nomenclature which is still more or less in general use. According to the Geneva classification, the aliphatic acid is regarded as a derivative of the hydrocarbon where a terminal " $-\text{CH}_3$ " group is replaced by a carboxyl group " $-\text{COOH}$." The name of the acid is derived from that of the hydrocarbon by replacing the terminal *e* by the suffix *oic*. Thus, the fatty acid related to the hydrocarbon, butane ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$), is named butanoic acid and has the formula $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$. The chief advantage of the Geneva system, other than that of furnishing a uniform system which also relates the acid to the corresponding hydrocarbon, is that, in all cases above the 4-carbon members, the name of the hydrocarbon (and consequently of the acid) is derived from the Latin word for the number which corresponds to the number of carbon atoms in the specific compound. This affords an easy method for the recognition of the formula

⁷ A. Pictet, *Arch. sci. phys. nat.*, 27, 485-520 (1892).

⁸ F. Tiemann, *Ber.*, 26, 1595-1631 (1893).

from the name. Thus, the 12-carbon acid commonly known as lauric acid ($\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$) is called by the Geneva system *dodecanoic*, since it is derived from the hydrocarbon *dodecane* ($\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3$).

The system for naming the unsaturated acids is simple when once understood. In all cases, the same rule holds as for the saturated acids, namely that they are named after the corresponding hydrocarbon. It should be recalled that in the case of the unsaturated hydrocarbons with one double bond the suffix *ane* is replaced by *ene*. Thus *butenoic* acid has the formula $\text{CH}_3\text{CH}:\text{CHCOOH}$ (or $\text{CH}_2:\text{CHCH}_2\text{COOH}$) and is to be regarded as a derivative of the corresponding *butene*. Since the position of the double bond is also of importance in characterizing the compound, this must be designated if the exact substance is to be defined. According to the Geneva system, the successive carbon atoms are numbered, starting with the carboxyl carbon as "1." Thus, $\text{CH}_3\text{CH}:\text{CHCOOH}$ would be 2-butenoic acid, while $\text{CH}_2:\text{CHCH}_2\text{COOH}$ would be referred to as 3-butenoic acid. Oleic acid is thus systematically designated as 9-octadecenoic acid, although some authors prefer to use the designation $\Delta^{9,10}$ -octadecenoic acid, or simply Δ^9 -octadecenoic acid. In the latter case only the number of the carbon atom in the unsaturated bond nearest the carboxyl group is indicated. Another earlier practice for the designation of double bonds by the Greek letters α , β , γ , etc., is entirely impractical for the long-chain fatty acids with which one is concerned in natural fats.

The procedure for the polyethenoid acids (those with more than one unsaturated linkage) should also be understood, since a number of such acids are of a far greater importance than would be indicated by their quantitative distribution. Acids with two double bonds have the suffix *dienoic* replacing the terminal *e* on the saturated hydrocarbon. Similarly, the suffixes *trienoic*, *tetraenoic*, and *pentaenoic* refer to acids with three, four, or five double bonds, respectively. Linoleic acid ($\text{CH}_3(\text{CH}_2)_4\text{CH}:\text{CHCH}_2\text{CH}:\text{CH}(\text{CH}_2)_7\text{COOH}$) is referred to as 9,12-octadecadienoic acid or as $\Delta^{9,10,12,13}$ (or $\Delta^{9,12}$)-octadecadienoic acid. Linolenic acid ($\text{CH}_3\text{CH}_2\text{CH}:\text{CHCH}_2\text{CH}:\text{CHCH}_2\text{CH}:\text{CH}(\text{CH}_2)_7\text{COOH}$) is called 9,12,15-octadecatrienoic acid (or $\Delta^{9,10,12,13,15,16}$ -octadecatrienoic acid or more simply $\Delta^{9,12,15}$ -octadecatrienoic acid).

(2) Saturated Fatty Acids

The empirical formulas for all fatty acids in this group agree with the type formula, $\text{C}_n\text{H}_{2n}\text{O}_2$. In the case of natural fats, n is, in almost every case, an even number. However, this is not invariably true, as a number of odd-carbon acids occur in the case of the iso acids in wool fat.⁹ In line with this is the interesting observation of Weitkamp, Smiljanic, and

⁹ A. W. Weitkamp, *J. Am. Chem. Soc.*, 67, 447-454 (1945).

Rothman¹⁰ that the free fatty acids obtained from human hair vary in length from C₇ to C₂₂; traces of C₇, C₉, and C₁₁ fatty acids are present, while appreciable proportions of the C₁₃, C₁₅, and C₁₇ acids are also found. Both saturated and unsaturated odd-carbon fatty acids are present.

The fatty acids found most commonly as components of the fat molecule are listed in Table 1, together with data on their discovery and also their chief natural sources.

The lower members of the fatty acids are liquid at ordinary temperatures, while those with 10 carbons and more are solids having progressively higher melting points with an increase in the length of the fatty acid chain. It is interesting that the even progression of melting points and the length of the carbon chain give a smooth curve only if one considers the fatty acids with an even number of carbon atoms. When all the fatty acids are included, the curve becomes a step-like one, each odd-carbon acid having a melting point somewhat lower than that of the even-chain acid immediately preceding it.

Like other members of the lipids, the fatty acids are characterized, in the main, by their solubility in fat solvents and their insolubility in water. However, butyric acid is completely miscible with water in all proportions. Caproic acid is soluble to the extent of 0.9%, while the higher acids (above lauric acid) are practically insoluble in water. On the other hand, the alkali salts or soaps of the fatty acids are in general quite soluble in water. However, other metallic salts do not possess a similar property. Magnesium and calcium salts are largely insoluble in water, while lead soaps of the saturated acids are insoluble in alcohol. This fact affords a method of separation of the saturated from the unsaturated acids, since the lead soaps of the latter are alcohol-soluble.

Most of the fatty acids dissolve in absolute or 95% ethyl alcohol, although this is the case only for palmitic acid and higher members of the series when the alcohol is hot. All fatty acids are soluble in diethyl ether, chloroform, benzene, and petroleum ether, although the last solvent is not a satisfactory one for hydroxy-acids.

(3) *Monoethenoid Acids*

This series is comprised of the simplest group of unsaturated acids which contain only one double bond. The empirical formula for the series is C_nH_{2n-2}O₂. It includes only acids having an even number of carbon atoms. The acids having a chain shorter than 10 carbon atoms are not represented among the acids found naturally. A number of isomers of several of the acids occur naturally where the unsaturated bond is located at different positions in the molecule. Table 2 lists the common acids in this group.

¹⁰ A. W. Weitkamp, A. M. Smiljanic, and S. Rothman *J. Am. Chem. Soc.*, 69, 1936-1939 (1947).

TABLE I
 SATURATED FATTY ACIDS MOST COMMONLY FOUND IN NATURAL FATS^a

Common name	Systematic name	Formula	Discovery			Additional common sources
			Date	Investigator	Source	
Butyric	<i>n</i> -Butanoic	$\text{C}_4\text{H}_7(\text{CH}_2)_2\text{COOH}$	1819	Chevreul ^b	Butter fat	Coconut oil, babassu fat, palm oil Coconut oil, palm oil, seed oils Coconut oil, head oil of sperm whale Seed fats of laurel family and <i>Palmae</i> ; milk fat, coconut oil Most animal and vegetable fats; nutmeg butter, fatty acids of <i>Myristicaceae</i> In practically all animal and vegetable fats examined Usually wherever palmitic acid is present Rambutan tallow (<i>Nephelium lappa- ceum</i>), pulasari tallow (<i>N. mutabile</i>), macassar nut fat (<i>Schleichera trijuga</i>), fish oils Peanut, rapeseed, and mustard oils
Caproic	<i>n</i> -Hexanoic	$\text{C}_6\text{H}_{11}(\text{CH}_2)_4\text{COOH}$	1819	Chevreul ^b	Butter fat	
Caprylic	<i>n</i> -Octanoic	$\text{C}_8\text{H}_{15}(\text{CH}_2)_6\text{COOH}$	1844	Lerch ^c	Butter fat	
Capric	<i>n</i> -Decanoic	$\text{C}_{10}\text{H}_{19}(\text{CH}_2)_8\text{COOH}$	1819	Chevreul ^b	Butter fat	
Lauric	<i>n</i> -Dodecanoic	$\text{C}_{12}\text{H}_{23}(\text{CH}_2)_{10}\text{COOH}$	1842	Marsson ^d	Laurel kernel oil	
Myristic	<i>n</i> -Tetradecanoic	$\text{C}_{14}\text{H}_{27}(\text{CH}_2)_{12}\text{COOH}$	1841	Playfair ^e	Nutmeg fat	
Palmitic	<i>n</i> -Hexadecanoic	$\text{C}_{16}\text{H}_{31}(\text{CH}_2)_{14}\text{COOH}$	1816	Chevreul ^b	Lard	
Stearic	<i>n</i> -Octadecanoic	$\text{C}_{18}\text{H}_{35}(\text{CH}_2)_{16}\text{COOH}$	1816	Chevreul ^b	Mutton tallow	
Arachidic	<i>n</i> -Eicosanoic	$\text{C}_{20}\text{H}_{39}(\text{CH}_2)_{18}\text{COOH}$	1854	Gössmann ^f	Peanut oil	
Behenic	<i>n</i> -Docosanoic	$\text{C}_{22}\text{H}_{43}(\text{CH}_2)_{20}\text{COOH}$	1848	Völkero ^g	Behen oil from horse-radish tree (<i>Moringa oleifera</i> Lam.)	
Lignoceric	<i>n</i> -Tetracosanoic	$\text{C}_{24}\text{H}_{47}(\text{CH}_2)_{22}\text{COOH}$	1880	Hell and Hermanns ^h	Beech-tar paraffin	
Cerotic	<i>n</i> -Hexacosanoic	$\text{C}_{26}\text{H}_{51}(\text{CH}_2)_{24}\text{COOH}$	1848	Brodie ⁱ	Chinese insect wax (<i>Coccus ceriferus</i>)	
Montanic	<i>n</i> -Octacosanoic	$\text{C}_{28}\text{H}_{55}(\text{CH}_2)_{26}\text{COOH}$	1884	Nafziger ^j	Beeswax	
Melissic	<i>n</i> -Triacontanoic	$\text{C}_{30}\text{H}_{59}(\text{CH}_2)_{28}\text{COOH}$	1884	Nafziger ^j	Beeswax	
Lacceroic	<i>n</i> -Dotriacontanoic	$\text{C}_{32}\text{H}_{63}(\text{CH}_2)_{30}\text{COOH}$	1914	Gascard ^k	Stick-lac wax (<i>Ta- chardia lacca</i>)	

^a Adapted from K. S. Markley, *Fatty Acids*, Interscience, p. 21.

^b M. E. Chevreul, *Recherches chimiques sur les corps gras d'origine animale*, Levrault, Paris, 1823; cited by A. W. Ralston, *op. cit.*, pp. 19, 27, 35, 40.

^c J. N. Lerch, *Ann.*, 49, 212-231 (1844).

^d T. Marsson, *Ann.*, 41, 329-336 (1842).

^e L. Playfair, *Ann.*, 37, 152-164 (1841).

^f A. Gössmann, *Ann.*, 89, 1-11 (1854).

^g A. Völkero, *Ann.*, 64, 342-346 (1848).

^h C. Hell, *Ber.*, 13, 1709-1713 (1880).

ⁱ C. Hell and O. Hermanns, *Ber.*, 13, 1713-1721 (1880).

^j B. C. Brodie, *Ann.*, 67, 180-214 (1848).

^k F. Nafziger, *Ann.*, 224, 225-258 (1884).

^l A. Gascard, *Compt. rend.*, 159, 258-260 (1914).

All of the members of this series have melting points considerably lower than those of the corresponding saturated acids. The main reason for the fact that vegetable oils are liquid while most animal fats are solid is to be traced to the much greater proportion of unsaturated acids in the former.

In addition to the acids listed in Table 2, one probably should include crotonic acid ($\text{CH}_3\text{CH}=\text{CHCOOH}$ or 2-butenoic acid). This is the simplest monoethenoid acid found naturally, but because it is present only in the inedible croton oil (from which it is named) it is not of sufficient importance to be listed. However, it is an acid which is readily metabolized.¹¹ It can be synthesized from the important physiological compound, β -hydroxybutyric acid ($\text{CH}_3\text{CHOHCH}_2\text{COOH}$), while Blunden¹¹ found that the reverse transformation could be demonstrated in the fasting rat.

a. Octadecenoic Acids. Oleic acid, or 9-octadecenoic acid, is the commonest one of the monoethenoid series. This acid is so widely distributed in natural oils as to be considered the predominant acid. It frequently comprises as much as 50% of the total acid content of oils, and seldom constitutes less than 10% of the total fatty acid content. It is present to the extent of nearly 85% in olive oil,¹² and of 74% in cashew kernel oil (*Anacardium occidentale*).¹³ Oleic acid is widely distributed in animal fats, as well as in the vegetable oils. It seems to be the chief component of the fats of warm-blooded animals, in contrast to 9-hexadecenoic acid (palmitoleic acid) which is largely present in the fat of cold-blooded animals. The favored position for the unsaturated linkage would seem to be between carbons 9 and 10. Not only does the double bond occur in this position in oleic and palmitoleic acids, which from a quantitative standpoint account for most of the monoethenoid acids, but it is found at the same position in four other acids (including gadoleic acid) where the chain length varies from 10 to 20 carbon atoms.

Because of the presence of a double bond in the molecule, the monoethenoid acids can exist in either a *cis* or a *trans* form. Oleic acid is the *cis* isomer of 9-octadecenoic acid, while elaidic acid is its *trans* isomer. Although the *cis* \rightarrow *trans* rearrangement may be readily accomplished by chemical means, it apparently cannot be brought about in the animal organism. Elaidic acid is never found in natural products; however, if fed to animals, it may be laid down in the depot fat. Most of the monoethenoid acids occur only in the *cis* form. This is the case with palmitoleic,

¹¹ H. D. Blunden, *The Intermediary Metabolism of Butyric Acid as Determined from the Physiological Behavior of the Various Four-Carbon Acids*, Dissertation, Dept. Biochemistry, Univ. Southern California, April, 1938.

¹² G. S. Jamieson and W. F. Baughman, *J. Oil & Fat Ind.*, 2, 40-44 (1925); *Chem. Abst.*, 20, 2083-2084 (1926).

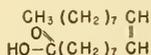
¹³ C. K. Patel, J. J. Sudborough, and H. E. Watson, *J. Indian Inst. Sci.*, Part 6, 8, 111-129 (1923); *Chem. Abst.*, 17, 3616 (1923).

TABLE 2
MONOETHENOIC ACIDS MOST COMMONLY FOUND IN NATURAL FATS

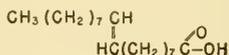
Common name	Systematic name	Empirical formula	Discovery		Additional common sources
			Date	Investigator	
Obtusilic	4-Decenoic	$C_{16}H_{32}O_2$	1937	Toyama ^a	Tohaku from <i>Lindera obtusiloba</i> (Korean spice bush, benzoin) Butter fat
Caprolic	9-Decenoic	$C_{16}H_{32}O_2$	1912	Shredley ^b	Milk fat of human, goat; sperm head oil
Linderic	4-Dodecenoic	$C_{12}H_{24}O_2$	1921	Iwamoto ^c	Tohaku oil Formosan seed oils; <i>Lindera obtusiloba</i> (spice-bush)
Laurolic	5-Dodecenoic	$C_{12}H_{24}O_2$	1918	Lexow ^d	Sperm blubber and head oil
—	9-Dodecenoic	$C_{12}H_{24}O_2$	1885	Raimann ^e	Cows milk fat
Tsuzuic	4-Tetradecenoic	$C_{14}H_{28}O_2$	1926	Tsujimoto ^{f,g}	<i>Lindera hipoglauca</i> (benzoin), <i>L. obtusiloba</i> (spice-bush)
—	5-Tetradecenoic	$C_{14}H_{28}O_2$	1923	Tsujimoto ^h	Whale head oil, sardine
Myristolic	9-Tetradecenoic	$C_{14}H_{28}O_2$	1925	Armstrong and Hilditch ⁱ	Shark liver oil, cet oil, turtle oil, human milk fat, depot fats
Palmitolic ("Phytolic")	9-Hexadecenoic	$C_{16}H_{32}O_2$	1854	Hofstädter ^j	Milk fat, seed oils, marine oils
Petroselinic	6-Octadecenoic	$C_{18}H_{36}O_2$	1909	Vongerichten and Köhler ^k	Sperm head oil (<i>Physeter macrocephalus</i> Shaw) Parsley seed oil
Oleic	9-Octadecenoic	$C_{18}H_{36}O_2$	1815	Chevreul ^l	Coriander, <i>Umbelliferae</i>
Vaccenic	11-Octadecenoic	$C_{18}H_{36}O_2$	1844	Lereh ^m	Most fats and oils
—	12-Octadecenoic	$C_{18}H_{36}O_2$	1928	Bertram ⁿ	Mutton fat, lard, margarine
—	—	—	1928	Bauer and Mitsotakis ^o	Partially hydrogenated peanut oil Hydrogenated oils

Common name	Systematic name	Empirical formula	Date	Investigator	Source	Additional common sources
Gadoleic	9-Eicosenoic	$C_{20}H_{38}O_2$	1906	Bull ¹	Cod-liver oil	Many fish and marine oils
—	11-Eicosenoic	$C_{20}H_{38}O_2$	1933	Greene and Foster ²	Jojoba oil (<i>Simmondsia chinensis</i> (californica))	
Cetoleic	11-Docosenoic	$C_{22}H_{42}O_2$	1927	Toyama ³	Marine oil	Shark liver, herring, sardine and other marine oils
Erucic	13-Docosenoic	$C_{22}H_{42}O_2$	1849	Darby ⁴	Mustardseed oil (<i>Brassica alba</i> , <i>B. nigra</i>)	Other <i>Cruciferae</i>
Selaeholic	15-Tetracosenoic	$C_{24}H_{46}O_2$	1926	Tsujiimoto ⁵	Shark liver oil from spiny dog-fish (<i>Centrophorus lusitanicus</i>)	Brain cerebrosides (nervonic acid)
Ximenic	17-Hexacosenoic	$C_{26}H_{50}O_2$	1937	Puntambekar and Krishna ⁶	<i>Ximenia americana</i> (tallow-wood)	
Lumecic	21-Triacontenoic	$C_{30}H_{58}O_2$	1939	Boekenoogen ⁷	<i>Ximenia americana</i>	
^a Y. Toyama, <i>J. Soc. Chem. Ind., Japan</i> , 40, suppl., 285-289 (1937); <i>Chem. Abst.</i> , 31, 8969 (1937).						
^b I. Smedley, <i>Biochem. J.</i> , 6, 451-461 (1912).						
^c Y. Iwamoto, <i>J. Soc. Chem. Ind., Japan</i> , 24, 1143-1160 (1921).						
^d T. Lexow, <i>Tids. Kemi</i> , 15, 309-324 (1918); <i>Chem. Abst.</i> , 13, 1648 (1919).						
^e E. Rainmann, <i>Monatsh.</i> , 6, 891-898 (1885).						
^f M. Tsujimoto, <i>J. Soc. Chem. Ind. Japan</i> , 29, 105-108 (1926); <i>Chem. Abst.</i> , 20, 2420 (1926).						
^g M. Tsujimoto, <i>Bull. Inst. Tokyo Ind. Research Lab.</i> [3], 23, 53-60 (1928); <i>Chem. Abst.</i> , 23, 1109 (1929); <i>Chem. Umschau</i> , 35, 225-227 (1928); <i>Chem. Abst.</i> , 22, 4470 (1928).						
^h M. Tsujimoto, <i>Chem. Umschau</i> , 30, 33-36 (1923); <i>Chem. Abst.</i> , 17, 3618 (1923).						
ⁱ E. F. Armstrong and T. P. Hilditch, <i>J. Soc. Chem. Ind.</i> , 44, 180-189T (1925).						
^j P. G. Hofstädter, <i>Ann.</i> , 91, 177-185 (1854).						
^k E. Vongerichten and A. Köhler, <i>Ber.</i> , 42, 1638-1639 (1909).						
^l M. E. Chevreul, <i>Recherches chimiques sur les corps gras d'origine animale</i> , Levrault, Paris, 1823. Cited by A. W. Ralston. ⁸						
^m J. N. Lerch, <i>Ann.</i> , 49, 212-231 (1844).						
ⁿ S. H. Bertram, <i>Biochem. Z.</i> , 197, 433-441 (1928).						
^o K. H. Bauer and J. Mitsotakis, <i>Chem. Umschau</i> , 35, 137-139 (1928); <i>Chem. Abst.</i> , 22, 3133 (1928).						
^p H. Bull, <i>Ber.</i> , 39, 3570-3576 (1906).						
^q R. A. Greene and E. O. Foster, <i>Botan. Gaz.</i> , 94, 826-828 (1933). T. G. Green, T. P. Hilditch, and W. J. Stainsby, <i>J. Chem. Soc.</i> , 1936, 1750-1755.						
^r Y. Toyama, <i>J. Soc. Chem. Ind. Japan</i> , 30, 597-602 (1927); <i>Chem. Abst.</i> , 22, 575 (1928).						
^s S. Darby, <i>Ann.</i> , 69, 1-8 (1849).						
^t M. Tsujimoto, <i>J. Soc. Chem. Ind. Japan</i> , 29, 67-71 (1926); <i>Chem. Abst.</i> , 20, 2421 (1926).						
^u S. V. Puntambekar and S. Krishna, <i>J. Indian Chem. Soc.</i> , 14, 268-274 (1937); <i>Chem. Abst.</i> , 31, 8970 (1937).						
^v H. A. Boekenoogen, <i>Fette u. Seifen</i> , 46, 717-719 (1939); <i>Chem. Abst.</i> , 34, 3521 (1940).						

petroselinic, erucic, and selacholeic acids. Since the *cis* compounds are shorter than the *trans* ones, it is known that they are bent back on themselves as indicated in the formulas for oleic and elaidic acids. For a further discussion of *cis-trans* isomerism in the fatty acid, the reader is referred to page 85.



Oleic acid



Elaidic acid

b. Vaccenic Acid. An isomer of oleic acid, vaccenic acid (11-octadecenoic acid), is of especial interest because of its occurrence in animal fats such as butter, lard, mutton, and beef fats¹⁴⁻¹⁶ and its absence from such vegetable oils as corn, cottonseed, soybean, and coconut.¹⁶ However, it is present in hydrogenated vegetable fats. Grossfeld and Simmer¹⁵ report 1.49% of vaccenic acid in margarine, which is in the range of values found by these authors for butter. It has been suggested that vaccenic acid possesses growth-stimulating properties,¹⁷ but this hypothesis has been shown by later work to be fallacious.^{18-20, 20a}

Bertram¹⁴ was of the opinion that natural vaccenic acid possesses a *trans* configuration. This hypothesis has been shown to be correct, on the basis of a recent study of Rao and Daubert.²¹

c. Hexadecenoic Acid. Palmitoleic acid so named because of its analogy to palmitic acid. It is found in marine animal oils in amounts of 15 to 20%, while the depot fats of amphibia and reptiles contain 8 to 15% of this compound. The depot fats of birds are reported to have 6 to 8% of this acid. Mammalian fats contain much less (liver fats 6-8%; depot fats 2-3%), while the vegetable oils have only a small proportion of the acid (usually less than 1%). Palmitoleic acid has also been shown to occur in

¹⁴ S. H. Bertram, *Biochem. Z.*, 197, 433-441 (1928).

¹⁵ J. Grossfeld and A. Simmer, *Z. Untersuch. Lebensm.*, 59, 237-258 (1930).

¹⁶ R. P. Geyer, H. Nath, V. H. Barki, C. A. Elvehjem, and E. B. Hart, *J. Biol. Chem.*, 169, 227-228 (1947).

¹⁷ J. Boer, B. C. P. Jansen, and A. Kentie, *Nature*, 158, 201 (1946).

¹⁸ H. J. Deuel, Jr., S. M. Greenberg, E. Straub, D. Jue, C. M. Gooding, and C. F. Brown, *J. Nutrition*, 35, 301-314 (1948).

¹⁹ B. v. Euler, H. v. Euler, and G. Lindeman, *Arkiv Kemi Mineral. Geol.*, B26, No. 3, 1-5 (March 19, 1948).

²⁰ H. Nath, V. H. Barki, C. A. Elvehjem, and E. B. Hart, *J. Nutrition*, 36, 761-772 (1948).

^{20a} J. Boer, E. H. Groot, and B. C. P. Jansen, *Voeding*, 9, 60-62 (1948); *Chem. Abst.*, 42, 7847 (1948).

²¹ P. C. Rao and B. F. Daubert, *J. Am. Chem. Soc.*, 70, 1102-1104 (1948).

²² T. P. Hilditch and H. Jaspersen, *Biochem. J.*, 37, 238-243 (1943).

²³ J. B. Brown and B. M. Orians, *Arch. Biochem.*, 9, 201-219 (1946).

²⁴ R. W. Riemenschneider and N. R. Ellis, *J. Biol. Chem.*, 114, 441-447 (1936).

²⁵ T. P. Hilditch and M. L. Meara, *Biochem. J.*, 38, 29-31 (1944).

milk fats,²²⁻²⁵ butter fat,²⁴ and in egg-yolk oil.²⁶ Bosworth and Brown,²⁷ however, feel that the evidence for the occurrence of palmitoleic acid in milk fatty acids is doubtful. The vegetable phosphatides contain 5 to 10% of this fatty acid.²⁸ Riebsomer and Johnson²⁹ found as much as 35% of the unsaturated acids of *Lycopodium clavatum* (ground pine) spores to consist of palmitoleic acid, and 60% of 9-octadecenoic acid, while another primitive form, a sea alga (*Cystophyllum hakodatense* Yendo), has been shown to contain more than 20% of palmitoleic acid.³⁰

d. Other Monoethenoid Acids. The chief sources of the other monoethenoid acids are the marine oils. Thus, the head oil of the sperm whale (*Physeter macrocephalus*) contains decenoic, dodecenoic, and tetradecenoic acids, while 9-eicosenoic acid, or gadoleic acid, has been reported in cod liver oil, whale, herring, and sardine oils. It may also be obtained from many other marine sources. The elasmobranch fish oils contain one of the C₂₄ monoethenoid acids, 15-tetracosenoic acid, while a hexacosenoic acid has been reported in the body oil of the castor oil fish. Milk fat is the source of fats of the C₁₀, C₁₂, and C₁₈ series. Erucic acid, 13-docosenoic acid, makes up 40 to 50% of rapeseed, mustardseed, and wallflowerseed oils, while it almost completely accounts for the fatty acid content of nasturtium oil (80%).

(4) Polyethenoid Acids

The acids which have more than one double bond are of great importance in animal nutrition, as well as for industrial uses. They include the so-called "essential" fatty acids which are required by the animal since they cannot be synthesized from other fatty acids or from carbohydrates. They are of considerable importance in the paint industry, as well as wherever drying oils are employed. Moreover, it is by virtue of the relative ease with which such acids can be hydrogenated that the margarine and vegetable shortening industries have been able to develop.

Only one representative of the diethenoid series is found ordinarily in fats. It is linoleic acid, CH₃(CH₂)₄CH:CHCH₂CH:CH(CH₂)₇COOH, which has the empirical type formula C_nH_{2n-4}O₂. Three triethenoid acids are fairly common, one of which is a C₁₆ (hiragonic) and the other two C₁₈ acids (linolenic and elaeostearic). These have empirical formulas of C_nH_{2n-6}O₂. Of the two tetraethenoid acids, arachidonic acid is better known as a component of depot fats. Two more highly unsaturated acids

²⁶ F. Trost and B. Doro, *Ann. chim. applicata*, 27, 233-242 (1937); *Chem. Abst.*, 31 8233 (1937).

²⁷ A. W. Bosworth and J. B. Brown, *J. Biol. Chem.*, 103, 115-134 (1933).

²⁸ T. P. Hilditch and W. H. Pedely, *Biochem. J.*, 31, 1964-1972 (1937).

²⁹ J. L. Riebsomer and J. R. Johnson, *J. Am. Chem. Soc.*, 55, 3352-3357 (1933).

³⁰ E. Takahashi, K. Shirahama, and N. Ito, *J. Chem. Soc. Japan*, 59, 622-666 (1938).

TABLE 3
DIETHENOID AND POLYETHENOID ACIDS MOST COMMONLY FOUND IN NATURAL FATS

Common name	Systematic name	Empirical formula	First isolated			Source	Additional common sources
			Date	Investigator	Source		
Linoleic	9,12-Octadecadienoic	$C_{18}H_{32}O_2$	1844 1886	Sacc ^a Peters ^b	Linseed oil	Most seed fats	
Hiragonic	6,10,14-Hexadecatrienoic	$C_{16}H_{28}O_2$	1929	Toyama and Tsuchiya ^c	Sardine oil		
Linolenic	9,12,15-Octadecatrienoic	$C_{18}H_{30}O_2$	1887	Hazura and Friedreich ^d	Hempseed oil	Linseed oil, walnut oil, soybean oil, seed oils	
Elaeostearic	9,11,13-Octadecatrienoic	$C_{18}H_{30}O_2$	1876	Cloëz ^e	Japanese tung oil (<i>Elaeococca vernicia</i>)	Florida tung oil, baglumbang nut (<i>Aleurites trisperma</i>), esung-seed oil (<i>Ricinodendron africanum</i>)	
Moroctic	4,8,12,15-Octadecatetraenoic	$C_{18}H_{28}O_2$	1906	Tsujimoto, ^f Toyama, and Tsuchiya ^g	Fish oil	Sardine oil	
Parinaric	9,11,13,15-Octadecatetraenoic	$C_{18}H_{28}O_2$	1933	Tsujimoto and Koyanagi ^h	(<i>Parinarium lativinum</i>) "Akamittom" seed fat		
Arachidonic	5,8,11,14-Eicosatetraenoic	$C_{20}H_{32}O_2$	1909	Hartley ⁱ	Liver lipid	Brain, egg lecithin, glandular organs	
—	4,8,12,16-Eicosatetraenoic	$C_{20}H_{32}O_2$	1935	Toyama and Tsuchiya ^j	Sardine oil	Whale oil	

Common name	Systematic name	Empirical formula	First isolated		Additional common source
			Date	Investigator	
Timnodonic	4,8,12,15,18-Eicosapentaenoic	$C_{20}H_{30}O_2$	1935	Toyama and Tsuchiya ^a	Sardine oil
Clupanodonic	4,8,12,15,19-Docosapentaenoic	$C_{22}H_{34}O_2$	1926	Tsujimoto ^b	Sardine oil
Nisimic	4,8,12,15,18,21-Tetracosahexaenoic	$C_{24}H_{36}O_2$	1935	Toyama and Tsuchiya ^c	Sardine oil
Thynninc	?-Hexacosahexaenoic	$C_{26}H_{40}O_2$	1936	Ueno and Yonase ^d	Tunny oil

^a F. Sacc, *Ann.*, 51, 213-230 (1844)

^b K. Peters, *Monatsh.*, 7, 552-555 (1886)

^c Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 4, 83-91 (1929)

^d K. Hazura, *Monatsh.*, 8, 147-155 (1887); K. Hazura and A. Friedreich, *ibid.*, 8, 156-164 (1887); K. Hazura, *ibid.*, 8, 260-270 (1887).

^e S. Clôz, *Compt. rend.*, 82, 501-504 (1876); *Bull. soc. chim.* [2], 26, 286-287 (1876); [2], 28, 23-24 (1877).

^f M. Tsujimoto, *J. Coll. Eng. Tokyo Imp. Univ.*, 4, No. 1 (1906), cited by A. W. Ralston, p. 143.

^g Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 10, 232-241 (1935).

^h M. Tsujimoto and H. Koyanagi, *J. Soc. Chem. Ind. Japan*, 36, suppl., 110-113 (1933); *Chem. Abst.*, 27, 3099 (1933), cited by A. W. Ralston, p. 150.

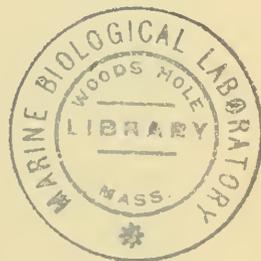
ⁱ P. Hartley, *J. Physiol.*, 38, 353-374 (1909).

^j Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 10, 241-248, 296-300, 301-304 (1935).

^k M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, 23, 1007-1010 (1920); *Chem. Abst.*, 15, 1227 (1921).

^l Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 10, 543-547, 547-551 (1935).

^m S. Ueno and C. Yonase, *J. Chem. Soc. Japan*, 57, 180-182 (1936); *Chem. Abst.*, 30, 5061 (1936).



from marine oils have been prepared, namely clupanodonic acid, which has 5 double bonds, and nisinic acid, a hexaethenoid acid from Japanese sardine oil (*Clupanodon melanostica*). The term, clupanodonic acid, was first applied by Tsujimoto³¹ to a $C_{18}H_{28}O_2$ acid; he later concluded that it should be assigned to the $C_{22}H_{34}O_2$ compound.³² Toyama and Tsuchiya³³ gave the name *moroctic acid* to the C_{18} acid. The data on these acids are given in Table 3.

a. Linoleic Acid. From a quantitative standpoint, linoleic acid is the most important of the polyethenoid acids found in vegetable oils. In a number of the less common vegetable oils, this acid may comprise as high as 70 to 80% of the total fatty acid (grapeseed, hackberry tree acid, tobaccoseed, Thom apple, and walnut), while many of the common food oils may have 50% or more of this acid (hempseed, poppyseed, safflowerseed, sunflowerseed, cottonseed, and soybean oils). Contrasted with these values, the amount which occurs in animal fats is quite low unless diets containing liberal amounts of linoleic acid are ingested (see Table 22, Chapter III).

According to Bosworth and Brown,²⁷ linoleic acid is probably absent from butter fat. Neither linoleic acid nor any other diethenoid acid is normally found in the fat of marine animals. The absence of large amounts of this acid in animal fats is apparently related to the inability of the animal cells to synthesize acids with more than one unsaturated linkage or, at least, to form it at a rate demanded by the growing organism.³⁴

The generally accepted formula for linoleic acid, 9,12-octadecadienoic acid, has been arrived at by a study of its oxidation products. Tetrahydroxystearic acid is first formed, but this breaks down to cleavage products.³⁵⁻³⁷ The suggestion that this acid is 9,14-octadecadienoic acid³⁸ has been adequately refuted by Arcus and Smedley-MacLean,³⁹ who used an ozonolysis technic similar to that of the Japanese investigator, and still obtained results in accord with the generally accepted structure.

Since linoleic acid has two ethylenic bonds, there are four possible

³¹ M. Tsujimoto, *J. Coll. Eng. Tokyo Imp. Univ.*, 4, No. 1 (1906); cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 143.

³² M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, 23, 1007-1010 (1920); *Chem. Abst.*, 15, 1227 (1921).

³³ Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 10, 232-241 (1935).

³⁴ H. H. Williams and W. E. Anderson, *Oil & Soap*, 12, 42-44 (1935).

³⁵ G. L. Goldsobel, *J. Russ. Phys. Chem. Soc.*, 38, 900-901 (1906); cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 126.

³⁶ T. Maruyama, *J. Chem. Soc. Japan*, 54, 1073-1081 (1933); *Chem. Abst.*, 28, 1015 (1934).

³⁷ L. C. A. Nunn and I. Smedley-MacLean, *Biochem. J.*, 29, 2742-2745 (1935).

³⁸ K. Takahashi, *J. Chem. Soc. Japan*, 42, 130-141 (1921); *Abst. Chem. Papers*, 120, Part I, 303 (1921); *Chem. Abst.*, 15, 2273-2274 (1921). Cited by C. L. Arcus and I. Smedley-MacLean, *Biochem. J.*, 37, 1-6 (1943).

³⁹ C. L. Arcus and I. Smedley-MacLean, *Biochem. J.*, 37, 1-6 (1943).

geometrical isomers: *cis-cis*; *cis-trans*; *trans-cis*; and *trans-trans*. Naturally occurring linoleic acid (α -linoleic acid) is the *cis-cis* isomer. The results of Brown and Frankel⁴⁰ seem to indicate that only one type of linoleic acid is present in the natural seed oils; this view is supported by the findings of McCutcheon.⁴¹ The β -acid, which is also known as isolinoleic acid, has a *trans-trans* structure, according to Suzuki *et al.*^{42,43} and Maruyama.⁴⁴ On elaidinization with Poutet's reagent or selenium, according to Kass and Burr,⁴⁵ two geometrical isomers resulted, namely *trans-9-trans-12*-linolelaidic acid and *trans-9-cis-12*-linolelaidic acid. This indicates that the double bond on carbon 12 cannot elaidinize without a previous change on the 9 carbon. Since no *cis-9-cis-12*-octadecadienoic acid was found, Kass and Burr concluded that the change does not involve an equilibrium reaction.

The presence of the unsaturated bonds enables linoleic acid to take up oxygen from the air. The fat, especially when spread in a thin layer, forms a hard and glossy surface, which is the chemical reaction taking place when paint hardens. Linoleic acid is generally credited with being the most important component in drying oils. In order to serve satisfactorily in this capacity, however, commercial oils should contain 40 to 65% of linoleic acid. Fats having less than 25% of linoleic acid are considered unsuitable for use in paints. Another requirement for a satisfactory drying oil is that it also contain appreciable amounts of the triethenoid acid, linolenic, in addition to the necessary proportion of linoleic acid. Only linseed, perilla, and hempseed oils meet these qualifications. When less than 15% of linolenic acid is present, the oils are unsatisfactory for paints, even though the diethenoid acid constitutes a considerable proportion of the fatty acids. Even walnut oil, in which linoleic acid values as high as 75% have been reported, but in which the linolenic acid content is only 2 to 10%,⁴⁶⁻⁴⁹ has a limited application in the drying oil industry.

Vegetable oils having the requisite quantity of linoleic acid but lacking a sufficient quota of linolenic acid have only limited application as drying oils, and hence are referred to as *semidrying oils*.

⁴⁰ J. B. Brown and J. Frankel, *J. Am. Chem. Soc.*, **60**, 54-56 (1938).

⁴¹ J. W. McCutcheon, *Can. J. Research*, **B16**, 158-175 (1938).

⁴² Y. Inoue and B. Suzuki, *Proc. Imp. Acad. Tokyo*, **7**, 15-18 (1931).

⁴³ T. Maruyama and B. Suzuki, *Proc. Imp. Acad. Tokyo*, **7**, 379-382 (1931); **8**, 186-189 (1932).

⁴⁴ T. Maruyama, *J. Chem. Soc. Japan*, **54**, 1082-1087 (1933); *Chem. Abst.*, **28**, 1015 (1934).

⁴⁵ J. P. Kass and G. O. Burr, *J. Am. Chem. Soc.*, **61**, 1062-1066 (1939).

⁴⁶ S. L. Ivanov and E. Berdichevski, *Schriften zentral. biochem. Forsch. Inst. Nahr. u. Genussmittelind., U. S. S. R.*, **3**, 246-250 (1933); *Chem. Abst.*, **28**, 2557 (1934).

⁴⁷ G. S. Jamieson and R. S. McKinney, *Oil & Soap*, **13**, 202 (1936).

⁴⁸ G. S. Jamieson and R. S. McKinney, *Oil & Fat Industries*, **6**, No. 2, 21-23 (1929).

⁴⁹ H. N. Griffiths and T. P. Hilditch, *J. Soc. Chem. Ind.*, **53**, 75-81T (1934).

b. Linolenic Acid. Another polyethenoid acid of especial importance frequently associated with linoleic acid is linolenic acid, which has three unsaturated linkages. According to the systematic nomenclature, this is 9,12,15-octadecatrienoic acid, which has the formula, $\text{CH}_3\text{CH}_2\text{CH}:\text{-CHCH}_2\text{CH}:\text{CHCH}_2\text{CH}:\text{CH}(\text{C}'\text{H}_2)_7\text{COOH}$.

It would appear that the oils which contain appreciable amounts of linolenic acid are extremely limited, although this acid may be present in small amounts in a number of oils. It is difficult to determine low concentrations of linolenic acid in the presence of large quantities of linoleic acid.

The best sources of linolenic acid are the vegetable fats. In the case of linseed oil, values as high as 60% have been reported, although the average is much lower and results as low as 26% have been recorded.¹ Appreciable amounts (25% or over) have been reported for hempseed and perilla oils. The animal fats are usually practically devoid of linolenic acid, as this substance is another acid which cannot be synthesized by the animal organism. However, as much as 17% of the fat in egg-yolk obtained after feeding linseed oil to chickens has been shown to be linolenic acid, while maximum values of 10% were found when hempseed oil was incorporated in the feed of the hens.⁵⁰ Small amounts of linolenic acid have also been reported in butter fat⁵¹ but the content in this case must be related to the diet of the cow.

There are eight possibilities for geometrical isomers of linolenic acid but only one form is known to occur naturally. This so-called α -linolenic acid is the all-*cis* form (*cis-cis-cis*). According to McCutcheon, Crawford, and Welch,⁵² the spectroscopic pattern in the infrared area indicates that natural linolenic acid contains only *cis* linkages. All-*trans*-linolenic acid (*trans-trans-trans*) can readily be prepared from natural linolenic acid by treatment with oxides of nitrogen. It is called linolenelaidic acid or elaidolinolenic acid. As far as it is known, it does not occur naturally.

Linolenic acid is just as satisfactory as linoleic acid in serving as a source of the essential fatty acid requirement in animal nutrition, but only when sparked by the latter acid.^{52a} The importance in the painting industry as a drying oil has been discussed under linoleic acid.

c. Elaeostearic Acid. This acid is another member of the triethenoid series which is an isomer of linolenic acid. However, in contradistinction to the latter acid, the double bonds are arranged in alternate (or conjugate) positions, a fact which seems to confer special properties on this

⁵⁰ E. M. Cruikshank, *Biochem. J.*, **28**, 965-977 (1934).

⁵¹ H. C. Eckstein, *J. Biol. Chem.*, **103**, 135-140 (1933).

⁵² J. W. McCutcheon, M. F. Crawford, and H. L. Welch, *Oil & Soap*, **18**, 9-11 (1941).

^{52a} S. M. Greenberg, C. E. Calbert, E. E. Savage, and H. J. Deuel, Jr., *J. Nutrition*, **41**, 473-486 (1950).

acid not possessed by linolenic acid. The structural formula for elaeostearic acid was a matter of dispute until Böesecken and Ravenswaay^{53,54} showed that it contains three ethylenic linkages, as was indicated by the high molecular refraction and the fact that it reacts with more than two molecules of hydrogen. These workers indicated that the acid is 9,11,13-octadecatrienoic acid, which is represented by the formula, $\text{CH}_3(\text{CH}_2)_3\text{CH}:\text{CHCH}:\text{CHCH}:\text{CH}(\text{CH}_2)_7\text{COOH}$. This structure was later confirmed by Eibner and Rossmann.⁵⁵

Elaeostearic acid makes up over 94% of the total fatty acids in Florida tung oil,⁵⁶ while values of 74.5 to 76.7% have been reported for China tung oil^{57,58} and 70.5% for Japanese tung oil.⁵⁹ It is also a major component of bagilumbang (*Aleurites trisperma*) nut oil,⁶⁰ and essang (*Ricinodendron africanum* Müller) seed oil.⁶¹ Elaeostearic acid is not a normal constituent of animal fats. However, when it is fed to animals, it may be stored in the depot fats, where it has been identified by its spectroscopic absorption. Apparently, the positions of the double bonds cannot be caused to shift to those of linolenic acid in the animal body, since elaeostearic acid, in contradistinction to linolenic acid, is entirely ineffective as a source of the essential fatty acids.⁶²

Eight geometrical isomers are possible in the case of elaeostearic acid, as is the case with linolenic acid. However, the natural compound, α -elaestearic acid, has in all probability an all-*cis* configuration.⁶³ Although most of the mono- and polyethenoid acids are liquid, the two common isomers of elaeostearic acid (α and β) are solids melting at 48–49°C. and 71°C., respectively. The higher melting point over that of linolenic acid, which melts at -11°C ., has been attributed to the arrangement of the double bonds in conjugated position. β -Elaeostearic acid is a natural *cis-trans* isomer. Although the α -isomer can be easily converted to the β -form by light, heat, or such catalysts as iodine or sulfur, the opposite change cannot be effected.

⁵³ J. Böesecken and H. J. Ravenswaay, *Rec. trav. chim.*, *44*, 241–243 (1925).

⁵⁴ J. Böesecken and H. J. Ravenswaay, *Verslag. Akad. Wetenschap. Amsterdam*, *34*, 204–207 (1925); *Proc. Acad. Sci. Amsterdam*, *28*, 386–389 (1925).

⁵⁵ A. Eibner and E. Rossmann, *Chem. Umschau*, *35*, 197–198 (1928); *Chem. Abstr.*, *22*, 4839 (1928); cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 138.

⁵⁶ R. S. McKinney and G. S. Jamieson, *Oil & Soap*, *12*, 92–93 (1935).

⁵⁷ A. Steger and J. Van Loon, *J. Soc. Chem. Ind.*, *47*, 361–363T (1928).

⁵⁸ H. P. Kaufmann and J. Baltes, *Ber.*, *69*, 2676–2679 (1936).

⁵⁹ R. S. McKinney and G. S. Jamieson, *Oil & Soap*, *14*, 2–3 (1937); *Chem. Abstr.*, *31*, 1641 (1937).

⁶⁰ G. S. Jamieson and R. S. McKinney, *Oil & Soap*, *12*, 146–148 (1935).

⁶¹ A. Steger and J. Van Loon, *Rec. trav. chim.*, *54*, 988–994 (1935).

⁶² G. O. Burr, M. M. Burr, and E. S. Miller, *J. Biol. Chem.*, *97*, 1–9 (1932).

⁶³ E. Rossmann, *Chem. Umschau*, *39*, 220–224 (1932); *Chem. Abstr.*, *27*, 702–703 (1933).

Elaeostearic acid and oxygen react with an increased activity as compared with the non-conjugated acids and oxygen. On the other hand, the addition of halogens is retarded by conjugation, and the iodine numbers obtained with elaeostearic acid are considerably below the theoretical value. Because of its ready oxidation, elaeostearic acid is highly prized as a drying oil. Tung oil finds wide application in rapidly drying varnishes and enamels, which are also alkali- and acid-resistant. In addition it has the property of being easily polymerized and of forming gels on heating.

d. Arachidonic Acid. Arachidonic acid is one of the most interesting of the polyethenoid acids. It has four double bonds in non-conjugated positions. Smedley-MacLean and associates,^{69,64} as well as Mowry *et al.*⁶⁵ have shown it to be 5,8,11,14-eicosatetraenoic acid, $\text{CH}_3(\text{CH}_2)_4\text{CH}:\text{CH}-\text{CH}_2\text{CH}:\text{CHCH}_2\text{CH}:\text{CHCH}_2\text{CH}:\text{CH}(\text{CH}_2)_3\text{COOH}$. The name was suggested by Lewkowitsch.⁶⁶

Arachidonic acid is present in animal but not in vegetable oils. It forms a part of liver,^{67,68} egg,⁶⁹ and brain lecithins,⁶⁹ as well as brain cephalin.^{69,70} Wesson⁷¹ has reported the presence of arachidonic acid in tissues and liver from rats, and in organs from dogs, including liver, pancreas, kidney, lung, spleen, and lymph-gland, as well as in muscle fats. This acid is also present in amounts as high as 2.1% in pig depot fat,^{72,73} and it has been found in ox,⁷⁴ duck, goose, chicken,⁷⁵ and human depot fat.⁷⁶⁻⁷⁸ It has likewise been reported as a component of butter fat.⁷⁹ Shinowara and Brown⁸⁰ have suggested that the arachidonic acid present in the phosphatides of beef adrenal tissue is 6,10,14,18-eicosatetraenoic acid; it comprises 20% of the total phosphatides. The latter authors stated that these phospholipids are the best starting material for the preparation of arachidonic acid. However, it should be noted that Arcus and Smedley-

⁶⁴ D. E. Dolby, L. C. A. Nunn, and I. Smedley-MacLean, *Biochem. J.*, *34*, 1422-1426 (1940).

⁶⁵ D. T. Mowry, W. R. Brode, and J. B. Brown, *J. Biol. Chem.*, *142*, 671-678 (1942).

⁶⁶ J. Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, and Waxes*, Vol. I, 6th ed., Macmillan, London (1921), pp. 28, 214, 215, 237, 239.

⁶⁷ R. A. Snider and W. R. Bloor, *J. Biol. Chem.*, *90*, 555-573 (1932).

⁶⁸ P. A. Levene and H. S. Simms, *J. Biol. Chem.*, *48*, 185-196 (1921); *51*, 285-294 (1922).

⁶⁹ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *51* 507-513 (1922); *54*, 91-98; 99-100 (1922); *67*, 659-666 (1926).

⁷⁰ L. G. Wesson, *J. Biol. Chem.*, *60* 183-187 (1924).

⁷¹ L. G. Wesson, *J. Biol. Chem.*, *65*, 235-250 (1925).

⁷² A. Banks and T. P. Hilditch, *Biochem. J.*, *26*, 298-308 (1932).

⁷³ H. K. Dean and T. P. Hilditch, *Biochem. J.*, *27*, 1950-1956 (1933).

⁷⁴ J. B. Brown and E. M. Deck, *J. Am. Chem. Soc.*, *52*, 1135-1138 (1930).

⁷⁵ J. B. Brown and C. C. Sheldon, *J. Am. Chem. Soc.*, *56*, 2149-2151 (1934).

⁷⁶ D. L. Cramer and J. B. Brown, *J. Biol. Chem.*, *151*, 427-438 (1943).

⁷⁷ H. C. Eckstein, *J. Biol. Chem.*, *64*, 797-806 (1925).

⁷⁸ O. Wagner, *Biochem. Z.*, *174*, 412-419 (1926).

⁷⁹ A. W. Bosworth and E. W. Sisson, *J. Biol. Chem.*, *107*, 489-496 (1934).

⁸⁰ G. Y. Shinowara and J. B. Brown, *J. Biol. Chem.*, *134*, 331-340 (1940).

MacLean³⁹ do not agree with the interpretation of the above investigators as to the structure of arachidonic acid.

Arachidonic acid is the only 20-carbon acid which is ordinarily detectable in normal animal fat. Its saturated derivative, arachidic acid, has been reported in hog fat, after the excessive ingestion of peanuts,⁸¹ since peanut oil is an excellent source of this fatty acid.

The source of the arachidonic acid normally present in the animal lipids is undetermined. It is not believed that the animal body can desaturate the ordinary saturated fats to this extent, and moreover arachidic acid is not ordinarily a component of food fats. Since arachidonic acid belongs to the category of essential fatty acids,⁸²⁻⁸⁶ it is obvious that it cannot be synthesized in the animal body *de novo*.

(5) Ethynoic Acids

This class of compounds includes acids which contain a triple bond. Although many ethynoic (or ethinoic) acids have been prepared synthetically, only two are components of natural fats. Most of the triple-bonded acids which are known have the unsaturated linkage either at the α or ω position in the hydrocarbon chain, as for example propyolic or α -propynoic acid, $\text{HC}:\text{CCOOH}$, and dehydroundecylinic or ω -undecynoic acid, $\text{HC}:\text{C}(\text{CH}_2)_8\text{COOH}$. In the case of the three naturally occurring acids which have 18 carbons, the unsaturated linkage (or linkages) occur elsewhere in the molecule. The octadecynoic acids are frequently referred to as stearolic acids.

a. Tariric Acid. Arnaud^{87,88} was the first to prepare tariric acid from *tariri* seed or bitterbush oil (*Picramnia Sow*). It is 6-octadecynoic acid; however, it is more frequently referred to as 6-stearolic acid. The position of the triple bond is established by the fact that on oxidation it yields lauric and adipic acids. The structural formula for this acid is $\text{CH}_3(\text{CH}_2)_{10}\text{C}:\text{C}(\text{CH}_2)_4\text{COOH}$.

The best source of tariric acid is the fat of the seeds of *Picramnia Sow*,⁸⁹ a plant indigenous to Guatemala. Vongerichten and Köhler⁹⁰ have

⁸¹ N. R. Ellis and H. S. Isbell, *J. Biol. Chem.*, 69, 219-238 (1926).

⁸² E. M. Hume, L. C. A. Nunn, I. Smedley-Maclean, and H. H. Smith, *Biochem. J.*, 34, 879-883 (1940).

⁸³ I. Smedley-MacLean and L. C. A. Nunn, *Biochem. J.*, 34, 884-902 (1940).

⁸⁴ O. Turpeinen, *J. Nutrition*, 15, 351-366 (1938).

⁸⁵ G. O. Burr, J. B. Brown, J. P. Kass, and W. O. Lundberg, *Proc. Soc. Exptl. Biol. Med.*, 44, 242-244 (1940).

⁸⁶ F. W. Quackenbush, F. A. Kummerow, and H. Steenbock, *J. Nutrition*, 24, 213-224 (1942).

⁸⁷ A. Arnaud, *Compt. rend.*, 114, 79-81 (1892).

⁸⁸ A. Arnaud, *Bull. soc. chim.* [3], 7, 233-234 (1892).

⁸⁹ A. Steger and J. Van Loon, *Rec. trav. chim.*, 52, 593-600 (1933).

⁹⁰ E. Vongerichten and A. Köhler, *Ber.*, 42, 1638-1639 (1909).

prepared a 6-octadecynoic acid (petroselinic acid) by the action of potassium hydroxide on 6,7-dibromostearic acid, which was obtained by the bromination of petroselinic acid. Although 9-stearolic acid (known simply as stearolic acid) has only been prepared synthetically by debromination of 9,10-dibromostearic acid,⁹¹ it has long been known as a representative of this group. Another triple-bonded acid, behenolic, $\text{CH}_3(\text{CH}_2)_7\text{C}:\text{C}(\text{CH}_2)_{11}\text{COOH}$, has been synthesized from erucic and brassidic acids, which are the *cis* and *trans* forms, respectively, of the monoethynoid C_{22} acid.

b. 6,9-Octadecenynoic Acid. A second naturally occurring triple-bonded acid which also contains a double bond has been isolated from the nut-bearing plant *Ongokea klaineana* (*Onguekoa Gore Engler*) by Steger and Van Loon⁹² in 1937. The positions of the double and triple bonds are not known with certainty, but the acid may be 6-octadecen-9-ynoic or 9-octadecen-6-ynoic acid.

c. Isamic Acid. Steger and Van Loon⁹² have also reported a second ethynoic acid in *Ongokea* oil which possesses two acetylenic and one ethylenic bond. Castille⁹³ confirmed this finding and named it erythro-genic acid. Steger and Van Loon⁹⁴ presented evidence that the structure was $\text{CH}_2:\text{CH}(\text{CH}_2)_4\text{C}:\text{CC}:\text{C}(\text{CH}_2)_7\text{COOH}$, which is one of the two structures suggested by Castille. These workers called the compound *isamic acid*.

(6) Hydroxy- and Keto-Acids

Although such compounds as β -hydroxybutyric acid ($\text{CH}_3\text{CHOHCH}_2\text{COOH}$) and acetoacetic (or diacetic) acid ($\text{CH}_3\text{COCH}_2\text{COOH}$) are well-known decomposition products of the fatty acids, the occurrence of such partially oxidized fatty acids as components of the natural fats is quite limited. The profound importance of such products in the animal is indicated by their presence in such structural lipids as the cerebrosides. The presence of the hydroxyl group confers some special properties upon such acids. Solubility in water is increased when sufficient hydroxyl groups are incorporated in the molecule, while their solubility in ether (especially petroleum ether) and to some extent in alcohol is depressed by the introduction of large numbers of hydroxy groups into the molecule. Moreover, they can be dehydrated to form unsaturated acids. In the case of castor oil, which contains a large proportion of the ester of ricinoleic acid ($\text{C}_2\text{H}_5(\text{CH}_2)_5\text{CHOHCH}_2\text{CH}:\text{CH}(\text{CH}_2)_7\text{COOH}$), conjugated or non-con-

⁹¹ O. Overbeck, *Ann.*, 140, 39-75 (1866).

⁹² A. Steger and J. Van Loon, *Fette u. Seifen*, 44, 243-246 (1937); *Chem. Abst.*, 32, 4366 (1938).

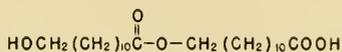
⁹³ A. Castille, *Ann.*, 543, 104-110 (1940).

⁹⁴ A. Steger and J. Van Loon, *Rec. trav. chim.*, 59, 1156-1164 (1940).

jugated linoleic acids may be produced by dehydration, which have considerable use in the paint industry.

The introduction of the hydroxyl group on one of the carbons results in the production of an asymmetric carbon atom. Hence, such acids may frequently be identified and quantitatively determined by their optical rotation. Table 4 lists some of the saturated and unsaturated hydroxy- and keto-acids.

a. Saturated Hydroxy-Acids. The presence of the hydroxyl radical on the fatty acids yields another group in addition to the carboxyl, which may unite with other substances. In the case of sabinic and juniperic acids, the naturally occurring products combine to form an *etholide* in which the properties of alcohols, esters, and acids are combined. They differ from the lactones, in which the union of acid and alcohol groups involves only a single molecule. A typical etholide of sabinic acid would have the structure indicated below.



Sabinic acid etholide

Sabinic acid appears only in the savin juniper (*Juniperus sabina*),⁹⁵ while juniperic acid is present in all the conifer waxes which have been examined. The structure of sabinic acid was established by Bougault⁹⁶ on the basis of its formation of dodecanedioic acid on oxidation and lauric acid on reduction with zinc and acetic acid. The same investigator also established the structure of juniperic acid.

No corresponding myristic acids with a hydroxyl on the terminal carbon are known to occur naturally. Ipurolic acid which is present in the seed fat of the *Ipomea purpurea* (South African morning glory)⁹⁷ was obtained by Asahina *et al.*^{98,99} by acid hydrolysis of pharbitic acid isolated from *Pharbitis nil* Choisy. (Japanese morning glory); it had the structure 3,11-dihydroxymyristic acid.⁵ This was later confirmed.¹⁰⁰ Another monohydroxymyristic acid was obtained by Müller¹⁰¹ from *Angelica archangelica* oil (garden angelica); it is believed to be the 11-hydroxy-acid, but the

⁹⁵ J. Bougault and L. Bourdier, *J. pharm. chim.* [6], 29, 561-573 (1908-1909); 30, 10-16 (1909); *Chem. Abst.*, 3, 2445 (1909).

⁹⁶ J. Bougault, *Compt. rend.*, 150, 874-876 (1910).

⁹⁷ F. B. Power and H. Rogerson, *Am. J. Pharm.*, 80, 251-286 (1908).

⁹⁸ Y. Asahina and S. X. Terada, *J. Pharm. Soc. Japan*, No. 452, 821 (1919); *Chem. Abst.*, 14, 310-311 (1920).

⁹⁹ Y. Asahina and T. Shimidzu, *J. Pharm. Soc. Japan*, No. 479, 1-18 (1922); *Chem. Abst.*, 16, 1936 (1922).

¹⁰⁰ Y. Asahina and S. Nakanishi, *J. Pharm. Soc. Japan*, No. 520, 515-520 (1925); *Chem. Abst.*, 19, 3479 (1925).

¹⁰¹ R. Müller, *Ber.*, 14, 2476-2484 (1881).

TABLE 4
HYDROXY- AND KETO-ACIDS PRESENT IN FOOD FATS

Common name	Systematic name	Empirical formula	Date	Investigator	First discovered		Additional common sources
					Source	Source	
Saturated Acids							
Sabinoic	12-Hydroxydodecanoic	$C_{12}H_{24}O_3$	1909	Bougault and Bourdier ^a	Wax from <i>Juniperus sabina</i> (savin juniper)		
Ipuroic	3,11-Dihydroxytetradecanoic	$C_{14}H_{28}O_4$	1908	Power and Rogerson ^b	Seed fat of <i>Ipomea purpurea</i> Roth (So. African morning glory)	Seed fat of <i>Pharbitis nil</i> Choisi. (Japanese morning glory)	
Convulvulinolic	11-Hydroxypentadecanoic	$C_{15}H_{30}O_3$	1894	Taverné ^c	<i>Convolvulus scammonia</i> L. (convolvulin resin)		
Jalapinolic	11-Hydroxyhexadecanoic	$C_{17}H_{32}O_3$	1912	Power and Rogerson ^d	<i>Ipomea orizabensis</i> (Mexican scammony root wax, or jalap)		
Juniperic	16-Hydroxyhexadecanoic	$C_{18}H_{32}O_3$	1909	Bougault and Bourdier ^a	Conifer waxes		
Dihydroxystearic	9,10-Dihydroxyoctadecanoic	$C_{18}H_{36}O_4$	1925	Eibner and Münzing ^e	Castor oil (<i>Ricinus communis</i>)		
Phellonic	2-Hydroxydocosanoic	$C_{22}H_{44}O_3$	1931	Zetzsche and Sonderegger ^f	Cork, suberin (<i>Quercus suber</i>)		Brain lipids ^g
Cerebronic	2-Hydroxytetraacosanoic	$C_{24}H_{48}O_3$	1901	Thudichum ^b	Brain lipids		
α -Hydroxycerotic	2-Hydroxyhexacosanoic	$C_{26}H_{52}O_3$	1936	Chibnall <i>et al.</i> ^g	Brain lipids		
Lactarinic	6-Keto-octadecanoic	$C_{18}H_{34}O_3$	1911	Bougault and Charaux ⁱ	Mushrooms of <i>Lactarius</i> family		

Common name	Systematic name	Empirical formula			First discovered		Additional common sources
		formula	Date	Investigator	Source	Source	
Unsaturated Acids							
Ricinoleic	12-Hydroxy-9-octadecenoic	$C_{18}H_{34}O_3$	1848	Saalh�uller ^j	Castor oil	Ergot oil, ivory wood oil	
Hydroxynervonic	2-Hydroxy-9-tetracosenoic	$C_{24}H_{46}O_3$	1926	Klenk ^k	Brain lipids		
Licanic	4-Keto-9,11,13-octadecatrienoic	$C_{18}H_{32}O_3$	1931	Wilborn ^l	Seed oil of rigid-nut <i>coucopia</i> (<i>Coucopia grandiflora</i>)	Oiticica oil	
^a J. Bougault and L. Bourdier, <i>J. pharm. chim.</i> , 29, 561-573 (1909); 30, 10-16 (1909); <i>Chem. Abst.</i> , 3, 2445 (1909). ^b F. B. Power and H. Rogerson, <i>Am. J. Pharm.</i> , 80, 251-286 (1908). ^c H. J. Taverne, <i>Rec. trav. chim.</i> , 13, 187-217 (1894). ^d F. B. Power and H. Rogerson, <i>J. Chem. Soc.</i> , 101, 1-26T (1912). ^e A. Eibner and E. M�unzing, <i>Chem. Umschau</i> , 32, 166-176 (1925); <i>Chem. Abst.</i> , 19, 3027 (1925). ^f F. Zetzsche and G. Sonderegger, <i>Helv. Chim. Acta</i> , 14, 632-641 (1931). ^g A. C. Chibnall, S. H. Piper, and E. F. Williams, <i>Biochem. J.</i> , 30, 100-114 (1936). ^h J. L. W. Thudichum, <i>Die chemische Konstitution des Gehirns der Menschen und der Tiere</i> , Pietzcker, T�ubingen, 1901; cited by A. W. Raalston, p. 195. ⁱ J. Bougault and C. Charaux, <i>Chem. Zentr.</i> , 1911, I, 1463, 1598; <i>Compt. rend.</i> , 153, 572-573 (1911). ^j L. Saalh�uller, <i>Ann.</i> , 64, 108-126 (1848). ^k E. Klenk, <i>Z. physiol. Chem.</i> , 157, 291-298 (1926). ^l F. Wilborn, <i>Chem.-Ztg.</i> , 55, 434 (1931).							

structure has not been established. Convolvulinolic acid is an unusual biological acid, since it contains an odd number of carbon atoms. Apparently it is present in the roots of plants of the *Convolvulaceae* family.¹⁰² As a result of the studies of Asahina and Akasu,¹⁰³ the structure was established as 11-hydroxypentadecanoic acid, $\text{CH}_3(\text{CH}_2)_5\text{CHOH}(\text{CH}_2)_9\text{COOH}$. An optically active monohydroxypalmitic acid has been separated from butter fat by Bosworth and Helz,¹⁰⁴ but the position of the hydroxyl group has not been established. The acid is believed to differ from any palmitic acid previously reported.

Dihydroxystearic acid is the only natural hydroxy-acid having two hydroxyl groups. It has been recognized as a component of a number of fats, but its chief source is castor oil.¹⁰⁵⁻¹⁰⁷ It is of especial physiologic interest because of the suggestion that it might have special nutritive properties.¹⁰⁸ This question has, however, been answered in the negative.

Several saturated acids and one unsaturated hydroxy-acid are present in the cerebrosides, which are largely found in the brain.^{109,110} The hydroxyl group is believed to be free in these compounds. It is not known whether or not this fact contributes anything to the functional activity of the nervous tissue.

b. Unsaturated Hydroxy- and Keto-Acids. (a) *Ricinoleic Acid.* Ricinoleic acid is the most important of the hydroxy-acids. It is the only one of this group which is present in the oils in a large enough proportion to be of economic importance. Bussy and Lecanu,¹¹¹ as early as 1827, noted that the products of distillation of castor oil differed from those of other fats. The structure of ricinoleic acid was established by Goldsobel,¹¹² in 1894, as 12-hydroxy-9-octadecenoic acid, $\text{CH}_3(\text{CH}_2)_5\text{CHOHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$.

Ricinoleic acid, as the triglyceride triricinolein, accounts for 80% of castor oil.¹⁰⁵ Ricinoleic acid is also present in oil of ergot (*Secale cornutum*),¹¹³

¹⁰² M. Hoehnel, *Arch. Pharm.*, 234, 647-685 (1896).

¹⁰³ Y. Asahina and M. Akasu, *J. Pharm. Soc. Japan*, No. 523, 779-786 (1925); *Chem. Abst.*, 20, 365 (1926).

¹⁰⁴ A. W. Bosworth and G. E. Helz, *J. Biol. Chem.*, 112, 489-492 (1936).

¹⁰⁵ A. Eibner and E. Münzing, *Chem. Umschau*, 32, 153-162; 166-176 (1925); *Chem. Abst.*, 19, 3027 (1925).

¹⁰⁶ P. Panjutin and M. Rapoport, *Chem. Umschau*, 37, 130-135 (1930); *Chem. Abst.*, 24, 3665 (1930).

¹⁰⁷ H. P. Kaufmann and H. Bornhardt, *Fette u. Seifen*, 46, 444-446 (1939); *Chem. Abst.*, 34, 3521 (1940).

¹⁰⁸ R. S. Harris, H. Sherman, and E. E. Lockhart, *Arch. Biochem.*, 5, 63-70 (1944).

¹⁰⁹ E. Klenk, *Z. physiol. Chem.*, 153, 74-82 (1926); 166, 287-293 (1927); 179, 312-319 (1928); 185, 169-182 (1929); 200, 51-68 (1931); 206, 25-40 (1932).

¹¹⁰ A. C. Chibnall, S. H. Piper, and E. F. Williams, *Biochem. J.*, 30, 100-114 (1936).

¹¹¹ A. Bussy and L. R. Lecanu, *J. pharm. chim.* [2] 13, 57-81 (1827).

¹¹² A. G. Goldsobel, *Ber.*, 27, 3121-3129 (1894).

¹¹³ H. Matthes and P. Schütz, *Arch. Pharm.*, 265, 541-546 (1927).

ivorywood oil (*Agonandra brasiliensis*)¹¹⁴ to the extent of 45%, argemone oil from the prickle-poppy (*Argemone mexicana*)¹¹⁵ in the amount of 9.8%, and also in seed oil from the Tonkinese "cay thu muc" (*Wrightia annamensis*).¹¹⁶ It also occurs in all seed fats of the *Ricinus* (castor bean) family.

c. Hydroxy-Acids in Wool Fat. In addition to the normal aliphatic fatty acids, wool fat contains a considerable proportion of hydroxy-acids. As early as 1892, Lewkowitsch^{117,118} called attention to the presence of hydroxy-acids in wool fat; he observed that the acids form lactones on heating, and also exhibit a reaction with acetic anhydride. Kuwata¹¹⁹ and Weitkamp⁹ have both identified 2-hydroxypalmitic acid in fat from this source, and the latter investigator also demonstrated the presence of 2-hydroxymyristic acid.⁹ Weitkamp is of the opinion that they are both optical isomers, and Kuwata showed that the C₁₆ member was levorotatory.

(a) *Lanoceric Acid.* Lanoceric acid was one of the first acids to be identified. Darmstaedter and Lifschütz¹²⁰ found that it was a dihydroxy-acid having an empirical formula of C₂₀H₆₀O₄, and melting at 104–105°C. The presence of lanoceric acid was later confirmed by Abraham and Hilditch¹²¹ and also by Kuwata.¹¹⁹

(b) *Lanopalmitic Acid.* A second acid identified by Darmstaedter and Lifschütz¹²⁰ had an empirical formula of C₁₆H₃₂O₃ and melted at 87–88°C. Its presence in wool fat was later confirmed.^{119,121} Kuwata¹¹⁹ showed it to be an isomer of 2-hydroxypalmitic acid, since it was converted to 2-ketopalmitic acid on oxidation, and was ultimately degraded to pentadecanoic acid on further oxidation. It is optically active ($[\alpha]_D = -1.5^\circ$).

d. Licanic Acid. Licanic acid was first called couepic acid by several workers,^{122–124} because of its occurrence in the seeds of *Couepia grandiflora* (couflor). The structure was established by Brown and Farmer¹²⁵ as 4-keto-9,11,13-octadecatrienoic acid, CH₃(CH₂)₃CH:CHCH:CHCH:CH-(CH₂)₄CO(CH₂)₂COOH, on the basis of the identification of 4-ketostearic acid when α -licanic acid was subjected to hydrogenation, and of the fact that double bonds were conjugated.

¹¹⁴ L. Gurgel and T. F. de Amorin, *Mem. inst. quim. Rio de Janeiro*, No. 2, 31–38 (1929); *Chem. Abst.*, 24, 3668 (1930).

¹¹⁵ S. N. Iyer, J. J. Sudborough and P. R. Ayyar, *J. Indian Inst. Sci.*, A8, 29–38 (1925); *Chem. Abst.*, 19, 3607 (1925).

¹¹⁶ L. Margaillan, *Compt. rend.*, 192, 373–374 (1931).

¹¹⁷ J. Lewkowitsch, *J. Soc. Chem. Ind.*, 11, 134–144 (1892).

¹¹⁸ J. Lewkowitsch, *J. Soc. Chem. Ind.*, 15, 14–15 (1896).

¹¹⁹ T. Kuwata, *J. Am. Chem. Soc.*, 60, 559–560 (1938).

¹²⁰ L. Darmstaedter, and J. Lifschütz, *Ber.*, 29, 1474–1477; 2890–2900 (1896).

¹²¹ E. E. U. Abraham and T. P. Hilditch, *J. Soc. Chem. Ind.*, 54, 398–404T (1935).

¹²² F. Wilborn, *Chem.-Ztg.*, 55, 434 (1931).

¹²³ J. Van Loon and A. Steger, *Chem. Umschau*, 24, 337–340 (1930); *Chem. Abst.*, 25, 1111 (1931).

¹²⁴ J. Van Loon and A. Steger, *Rec. trav. chim.*, 50, 936–942 (1931).

¹²⁵ W. B. Brown and E. H. Farmer *Biochem. J.*, 29, 631–639 (1935).

When α -licanic acid (m.p. 74–75°C.) is subjected to traces of iodine or sulfur or exposed to sunlight,⁵ it is converted to β -licanic acid^{125–127} which melts at a higher temperature (m.p. 96.5°C.). Licanic acid also readily undergoes polymerization because of its conjugate double bond system; under such conditions, it forms films resistant to both water and alkali, which resemble those produced by elaeostearic acid.

TABLE 5

BRANCHED-CHAIN ACIDS WHICH HAVE BEEN ISOLATED FROM BIOLOGICAL MATERIAL

Common name (systematic name)	Empirical formula	First isolated		
		Date	Ref.	Source
Isovaleric (3-Methylbutanoic)	C ₆ H ₁₀ O ₂	1823	a	Dolphin and porpoise oils (<i>Delphinus globiceps</i> , <i>Phocaena phocaena</i>)
Tuberculostearic (d-10-Methyloctadecanoic)	C ₁₉ H ₃₈ O ₂	1929	b	Wax of tubercle bacilli
Phthioic	C ₂₆ H ₅₂ O ₂	1936	c	Tubercle bacilli
	—	1944	d	
Mycolic	C ₈₃ H ₁₇₆ O ₄	1938	e	Human tubercle bacilli
Mycocerosic	C ₃₀ H ₆₀ O ₂	1944	f	Tubercle wax
Phytomonic	C ₂₀ H ₄₀ O ₂	1944	g	Crown gall bacilli (<i>Phytomonas tumefaciens</i>)

^a M. E. Chevreul, *Recherches chimiques sur les corps gras d'origine animale*, Levrault, Paris, 1823, p. 115. Cited in Beilstein, *Handbuch org. chem.*, 135, 4th ed., Vol. II, 1920, p. 309; also K. S. Markley,⁴ p. 38.

^b R. J. Anderson and E. Chargaff, *J. Biol. Chem.*, 85, 77–88 (1929).

^c M. A. Spielman and R. J. Anderson, *J. Biol. Chem.*, 112, 759–767 (1936).

^d L. G. Ginger and R. J. Anderson, *J. Biol. Chem.*, 156, 443–451 (1944).

^e F. H. Stodola, A. Lesuk, and R. J. Anderson, *J. Biol. Chem.*, 126, 505–513 (1938).

^f L. G. Ginger and R. J. Anderson, *J. Biol. Chem.*, 157, 203–211 (1945).

^g S. F. Velick and R. J. Anderson, *J. Biol. Chem.*, 152, 523–531 (1944).

The chief source of licanic acid is oiticica oil from the *Licania rigida* (oiticica),¹²⁵ where it makes up 75–80% of the fatty acid. Mexican oiticica oil from the *Licania arborea*^{4,128} (“cacahuananche”) is also a good source, as is po-yoak oil¹²⁹ obtained from *Parinarium sherbroense*⁴ from Sierra Leone. In the latter case, 45–50% of the total fatty acid consists of licanic acid and about 30% of elaeostearic acid.⁴

(7) Miscellaneous Substituted Acids

Although practically all of the fatty acids found in nature are straight-chain compounds, a few cases are known in which branched-chain acids

¹²⁶ R. S. Morrell and W. R. Davis, *J. Chem. Soc.*, 1936, 1481–1484.

¹²⁷ R. S. Morrell and W. R. Davis, *J. Oil Colour Chem. Assoc.*, 19, 359–362 (1936).

¹²⁸ H. A. Gardner, *Nat. Paint Varnish Lacquer Assoc.*, Circ. No. 654, 28–34 (1943).

¹²⁹ A. Steger and J. Van Loon, *Rec. trav. chim.*, 57, 620–628 (1938).

occur. This should not be too surprising, in view of the fact that several of the well-known essential amino acids such as valine ($(\text{CH}_3)_2\text{CHCH}(\text{NH}_2)\text{-COOH}$), leucine ($(\text{CH}_3)_2\text{CHCH}_2\text{CH}(\text{NH}_2)\text{COOH}$), and isoleucine ($\text{CH}_3\text{-CH}_2\text{-C}(\text{CH}_3)\text{CH}(\text{NH}_2)\text{COOH}$), which occur in practically all animal and plant proteins, contain branched chains. Moreover, many of the natural products such as the carotenoids, vitamin A, vitamin E, and vitamin K, have methyl groups replacing some of the hydrogen atoms in the side chain. The presence of the methyl group usually results in an asymmetry of one of the carbon atoms, and this accounts for an optical activity on the part of the acid. However, since the substitution occurs at the terminal carbon atom in isovaleric acid, this product has no asymmetric carbon and does not exhibit optical activity.

a. Isovaleric Acid. As early as 1823 this acid was identified as a component of dolphin and porpoise oils by Chevreul,¹³⁰ who called it *acide phocéniqne*. In the systematic nomenclature it is called 3-methylbutanoic acid, and the formula is $(\text{CH}_3)_2\text{CHCH}_2\text{COOH}$. Equally remarkable, in addition to the presence of the branched-chain acid in these fats, is the fact that such a short-chain acid should be present. The only common fat in which this has been observed is cow's milk fat, in which a considerable proportion of butyric acid occurs.

The identity of the isovaleric acid from dolphin and porpoise oil with the product synthetically prepared was proved by André,¹³¹ who also showed that it was not a 50:50 mixture of butyric and caproic acids. Gill and Tucker,¹³² more recently, have reported as much as 26.7% of isovaleric acid in a sample of porpoise jaw oil, while Klein and Stigol¹³³ in the same year demonstrated its presence in the oil of the Black Sea dolphin. The identification was satisfactory in both cases, being based in the first instance upon the distillation temperature and neutralization value of the free acid (coupled with a zero iodine number) and in the second case upon the boiling point of the ethyl ester as well as upon certain other qualitative tests. More recently, Tsujimoto and Koyanagi¹³⁴ have identified cetyl isovalerate as a distillation product of pilot-whale head oil (*Globicephala melaena*).

b. Tuberculostearic Acid. Anderson and Chargaff¹³⁵ were the first to isolate this acid from human tubercle wax. It was also presumably

¹³⁰ M. E. Chevreul, *Recherches chimiques sur les corps gras d'origine animale*, Levrault, Paris, 1823, p. 115; cited by B. F. Daubert and C. G. King, *Chem. Revs.*, 29, 269-285 (1941), p. 270.

¹³¹ E. André, *Bull. soc. chim.*, 35, 857-868 (1924); *Compt. rend.*, 178, 1188-1191 (1924).

¹³² A. H. Gill and C. M. Tucker, *Oil & Fat Industries*, 7, 101-102 (1930).

¹³³ A. Klein and M. Stigol, *Pharm. Zentralhalle*, 71, 497-500 (1930); *Chem. Abst.*, 24, 5522 (1930).

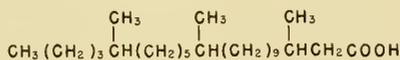
¹³⁴ M. Tsujimoto and H. Koyanagi, *J. Soc. Chem. Ind. Japan*, 40, suppl., 272-274 (1937); *Chem. Abst.*, 31, 7685 (1937).

¹³⁵ R. J. Anderson and E. Chargaff, *J. Biol. Chem.*, 85, 77-88 (1929).

isolated later by Anderson and Roberts¹³⁶ from bovine tubercle wax as well as from the leprosy bacilli.¹³⁷

Spielman¹³⁸ assigned to it the structure of 10-methylstearic acid, $\text{CH}_3\text{-(CH}_2)_7\text{CH(CH}_3\text{)(CH}_2)_8\text{COOH}$. Although later evidence seemed to disprove this structure, Velick¹³⁹ has recently concluded, largely on the basis of its x-ray diffraction patterns, that this structure is the correct one. The final proof has apparently been furnished by the brilliant studies of Prout, Cason, and Ingersoll.^{140,141} These workers compared natural tuberculostearic acid with *d*- and *l*-10-methylstearic acid, synthetically prepared. It was found, on the basis of melting points and mixed melting points of the natural and synthetic acids as well as of the amides and tribromanilides derived from them, that tuberculostearic acid is *l*-10-methyloctadecanoic acid.

c. Phthioic Acid. Another branched-chain acid, isolated by Spielman and Anderson¹⁴² and also by Ginger and Anderson¹⁴³ from tubercle bacilli, was named phthioic acid. It is a polymethylated compound with an empirical formula of $\text{C}_{26}\text{H}_{52}\text{O}_2$. Stenhagen and Ställberg¹⁴⁴ suggested that phthioic acid was ethyldecylododecylacetic acid, but Polgar and Robinson¹⁴⁵ showed that this was probably incorrect. The first structure proposed by the latter workers could be either 10,13,19- or 3,13,19-trimethyltricosanoic acid.¹⁴⁶ On the basis of the high rotation, and especially in view of the identity of the synthetic 3,13,19-trimethyltricosanoic acid with phthioic acid, the latter structure was indicated.¹⁴⁶ However, Cason and Prout¹⁴⁷ disagreed with the postulated structure, since they believed that such a compound should not have as high a specific rotation as that determined for phthioic acid.



Phthioic acid (Polgar and Robinson¹⁴⁶)

d. Mycolic Acid. Mycolic acid is an acid with a high molecular weight, containing hydroxyl or hydroxymethoxy groups. According to Stodola,

¹³⁶ R. J. Anderson and E. G. Roberts, *J. Biol. Chem.*, **89**, 599-610 (1930).

¹³⁷ R. J. Anderson and N. Uyei, *J. Biol. Chem.*, **97**, 617-637 (1932).

¹³⁸ M. A. Spielman, *J. Biol. Chem.*, **106**, 87-96 (1934).

¹³⁹ S. F. Velick, *J. Biol. Chem.*, **154**, 497-502 (1944).

¹⁴⁰ F. S. Prout, J. Cason, and A. W. Ingersoll, *J. Am. Chem. Soc.*, **69**, 1233 (1947).

¹⁴¹ F. S. Prout, J. Cason, and A. W. Ingersoll, *J. Am. Chem. Soc.*, **70**, 298-305 (1948).

¹⁴² M. A. Spielman and R. J. Anderson, *J. Biol. Chem.*, **112**, 759-767 (1936).

¹⁴³ L. G. Ginger and R. J. Anderson, *J. Biol. Chem.*, **156**, 443-451 (1944).

¹⁴⁴ E. Stenhagen and S. Ställberg, *J. Biol. Chem.*, **139**, 345-364 (1941).

¹⁴⁵ N. Polgar and R. Robinson, *J. Chem. Soc.*, **1943**, 615-619.

¹⁴⁶ N. Polgar and R. Robinson, *J. Chem. Soc.*, **1945**, 389-395.

¹⁴⁷ J. Cason and F. S. Prout, *J. Am. Chem. Soc.*, **70**, 879-880 (1948).

Lesuk, and Anderson,¹⁴⁸ it has a probable empirical formula of $C_{88}H_{176}O_4$. It occurs not only in the human tubercle bacilli¹⁴⁷ but also in the bovine type.¹⁴⁹ These mycolic acids were found to have molecular weights of 1284 and 1200, respectively; they are both slightly dextrorotatory ($[\alpha]_D^{25} = 1.8^\circ$), and melt at 54–56°C. On heating to 300–350°C. under reduced pressure, *n*-hexacosanoic acid distills off in both cases.

There are also three avian mycolic acids, isolated from avian tubercle wax.^{150,151} On pyrolysis, the α -acid decomposes to a branched-chain pentacosanoic acid, while the β -variety gives rise to a normal tetracosanoic acid. The third type of avian mycolic acid, γ -mycolic acid, isolated by Anderson, Creighton, and Peck¹⁵¹ on pyrolysis at 270–280°C., yielded a tetracosanoic acid which, however, differed in crystalline form from the ordinary straight-chain fatty acid. In a more recent study, Lesuk and Anderson¹⁵² prepared a hydroxynormycolic acid, after demethylation with hydrogen iodide, which splits in a normal manner, on pyrolysis, to *n*-hexacosanoic acid. Since a small amount of a hydroxy-acid having an approximate formula of $C_{104}H_{208}O_3$ was separated from the hydroxynormycolic and normycolic acids, it is believed that mycolic acid is a mixture of two acids. That further revisions in our ideas on the mycolic acids must be expected is indicated by the recent report of Ställberg-Stenhagen and Stenhagen¹⁵³ in which it is stated that the pentacosanoic and tetracosanoic acids, reported as pyrolysis products of α - and β -mycolic acids, respectively, are in actuality mixtures of closely related normal chain homologues.

e. Mycocerosic Acid. In addition to dextrorotatory acids analogous to phtioic acid and the weakly levorotatory tuberculostearic acid, Ginger and Anderson¹⁵⁴ have recently isolated a strongly levorotatory acid, which they call mycocerosic acid, from the ether-soluble lead salt fraction of tubercle wax. This appears to be a constant constituent of all wax fractions of human tubercle bacilli. It is a non-crystalline waxy solid, melting at 27–28°C., with a levorotation ($[\alpha]_D = -5$ to -6° (in chloroform)), and a composition indicated by the formula, $C_{30}H_{60}O_2$. The relatively low melting point indicates that this is probably a branched-chain acid.

f. Phytomonic Acid. Velick and Anderson¹⁵⁵ have described another similar acid from the acetone-soluble fat and from the phosphatide of the

¹⁴⁸ F. H. Stodola, A. Lesuk, and R. J. Anderson, *J. Biol. Chem.*, **126**, 505–513 (1938).

¹⁴⁹ J. Cason and R. J. Anderson, *J. Biol. Chem.*, **126**, 527–541 (1938).

¹⁵⁰ R. J. Anderson and M. M. Creighton, *J. Biol. Chem.*, **129**, 57–63 (1939).

¹⁵¹ R. J. Anderson, M. M. Creighton, and R. L. Peck, *J. Biol. Chem.*, **133**, 675–693 (1940).

¹⁵² A. Lesuk and R. J. Anderson, *J. Biol. Chem.*, **136**, 603–613 (1940).

¹⁵³ S. Ställberg-Stenhagen and E. Stenhagen, *J. Biol. Chem.*, **165**, 599–604 (1946).

¹⁵⁴ L. G. Ginger and R. J. Anderson, *J. Biol. Chem.*, **157**, 203–211 (1945).

¹⁵⁵ S. F. Velick and R. J. Anderson, *J. Biol. Chem.*, **152**, 523–531 (1944).

crown-gall bacillus (*Phytomonas tumefaciens*); they designate this acid as phytomonic acid. It is a saturated acid, liquid at ordinary temperature, and has a composition which corresponds to an empirical formula of $C_{20}H_{40}O_2$. On the basis of the low melting point of $24^\circ C.$ and of other physical properties, it is believed to be a branched-chain acid.¹⁵⁶ More recently Velick¹⁵⁷ suggested that phytomonic acid is either 10- or 11-methylnonadecanoic acid.

g. Other Substituted Acids in Tubercle Bacilli. Ginger and Anderson¹⁵⁴ recently isolated four different dextrorotatory acids from tubercle bacilli which differed from each other in molecular weight and in specific rotation. Phthioic acid, $C_{26}H_{52}O_2$, was included in this group; the composition of the other branched-chain acids corresponded to the empirical formulas of $C_{24}H_{48}O_2$, $C_{25}H_{50}O_2$, and $C_{27}H_{54}O_2$.

h. Unsaturated Substituted Acid. Hilditch and his co-workers¹⁵⁸ demonstrated the presence of a methylated unsaturated odd-carbon acid in the Java "olive" oil from the gum tree (*Sterculia foetida*). This acid accounts for 70% of the total fatty acids in the oil. The acid has been shown to be 12-methyl-9,11-octadecadienoic acid, which is represented by the formula, $CH_3(CH_2)_5C(CH_3):CHCH:CH(CH_2)_7COOH$.

i. Branched-Chain Fatty Acids in Wool Wax. It has long been known that the lipid make-up of wool wax differs markedly from that of other animal fats. Instead of containing glycerol to serve as the alcohol for combination with the fatty acids, wool fat contains exclusively the monoatomic alcohols, cholesterol, isocholesterol, and other high aliphatic alcohols. The major portion of the fatty acids are not combined with these sterols, however, as is indicated by Weitkamp,⁹ but rather with complex triterpenoid¹²¹ alcohols which are not found in animal fats.¹⁵⁹ Weitkamp⁹ classified the fatty acids present in the following categories: (1) normal saturated acids, (2) 2-hydroxy-acids, (3) iso- (or branched-chain) acids corresponding to the general formula, $CH_3CH(CH_3)(CH_2)_{2n}COOH$, in which n is 3 to 11 inclusive, and (4) anteiso-acids with the general structure, $CH_3CH_2CH(CH_3)(CH_2)_{2n}COOH$, in which n is 2 to 13 inclusive.

The normal saturated acids were found to consist of acids with an even number of carbon atoms from 10 to 26. The hydroxy-acids present are described earlier (see page 31).

The first of the series of homologous iso-acids consist of members with an even number of carbon atoms with the single methyl side chain attached to the penultimate (next to last) carbon atom. It is evident that the fatty

¹⁵⁶ S. F. Velick, *J. Biol. Chem.*, 152, 533-538 (1944).

¹⁵⁷ S. F. Velick, *J. Biol. Chem.*, 156, 101-107 (1944).

¹⁵⁸ T. P. Hilditch, M. L. Meara, and Y. A. H. Zaky, *J. Soc. Chem. Ind.*, 60, 198-203 (1941).

¹⁵⁹ H. Schulze, *Z. physiol. Chem.*, 238, 35-53 (1936).

acid chains under such conditions have an odd number of carbons. The C_{10} to C_{28} members of this series were isolated. As a result of a study of the x-ray diffraction patterns of a series of these acids (12-methyltridecanoic, 14-methylpentadecanoic, 16-methylheptadecanoic, 18-methylnonadecanoic, 20-methylheneicosanoic, and 22-methyltricosanoic acids) Velick¹⁶⁰ clearly demonstrated homology in the series. The long crystal spacings were shown to increase linearly with the carbon content.

The second of the series of homologous acids, the so-called *anteiso* series, consists of members with an odd number of carbon atoms containing a single methyl side chain attached to the antepenultimate (second from the last) carbon atom. In this case the fatty acid chains are composed of an even number of carbon atoms. The C_9 to C_{31} members in this series were isolated by Weitkamp.⁹ Velick and English¹⁶¹ succeeded in synthesizing *d*-14-methylpalmitic acid, $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)(\text{CH}_2)_{12}\text{COOH}$, which was shown to be identical with the natural product from wool wax. It is therefore believed that the *anteiso* series consists of the *d*-isomers of even-chained acids with a single methyl group in the antepenultimate position. On the basis of x-ray diffraction studies, the series was shown to be homologous.¹⁶⁰

(8) Cyclic Acids

Interest in the cyclic fatty acids has been stimulated by the report of their use in the treatment of leprosy. Several such cyclic acids are present in the seed oils of tropical plants of the family *Flacourtiaceae*, and especially of the genus *Hydnocarpus*. The principal sources are chaulmoogra oil, lukrabo oil, and gorli seed oil. The best known cyclic fatty acids are chaulmoogric, $C_{18}H_{32}O_2$, hydnoearpic, $C_{16}H_{28}O_2$, and gorlic, $C_{18}H_{30}O_2$. All of these acids have a terminal cyclopentenyl ring, and they vary from each other only in the nature of the side chain.

Although it has been recognized for many years that such oils are specific for the treatment of leprosy, no satisfactory method of biological testing has been available until recently. It has been shown¹⁶² that the therapeutic activity can be judged by the bactericidal action upon the leprosy bacillus, *B. leprae*. Such an *in vitro* test has been widely employed by the Adams group in testing the structural requirements for activity in a number of naturally occurring and synthetic acids.

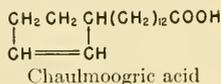
a. Chaulmoogric Acid. Although the specific action of chaulmoogra oil (*Gynocardia odorata*) upon leprosy was known to the Chinese, the nature of the fatty acids present in this oil was not understood until the

¹⁶⁰ S. F. Velick, *J. Am. Chem. Soc.*, **69**, 2317-2322 (1947).

¹⁶¹ S. F. Velick and J. English, Jr., *J. Biol. Chem.*, **160**, 473-480 (1945).

¹⁶² E. L. Walker and M. A. Sweeney, *J. Infectious Diseases*, **26**, 238-264 (1920).

studies of Power and his collaborators^{163,164} between 1904 and 1907. Even then, the formulas for the cyclic acids which were proposed proved to be erroneous. Their structure was not completely clarified until the work of Shriner and Adams¹⁶⁵ in 1925. Chaulmoogric acid was shown to be 13-[2-cyclopentenyl]tridecanoic acid, the formula of which is illustrated below:



Because of the asymmetry of the ring carbon joined to the side chain, chaulmoogric and related acids are optically active. They all possess a dextrorotation. When they are reduced to dihydrochaulmoogric acid, the optical activity is lost.

Racemic chaulmoogric acid was first synthesized by Perkins and Cruz¹⁶⁶ by condensation of the acetoacetic ester with 11-cyanoundecanoic acid. Hinegardner¹⁶⁷ was the first to prepare *dl*-chaulmoogric acid from the natural *d*-isomer. However, *l*-chaulmoogric acid has not been isolated. The conversion of hydnocarpic acid to chaulmoogric acid has been accomplished by Stanley and Adams.¹⁶⁸ Hydnocarpic and chaulmoogric acids can be separated from the other acids and from each other, in the acid mixture obtained by extraction of saponified chaulmoogra oil, by vacuum distillation.^{165,169} The first fraction is largely hydnocarpic acid, while the third fraction is essentially pure chaulmoogric acid.

Hydrogenation of chaulmoogric and hydnocarpic acids to the corresponding dihydro-compounds proceeds smoothly when platinum or palladium is used as a catalyst,¹⁷⁰⁻¹⁷² or with a mixed platinum-palladium oxide catalyst.¹⁶⁵ Zinc and ethanol may also be employed to produce the dihydro-compounds. However, when phosphorus and hydrogen iodide are used, the reduction proceeds to the hydrocarbon, chaulmoogrene, C₁₃H₃₄. Some chaulmoogryl alcohol is formed with sodium and ethanol, resulting in condensation to chaulmoogryl chaulmoograte.

¹⁶³ F. B. Power and F. H. Gornall, *J. Chem. Soc.*, 85, 838-851, 851-861 (1904).

¹⁶⁴ F. B. Power and M. Barrowcliff, *J. Chem. Soc.*, 87, 884-896 (1905). M. Barrowcliff and F. B. Power, *ibid.*, 91, 557-578 (1907). M. Barrowcliff and F. B. Power, *Proc. Chem. Soc.*, 23, 70-71 (1907); *Chem. Abst.*, 1, 1561 (1907).

¹⁶⁵ R. L. Shriner and R. Adams, *J. Am. Chem. Soc.*, 47, 2727-2739 (1925).

¹⁶⁶ G. A. Perkins and A. O. Cruz, *J. Am. Chem. Soc.*, 49, 1070-1077 (1927).

¹⁶⁷ W. S. Hinegardner, *J. Am. Chem. Soc.*, 55, 2831-2834 (1933).

¹⁶⁸ W. M. Stanley and R. Adams, *J. Am. Chem. Soc.*, 51, 1515-1518 (1929).

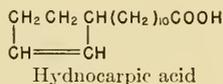
¹⁶⁹ T. Hashimoto, *J. Am. Chem. Soc.*, 47, 2325-2333 (1925).

¹⁷⁰ A. L. Dean and R. Wrenshall, *U. S. Pub. Health Service Pub. Health Repts.*, 37, No. 23, 1395-1399 (1922).

¹⁷¹ R. Wrenshall and A. L. Dean, *U. S. Pub. Health Service Bull.*, No. 141, 12-23 (1924).

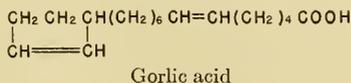
¹⁷² A. L. Dean, R. Wrenshall, and G. Fujimoto, *U. S. Pub. Health Service Bull.*, No. 141, 24-27 (1924).

b. Hydnocarpic Acid. Hydnocarpic acid is usually associated with chaulmoogric acid. The structure of this acid was first suggested by the investigations of Power *et al.*,^{163,164} but was only completely understood after the experimental work of Shriner and Adams.¹⁶⁵ It was shown to be 11-[2-cyclopentenyl]undecanoic acid, which has the structure shown in the accompanying formula.



Hydnocarpic acid thus is related to palmitic acid, while chaulmoogric acid has the same number of carbons as stearic acid. The properties of hydnocarpic acid are analogous to those of chaulmoogric acid.

c. Gorlic Acid. Dean, Wrenshall, and Fujimoto^{171,172} originally suggested that chaulmoogra and similar oils contained a liquid cyclic acid more unsaturated than chaulmoogric acid. On hydrogenation of the oil, it was found that a dihydrochaulmoogric acid was formed in which the 5-carbon ring was saturated but in which the side chain had an unsaturated bond. This acid was first separated from the oil of the seed of *Oncoba echinata* (commonly called gorli seed) by André and Jouatte,¹⁷³ who named it gorlic acid. The same acid was later obtained by Paget¹⁷⁴ and by Cole and Cardoso¹⁷⁵ from the oil of the sapucainha tree (*Carpotroche brasiliensis*). Paget called it dehydrochaulmoogric acid. Since it yielded adipic acid and 4-carboxyundecanedioic acid upon oxidation, its structure was established as 13-(2-cyclopentenyl)-6-tridecenoic acid. In the gorli seed, gorlic acid makes up 14.7% of the total fatty acids.¹⁷⁵



d. Other Cyclic Acids. A number of homologues of chaulmoogric acid have been isolated from the oil of *Hydnocarpus wightiana* and *H. anthelmintica* by Cole and Cardoso.^{175,176} These are called aleptic, aleprylic, aliprestic, and aleprolic acids. They differ from chaulmoogric acid in the length of the side chain.

e. Structure as Related to Antileprosy Action. The development of the *in vitro* test for activity based upon the bactericidal action on *Bacillus*

¹⁷³ E. André and D. Jouatte, *Bull. soc. chim.* [4], 43, 347-360 (1928).

¹⁷⁴ H. Paget, *J. Chem. Soc.*, 1937, 955-960.

¹⁷⁵ H. I. Cole and H. T. Cardoso, *J. Am. Chem. Soc.*, 60, 612-614; 614-617; 617-619 (1938).

¹⁷⁶ H. I. Cole and H. T. Cardoso, *J. Am. Chem. Soc.*, 61, 2349-2351; 2351-2353; 3442-3445 (1939).

leprae (*Mycobacterium leprae*) has rendered possible the determination of the particular linkages responsible for activity. It was first found that the therapeutic action of chaulmoogra oil was due to the cyclic acids present.^{162,177} Homochaulmoogric and homohydrocarpic acids, each of which contains an additional methylene group in the side chain as compared with its parent acid, were both found to be inactive when tested with *B. leprae*.¹⁷⁸ On the other hand, chaulmoogrylacetic acid prepared by Van Dyke and Adams¹⁷⁹ had some activity, while a series of synthetic 2-cyclopentenyl-2-alkylacetic acids had an increasing bactericidal effect as the alkyl group was lengthened from the pentyl to the nonyl residue.¹⁸⁰

Dean and his collaborators¹⁸¹⁻¹⁸³ reported that the ethyl esters of the chaulmoogra oil fatty acids were also biologically active. Although no relationship was found to exist between unsaturation of non-cyclic acids and growth-inhibiting action toward another acid-fast organism, the tubercle bacillus,¹⁸⁴ the activity of completely hydrogenated chaulmoogra oil was reduced to zero¹⁸⁵; this would seem to indicate that the cyclopentene nucleus is essential. However, Hasseltine¹⁸⁶ has reported the successful treatment of human leprosy with dihydrochaulmoogric acid, which would appear to be contradictory. Possibly this may indicate that the conditions inhibiting the growth of *Bacillus tuberculosis* (*Mycobacterium tuberculosis*) and of *Bacillus leprae*, respectively, are not identical.

Furthermore, the ring structure need not be limited to a 5-carbon one, as cyclohexyl-, cyclobutyl-, and even cyclopropyl-derivatives have anti-bacterial potency. A series of cyclohexyl acids prepared by Hiers and Adams^{187,188} showed activity similar to that of the cyclopentyl acids. Maximum efficacy was noted, however, with the C₁₆ and C₁₈ acids, and the bactericidal potency fell off when a greater or smaller number of carbon atoms was present. Similarly, no activity was noted with the cyclopropyl-methyl alkylacetic acids prepared by Arvin and Adams¹⁸⁹ until at least 16 carbon atoms were present in the molecule; the cyclobutylmethyl alkyl-

¹⁷⁷ O. Schöbl, *Philippine J. Sci.*, 23, 533-542 (1923).

¹⁷⁸ J. Sacks and R. Adams, *J. Am. Chem. Soc.*, 48, 2395-2399 (1926).

¹⁷⁹ R. H. Van Dyke and R. Adams, *J. Am. Chem. Soc.*, 48, 2393-2395 (1926).

¹⁸⁰ J. A. Arvin and R. Adams, *J. Am. Chem. Soc.*, 49, 2940-2942 (1927).

¹⁸¹ H. T. Hollman and A. L. Dean, *J. Cutan. Diseases*, 37, 367-386 (1919).

¹⁸² A. L. Dean and R. Wrenshall, *J. Am. Chem. Soc.*, 42, 2626-2645 (1920).

¹⁸³ J. T. McDonald and A. L. Dean, *J. Am. Med. Assoc.*, 76, 1470-1474 (1921).

¹⁸⁴ O. Schöbl, *Philippine J. Sci.*, 25, 123-134 (1924).

¹⁸⁵ O. Schöbl, *Philippine J. Sci.*, 25, 135-150 (1924).

¹⁸⁶ H. E. Hasseltine, *U. S. Pub. Health Service Bull.*, No. 141, 1-11 (1924).

¹⁸⁷ G. S. Hiers and R. Adams, *J. Am. Chem. Soc.*, 48, 1089-1093 (1926).

¹⁸⁸ G. S. Hiers and R. Adams, *J. Am. Chem. Soc.*, 48, 2385-2393 (1926).

¹⁸⁹ J. A. Arvin and R. Adams, *J. Am. Chem. Soc.*, 50, 1983-1985 (1928).

¹⁹⁰ S. G. Ford and R. Adams, *J. Am. Chem. Soc.*, 52, 1259-1261 (1930).

¹⁹¹ R. Adams, W. M. Stanley, and H. A. Stearns, *J. Am. Chem. Soc.*, 50, 1475-1478 (1928).

acetic acids containing 16 or 18 carbon atoms were likewise found to be the most effective of this series against *B. leprae*.^{190,191}

The introduction of a second ring into the aliphatic chain was found to have no effect in increasing the antibacterial activity. Davies and Adams¹⁹² investigated this point by studying the effectiveness against *B. leprae* of several di(cyclohexylalkyl)-acetic acids which they had synthesized.

The position of the carboxyl group is of secondary importance in establishing the antibacterial potency insofar as the cyclic acids are concerned.¹⁹³ However, in a series of non-cyclic hexadecanoic and octadecanoic acids synthesized by Stanley, Jay, and Adams,¹⁹⁴ in which the position of the carboxyl group varied from the end of the molecule to the center, the location of the acid group was found to be of primary importance. Maximum activity obtained when the carboxyl group was in the middle of the molecule, while no activity was noted when it occupied a terminal position. These results indicated that a ring is not essential for antibacterial action but that the configuration of the acid has the greater importance.

As is the case with the cyclic acids, it has been found that molecular weight plays an important role with the non-cyclic acids. In the earlier work¹⁹⁴ it was shown that the hexadecanoic acids were somewhat more effective than the octadecanoic acids. Armendt and Adams¹⁹⁵ reported that the dialkylacetic acids containing only 12 carbons were completely inactive as bactericidal agents while the C₁₃ and C₁₄ acids were only slightly effective. Corresponding C₁₅ and C₁₇ acids were found to be less effective in this respect than was C₁₆, while the C₁₉ was less potent than the C₁₈ acid.¹⁹⁶ These data emphasize the importance of the molecular weight as a determining factor in conferring potency against the leprosy bacillus.

The presence of an ethylenic linkage in the aliphatic side chain does not influence the biological activity of the acids. It is recalled that the saturation of the double bond in the pentenyl ring of chaulmoogric acid had little effect upon its ability to destroy *B. leprae*. Browning, Woodrow, and Adams¹⁹⁷ have investigated a number of such compounds synthesized by them and have reported an activity similar to that of saturated compounds of the same molecular weight. The series studied by them included undecenyl alkylacetic acids, α , β -unsaturated dialkylacetic acids, and alkyl alkylacetic acids.

¹⁹² L. A. Davies and R. Adams, *J. Am. Chem. Soc.*, *50*, 2297-2298 (1928).

¹⁹³ R. Adams, W. M. Stanley, S. G. Ford, and W. R. Peterson, *J. Am. Chem. Soc.*, *49*, 2934-2940 (1929).

¹⁹⁴ W. M. Stanley, M. S. Jay, and R. Adams, *J. Am. Chem. Soc.*, *51*, 1261-1266 (1929).

¹⁹⁵ B. F. Armendt and R. Adams, *J. Am. Chem. Soc.*, *52*, 1289-1291 (1930).

¹⁹⁶ C. M. Greer and R. Adams, *J. Am. Chem. Soc.*, *52*, 2540-2543 (1930).

¹⁹⁷ E. Browning, H. W. Woodrow, and R. Adams, *J. Am. Chem. Soc.*, *52*, 1281-1283 (1930).

TABLE 6
SATURATED DICARBOXYLIC ACIDS WHICH POSSESS BIOLOGICAL IMPORTANCE

Common name	Systematic names	First isolated		
		Formula	Date	Investigator
Oxalic	Ethanedioic	(COOH) ₂	1784	Scheele ^a
Malonic	Propanedioic	CH ₂ (COOH) ₂	1881	von Lippmann ^b
Succinic	Butanedioic	(CH ₂) ₂ (COOH) ₂	1675	Lemery ^c
Glutaric	Pentanedioic; trimethylene-1,3-dicarboxylic	(CH ₂) ₃ (COOH) ₂	1888	Buisine and Buishne ^d
Adipic	Hexanedioic; tetramethylene-1,4-dicarboxylic	(CH ₂) ₄ (COOH) ₂	1884	Dieterle and Hell ^e
Pimelic	Heptanedioic; pentamethylene-1,5-dicarboxylic	(CH ₂) ₅ (COOH) ₂	1884	Gantner and Hell ^f
Suberic	Octanedioic; hexamethylene-1,6-dicarboxylic	(CH ₂) ₆ (COOH) ₂	1841	Tilley ^g
Azelaic	Nonanedioic; heptamethylene-1,7-dicarboxylic	(CH ₂) ₇ (COOH) ₂	1881	Gantner and Hell ^h
Sebaic	Decanedioic; octamethylene-1,8-dicarboxylic	(CH ₂) ₈ (COOH) ₂	1886	Saytzeff ⁱ
Brassylic	Tridecanedioic; undecamethylene-1,11-dicarboxylic	(CH ₂) ₁₁ (COOH) ₂	1855	Bouis ^j
Thapsic	Hexadecanedioic; tetradecamethylene-1,14-dicarboxylic	(CH ₂) ₁₄ (COOH) ₂	1893	Fileti and Ponzio ^k
Japanese	Heptacosanedioic; nonadecamethylene-1,19-dicarboxylic	(CH ₂) ₁₉ (COOH) ₂	1883	Canzoneri ^l
			1888	Eberhardt ^m

^a C. W. Scheele, 1784; cited by Beilstein's *Handbuch*, 4th ed.,

Vol. 2, Springer, Berlin, 1920, p. 502.

^b E. O. von Lippmann, *Ber.*, 14, 1183-1185 (1881).

^c N. Lemery, 1675; cited by Beilstein's *Handbuch*, 4th ed.,

Vol. 2, Springer, Berlin, 1920, p. 601.

^d A. Buisine and F. Buisine, *Compt. rend.*, 107, 789-792 (1888).

^e W. Dieterle and C. Hell, *Ber.*, 17, 2221-2228 (1884).

^f F. Gantner and C. Hell, *Ber.*, 17, 2212-2217 (1884).

^g T. G. Tilley, *Ann.*, 39, 160-168 (1841).

^h F. Gantner and C. Hell, *Ber.*, 14, 1545-1552 (1881).

ⁱ A. Saytzeff, *J. prakt. Chem.*, [2], 33, 300-318 (1886).

^j J. Bouis, *Ann. chim. phys.*, [3], 44, 77-152 (1855).

^k M. Fileti and G. Ponzio, *J. prakt. Chem.*, [2], 48, 323-336 (1893).

^l F. Canzoneri, *Gazz. chim. ital.*, 13, 514-521 (1883).

^m L. E. Eberhardt, *Dissertation*, Strassburg (1888); cited by

A. W. Ralston, p. 245; also T. P. Hilditch, pp. 146, 152.

Biological source
Rhubarb roots
Incrustation on steam distilla-
tion of sugar beets
Distillation of amber
Washings of crude sheep wool
Nitric acid oxidation of castor
oil
Oxidation product of ricinoleic
acid
Nitric acid oxidation of castor
oil
Oxidation of oleic acid with
KMnO₄
Dry distillation of castor oil or
ricinoleic acid with NaOH
Nitric acid oxidation of erucic
and brassidic acid
Dried roots of the *Mediterra-*
nean "deadly carrot," *Thapsia*
garganica (*Umbelliferae* spp.)
Japan wax from sumac (*Rhus*
spp.)

Although it is not certain that the results obtained in the *in vitro* test with *B. leprae* will apply in all cases to human leprosy, they are most important as pilot experiments. The confirmation of the results by clinical studies will require a prolonged period and will in all probability be less decisive than the *in vitro* tests.

(9) Dicarboxylic Acids

Although the dicarboxylic acids do not occur in appreciable amounts as components of lipids, some of them are important metabolic products of these substances, inasmuch as they originate from them by ω -oxidation. Moreover, the lower members of the series which can be synthesized *de novo* by the animal are of great importance in the so-called tricarboxylic acid cycle. This series of reactions is responsible for the oxidation not only of carbohydrate but also of a considerable portion of the fatty acid intermediates. These acids will therefore be briefly described.

The saturated dicarboxylic acids (also known as the oxalic acid series) have the type formula $(\text{CH}_2)_n(\text{COOH})_2$. Since they have two carboxyl groups, they may form two series of esters, salts, or other derivatives, depending upon whether one or both carboxyl groups are in combination. They are considerably more soluble in water and less soluble in the fat solvents than are the members of the monocarboxylic fatty acids. In general they occur free, as salts, as amides, or as esters with short-chain alcohols. Combinations of the dicarboxylic acids with glycerol do not occur in nature.

The acids in this group are almost exclusively referred to in the literature by their common names. The systematic name, which has the advantage of indicating the hydrocarbon from which they may be considered to be derived, is made up by adding *dioic* to the name of the hydrocarbon which has the corresponding total number of carbon atoms. Thus, the C_2 acid, oxalic, is called *ethanedioic*, the C_6 acid, adipic, is referred to as *hexanedioic*. Some confusion exists in the older literature, as the names of the acids were derived from the number of methylene groups present. Thus, adipic acid in this system is tetramethylene-1,4-dicarboxylic acid.

The common and systematic names of the dicarboxylic acids of biological interest, together with data on their discovery, are included in Table 6.

The lower dicarboxylic acids occur chiefly as salts in vegetables. Thus, oxalic acid is present as the potassium salt in rhubarb, malonic acid occurs as the calcium salt in beet roots, while succinic acid is distributed in both plant and animal sources such as grapes, plant resins, amber, and in muscle tissue. The German terminology for succinic acid, Bernsteinsäure, is based upon the fact that it is the acid found in amber. Glutaric acid occurs

principally in muscle tissue, as the derivative, α -ketoglutaric acid. Adipic acid is present in beet extracts.¹⁹⁸ Thapsic acid, the C₁₆ dicarboxylic acid, is another example of this group of acids which occur in "death-carrot" root (*Thapsia garganica*).¹⁹⁹ Suberic acid obtains its name from the fact that it originates on oxidation of cork with nitric acid (suberose means cork-like).

The rather widespread occurrence of azelaic acid can, in some cases at least, be traced to its origin as an oxidation product of unsaturated fatty acid.²⁰⁰ This is obviously the explanation for the presence of this acid in the specimens of ointment removed from Egyptian tombs, where it had been exposed to the air for 5000 years.²⁰¹ A similar explanation should also hold for its presence in some samples of linseed oil.²⁰² It has been reported to result from the hydrolysis of a substance present in mold spores,²⁰³ as well as from the oxidation of keratin.²⁰⁴

The higher weight dicarboxylic acids occur in different plant waxes. In Japan wax, 5 to 7% of the fat is composed of dicarboxylic acids of high molecular weight; the C₂₀, C₂₁, C₂₂, and C₂₃ acids have been detected.²⁰⁵⁻²¹¹ Sumac berry wax has also been found to contain some of the higher dicarboxylic acids.²¹²

3. Physical Properties of Fatty Acids

(1) General Physical Properties

The physical properties of the fatty acids are of interest as regards their structure and molecular size. Melting point, boiling point, density, and refractive index are properties which are quite distinctive for the different types of fatty acids, and which vary progressively with the length of the carbon chain, as well as with the number and position of the double bonds. The values of these constants for the different acid series are included in Tables 7 to 11 (pp. 45-49), while the basic factors on which they depend are included in later sections. Crystal structure, polymorphism and spectral

¹⁹⁸ E. O. v. Lippmann, *Ber.*, 24, 3299-3306 (1891).

¹⁹⁹ F. Canzoneri, *Gazz. chim. ital.*, 13, 514-521 (1883).

²⁰⁰ B. H. Nicolet and L. M. Liddle, *J. Ind. Eng. Chem.*, 8, 416-417 (1916).

²⁰¹ A. Banks and T. P. Hilditch, *Analyst*, 58, 265-269 (1933).

²⁰² L. C. A. Nunn and I. Smedley-MacLean, *Biochem. J.*, 32, 1974-1981 (1938).

²⁰³ A. Kiesel, *Z. physiol. Chem.*, 149, 231-258 (1925).

²⁰⁴ T. Lissizin, *Z. physiol. Chem.*, 62, 226-228 (1909).

²⁰⁵ A. C. Geitel and G. van der Want, *J. prakt. Chem.* [2], 61, 151-156 (1900).

²⁰⁶ R. Majima and S. Chó, *Ber.*, 40, 4390-4397 (1907).

²⁰⁷ R. Schaal, *Ber.*, 40, 4784-4788 (1907).

²⁰⁸ E. Tassilly, *Bull. soc. chim.* [4], 9, 608-615 (1911).

²⁰⁹ G. J. Fels, *Seifenfabrik.*, 36, 141-142 (1916); *Chem. Abst.*, 10, 1443 (1916).

²¹⁰ B. Flaschenträger, and F. Halle, *Z. physiol. Chem.*, 190, 120-140 (1930).

²¹¹ M. Tsujimoto, *Bull. Chem. Soc. Japan*, 6, 325-337 (1931).

²¹² M. Tsujimoto, *Bull. Chem. Soc. Japan*, 6, 337-341 (1931).

TABLE 7
 PHYSICAL CONSTANTS OF SATURATED FATTY ACIDS MOST COMMONLY FOUND IN FATS

Systematic name	Number of carbon atoms	Molecular weight ^a	Neutralization value ^b	Melting point, °C.	Boiling point, °C. ^c	Density (d_4^{20}) ^d	Refractive index (n_D^{20}) ^d
<i>n</i> -Butanoic	4	88.10	636.79	- 7.9	162-162.5 ^{68,8}	0.9587 ²⁰	1.33906 ²⁰ ; 1.3775 ⁷⁰
<i>n</i> -Hexanoic	6	116.15	483.00	- 3.9	205.35 ⁷⁰⁰ ; 146 ¹⁰⁰ ; 100 ¹⁰	0.94423 ⁰ ; 0.93136 ¹⁵ ; 0.91832 ⁸⁰	1.41635 ³⁰
<i>n</i> -Octanoic	8	144.21	389.05	16.3	239.3 ¹⁰⁰ ; 124 ¹⁰	0.90884 ²⁰ ; 0.8708 ⁷⁰	1.4285 ²⁰ ; 1.4085 ⁷⁰
<i>n</i> -Decanoic	10	172.26	325.69	31.3	268.7 ²⁰⁰ ; 200 ¹⁰⁰ ; 149 ¹¹	0.8858 ¹⁰	1.42855 ¹⁰ ; 1.4201 ^{60,6}
<i>n</i> -Dodecanoic	12	200.31	280.08	44	225 ¹⁰⁰ ; 176 ¹⁵ ; 141-142 ^{0,7}	0.8690 ⁹⁰ ; 0.8373 ⁷⁵	1.4261 ^{60,1} ; 1.4225 ⁷⁰
<i>n</i> -Tetradecanoic	14	228.36	245.68	54.4	250.5 ¹⁰⁰ ; 199 ¹⁶	0.8622 ^{5,1} ; 0.8533 ⁷⁰	1.4273 ⁷⁰
<i>n</i> -Hexadecanoic	16	256.42	218.80	62.85	268.0 ¹⁰⁰ ; 219 ²⁰ ; 139 ¹	0.8527 ⁶² ; 0.8487 ⁷⁰ ; 0.8465 ^{75,8} ; 0.8347 ⁹⁰	1.4339 ⁶⁰ ; 1.4303 ⁷⁰ ; 1.4284 ^{74,5} ; 1.42691 ⁸⁰
<i>n</i> -Octadecanoic	18	284.47	197.23	69.6	291 ¹⁰⁰ ; 238 ¹⁷ ; 158-160 ^{0,25}	0.9408 ²⁰ ; 0.8451 ^{60,2} ; 0.8386 ⁸⁰	1.4332 ⁷⁰ ; 1.4300 ⁸⁰
<i>n</i> -Eicosanoic	20	312.52	179.52	75.35	203-205 ¹	0.8240 ¹⁰⁰	1.4250 ¹⁰⁰
<i>n</i> -Docosanoic	22	340.57	164.73	79.95	306 ⁶⁰	0.8221 ¹⁰⁰	1.4270 ¹⁰⁰
<i>n</i> -Tetracosanoic	24	368.62	152.00	84.15	—	—	1.4287 ¹⁰⁰
<i>n</i> -Hexacosanoic	26	396.68	141.44	87.7	—	0.8198 ¹⁰⁰	1.4301 ¹⁰⁰
<i>n</i> -Octacosanoic	28	424.73	132.09	90.9	—	0.8191 ¹⁰⁰	1.4313 ¹⁰⁰
<i>n</i> -Triacosanoic	30	452.78	123.91	93.6 ^e	—	—	1.4323 ¹⁰⁰
<i>n</i> -Dotriacontanoic	32	480.83	116.70	96.2	—	—	—

^a Calculated on the basis of the International Atomic Weights (1940).

^b The neutralization value of a monobasic acid is equal to the number of milligrams of KOH required to neutralize one gram of the acid (56.104×1000 divided by the molecular weight of acid).

^c Superior figure indicates the barometric pressure in millimeters of mercury at which the boiling point was determined.

^d Superior figure in each case represents temperature (°C.) usually recorded at *x*.

^e Synthetic triacontanoic acid.

TABLE 8. PHYSICAL CONSTANTS OF MONOETHENOID ACIDS MOST COMMONLY FOUND IN NATURAL FATS^a

Systematic name	Number of carbon atoms	Molecular weight ^b	Neutralization value ^c	Iodine number ^d	Melting point, °C.	Boiling point, °C.	Density (d_4^{20}) ^f	Refractive index (n_D^{20}) ^f
4-Decenoic	10	170.2	329.6	149.1	—	148-150 ¹³	0.9223 ¹⁵ ; 0.9197 ²⁰	1.4519 ¹⁵ ; 1.4497 ²⁰
9-Decenoic	10	170.2	329.6	149.1	—	143-148 ¹⁵	0.9238 ¹⁵	1.4507 ¹⁵ ; 1.4488 ²⁰
4-Dodecenoic	12	198.3	282.9	128.0	1.0-1.3	170-172 ¹³	0.9106 ¹⁵ ; 0.9081 ²⁰	1.4545 ¹⁵ ; 1.4529 ²⁰
5-Dodecenoic	12	198.3	282.9	128.0	—	—	0.9130 ¹⁵	1.4535 ¹⁵
9-Dodecenoic	12	198.3	282.9	128.0	—	—	—	—
4-Tetradecenoic	14	226.4	247.9	112.2	18-18.5	185-188 ¹³	0.9055 ¹⁵ ; 0.9024 ²⁰	1.4575 ¹⁵ ; 1.4557 ²⁰
5-Tetradecenoic	14	226.4	247.9	112.2	—	—	0.9081 ¹⁵ ; 0.9046 ²⁰	1.4571 ¹⁵ ; 1.4552 ²⁰
9-Tetradecenoic	14	226.4	247.9	112.2	Liquid	—	0.9018 ²⁰	1.4549 ²⁰
9-Hexadecenoic	16	254.4	220.5	99.8	-0.5 to 0.5	—	—	—
6-Octadecenoic	18	282.4	198.6	89.9	32-33(33-34) ^g	208-210 ^{10a}	0.8824 ^{35b} ; 0.8795 ^{10c}	1.4533 ^{40g}
9-Octadecenoic	18	282.4	198.6	89.9	13	200-201 ^{1,2} ; 215-216 ⁵ ; 225-226 ¹⁰ ; 234-235 ¹⁵	0.8681 ^{40g} ; 0.898 ¹⁵ ; 0.887 ³⁰ ; 0.875 ³⁰	1.4535 ^{47h} ; 1.4582 ³⁰ ; 1.4417 ¹⁰
11-Octadecenoic	18	282.4	198.6	89.9	6-8 <i>cis</i> 39.5 <i>trans</i>	—	—	—
12-Octadecenoic	18	282.4	198.6	89.9	—	—	—	—
9-Eicosenoic	20	310.5	180.7	81.8	—	—	—	—
11-Eicosenoic	20	310.5	180.7	81.8	—	—	—	—
11-Docosenoic	22	338.6	165.7	75.0	—	—	—	—
13-Docosenoic	22	338.6	165.7	75.0	33.5	241-243 ⁵ ; 252-254 ¹²ⁱ ; 254.5 ¹⁰ ; 264 ¹⁵ ; 281 ^{30j}	—	—
15-Tetracosenoic	24	366.6	153.0	69.2	42.5-43	—	—	—
17-Hexacosenoic	26	394.7	142.2	64.3	—	—	—	—
21-Triacontenoic	30	450.8	124.5	56.3	—	—	—	—

^a Most of the data collected here were obtained from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948.

^b Calculated on the basis of International Atomic Weights (1940).

^c The neutralization value of a monobasic acid is equal to the number of milligrams of KOH required to neutralize one gram of the acid (56.104 X 1000 divided by the molecular weight of acid).

^d Calculated values.

^e Superior figure indicates the barometric pressure in millimeters of mercury at which the boiling point was determined.
^f Superior figure in each case represents temperature (°C.) usually recorded at *x*.

^g E. Vongerichte and A. Köhler, *Ber.*, **42**, 1638-1639 (1909).
^h A. A. Chernoyarova, *J. Gen. Chem. U. S. S. R.*, **9**, 149-152 (1939); *Chem. Abst.* **33**, 6239 (1939).

ⁱ C. R. Noller and R. H. Talbot, *Organic Syntheses*, Coll. Vol. 2, Blatt, ed., Wiley, New York, 1943, pp. 258-260.

^j F. Kraft and H. Noerdlinger, *Ber.*, **22**, 816-820 (1889).

TABLE 9
PHYSICAL CONSTANTS OF DIETHENOID AND POLYETHENOID ACIDS MOST COMMONLY FOUND IN NATURAL FATS^a

Systematic name	Number of carbon atoms	Molecular weight ^b	Neutralization value ^c	Iodine number ^d	Melting point, °C.	Boiling point, °C. ^e	Density (d_4^{25})	Refractive index (n_D^{25}) ^f
9,12-Octadecadienoic	18	280.4	200.1	181.0	-5 to -12 ^g	202 ^{1,4}	0.9038 ¹⁸	1.4715 ^{11,5} 1.4699 ²⁰
6,10,14-Hexadecatrienoic	16	250.4	224.1	(310.6)	—	—	0.9007 ^{22,8}	1.4683 ^{21,5}
9,12,15-Octadecatrienoic	18	278.4	201.5	273.5	-14.4 (to -14.5	157- 158 ^{9,10,1-0.002}	0.9324 ¹⁵ 0.9288 ²⁰	1.4876 ¹⁵ 1.4855 ²⁰
9,11,13-Octadecatrienoic	18	278.4	201.5	273.5	48 (α) 71 (β)	235 ¹²	—	—
4,8,12,15-Octadecatetraenoic	18	276.4	203.0	(372.6)	—	—	0.9334 ¹⁵	1.4930 ¹⁵
9,11,13,15-Octadecatetraenoic	18	276.4	203.0	367.4	85-86	—	0.9297 ²⁰	1.4911 ²⁰
5,8,11,14-Eicosatetraenoic	20	304.5	184.3	(316.2)	-49.5	—	—	—
4,8,12,15,18-Eicosapentaenoic	20	302.5	185.5	335.7	—	—	—	1.5563 ^{23,4}
4,8,12,15,19-Docosapentaenoic	22	330.5	169.8	384.0	-78	—	0.9385 ¹⁶	1.5039 ¹⁵
4,8,12,15,18,21-Tetracosahexaenoic	24	356.5	157.4	427.2	—	—	0.9356 ²⁰	1.5020 ²⁰
?-Hexacosahexaenoic	26	382.6	140.6	(372.1)	—	—	0.9433 ²⁰	1.5022 ²⁰

^a Most of the data collected here were obtained from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948.

^b Calculated on the basis of International Atomic Weights.

^c The neutralization values of monobasic acid are equal to the number of milligrams of KOH required to neutralize one gram of the acid (56.104 \times 1000 divided by the molecular weight of the acid).

^d Calculated values, except actual determinations, are given in parentheses.

^e Superior figure in each case represents temperature (°C.) usually recorded at x . The boiling point was determined.

^f Superior figure in each case represents temperature (°C.) usually recorded at x . The boiling point was determined.

^g The following values have been recorded: -5.2 to -5.0° (N. L. Matthews, W. R. Brode, and J. B. Brown, *J. Am. Chem. Soc.*, 63, 1064-1067 (1941)). -8 to -7° (D. Holde and R. Gentner, *Ber.*, 58B, 1067-1071 (1925)). -12 to -11 (W. C. Smit, *Rec. trav. chim.*, 49, 539-551 (1930)).

^h J. B. Brown, *J. Biol. Chem.*, 80, 455-460 (1928).

ⁱ W. C. Ault and J. B. Brown, *J. Biol. Chem.*, 107, 615-622 (1934).

TABLE 10
CONSTANTS OF SOME NATURAL HYDROXY-ACIDS

Systematic name	Number of carbon atoms	Molecular weight ^a	Neutralization value ^b	Melting point, °C.	Density (d_{4}^{20})	Refractive index (n_D^{20})	Specific rotation (α_D^{25})
12-Hydroxydodecanoic	12	216.2	259.5	84	—	—	—
3,11-Dihydroxytetradecanoic	14	260.2	215.6	—	—	—	—
11-Hydroxypentadecanoic	15	258.2	217.3	—	—	—	—
11-Hydroxyhexadecanoic	16	272.3	206.0	68-69	—	—	—
16-Hydroxyhexadecanoic	16	272.3	206.0	95	—	—	—
9,10-Dihydroxyoctadecanoic	18	316.3	177.4	—	—	—	—
12-Hydroxy-9-octadecenoic	18	298.3	188.1	5.5	0.940	1.4711	+6.25

^a Calculated on the basis of International Atomic Weights (1940).

^b The neutralization values of the monobasic acids are equal to the number of milligrams of KOH required to neutralize one gram of acid (56.104 \times 1000 divided by the molecular weight of the acid).

TABLE 11
 PHYSICAL CONSTANTS OF SATURATED DICARBOXYLIC ACIDS WHICH POSSESS BIOLOGICAL IMPORTANCE^a

Common name	Number of carbon atoms	Molecular weight ^b	Neutralization value ^c	Melting point, °C.	Boiling point, °C. ^d	Solubility, parts per 100 ml. ^e		
						Benzene	Alcohol	Water
Oxalic	2	90.02	1246.5	—	—	—	—	—
Malonic	3	104.03	1078.5	132	—	—	—	—
Succinic	4	118.05	950.5	185	—	—	—	—
Glutaric	5	132.06	849.7	97.5	—	—	—	—
Adipic	6	146.08	768.1	153	265 ⁰⁰ ; 244.5 ⁵⁰ ; 216.5 ¹⁵ ; 205.5 ¹⁰	Easily	Easily	0.605 ¹⁹ Easily
Pimelic	7	160.10	700.9	105.7	272 ⁰⁰ ; 251.5 ⁵⁰ ; 223 ¹⁵ ; 212 ¹⁰	Insol.	in cold	2.52 ^{13,3}
Suberic	8	174.11	644.5	140	279 ⁰⁰ ; 258.5 ⁵⁰ ; 230 ¹⁵ ; 219.5 ¹⁰	—	Soluble	0.8 ¹⁵ 0.08 ⁰ ; 0.98 ⁵⁰ ; 2.22 ⁶⁵
Azelaic	9	188.13	596.4	107	286.5 ¹⁰⁰ ; 265 ⁵⁰ ; 237 ¹⁵ ; 225.5 ¹⁰	—	Extremely	2.68 ¹⁵ 0.1 ⁰ ; 0.212 ²² ; 1.648 ⁵⁵ ; 2.2 ⁶⁵
Sebacic	10	202.14	555.1	133	294.5 ¹⁰⁰ ; 273 ⁵⁰ ; 243.5 ¹⁵ ; 232 ¹⁰	—	Easily	0.004 ⁰ ; 0.42 ⁵⁵ ; 2.0 ¹⁰⁰
Brassylic	13	244.19	459.9	113.5	—	—	—	—
Thapsic	16	286.24	392.0	125	—	—	—	—
Japanic	21	356.32	314.9	—	—	—	—	—

^a Most of the data collected here were obtained from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948.

^b Calculated on basis of International Atomic Weights (1940).

^c The neutralization value of dibasic acids is equal to the number of milligrams of KOH required to neutralize one gram of the acid (112.208 × 1000 divided by the molecular weight of acid).

^d Superior figure indicates the barometric pressure in millimeters of mercury at which boiling point was determined.

^e Superior figure represents the temperature (°C.) at which the solubility figure was obtained.

absorption, refraction, and viscosity are also properties of considerable interest to the biochemist; these will be discussed later. For a more complete discussion of properties such as thermal activity, and specific conductivity, which are of more concern to the fatty acid or soap chemist than to the biochemist, one is referred to the recent treatise of Markley.⁴

(2) *Melting Points of Fatty Acids*

There are many factors which influence the melting points of the fatty acids. Probably the most important of these are the chain length and the degree of unsaturation. The position of the double bond in the molecule is also of significance, as well as the geometric form of the acid. These conditions will be discussed in successive sections.

TABLE 12

MELTING AND FREEZING POINTS OF EVEN- AND ODD-CHAIN FATTY ACIDS^a

Name of even-chain acid	No. of carbon atoms	M.p., °C.	F.p., °C.	Name of odd-chain acid	No. of carbon atoms	M.p., °C.	F.p., °C.
Acetic	2	16.6	—	Formic	1	8.4	—
Butyric	4	-7.9	-19	Propionic	3	-22	—
Caproic	6	-3.4	-3.2	Valeric	5	-59	—
Caprylic	8	16.7	16.3	Heptanoic	7	-10.5	-6.3
Capric	10	31.6	31.2	Pelargonic	9	12.5	12.2
Lauric	12	44.2	43.9	Undecanoic	11	29.3	28.1
Myristic	14	53.9	54.1	Tridecanoic	13	41.5	41.8
Palmitic	16	63.1	62.8	Pentadecanoic	15	52.3	52.5
Stearic	18	69.6	69.3	Margaric	17	61.3	60.9
Arachidic	20	75.3	74.9	Nonadecanoic	19	68.6	68.8
Behenic	22	79.7	79.7	Heneicosanoic	21	74.3	73.7
Lignoceric	24	84.2	83.9	Tricosanoic	23	79.1	78.7
Cerotic	26	87.7	87.4	Pentacosanoic	25	83.5	82.9
Montanic	28	90.0	90.4	Heptacosanoic	27	—	—
Melissic	30	93.6	93.2	Nonacosanoic	29	90.3	89.7
Dotriacontanoic	32	96.0	95.5	—	—	—	—
Tetatriacontanoic	34	98.2	98.0	—	—	—	—
Hexatriacontanoic	36	99.9	99.7	—	—	—	—
Octatriacontanoic	38	101.6	101.5	—	—	—	—

^a Adapted from K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 114.

a. Melting and Boiling Points as Functions of Chain Length. In general, it is well known that the melting points of fatty acids and of the triglycerides increase progressively with the extension of the carbon chain. When the melting points of the even-chain or of the odd-chain acids are plotted against the number of carbon atoms, a practically straight-line curve is obtained in either case. The odd-chain acids usually melt at a lower temperature than do the even-chain acids containing one less carbon

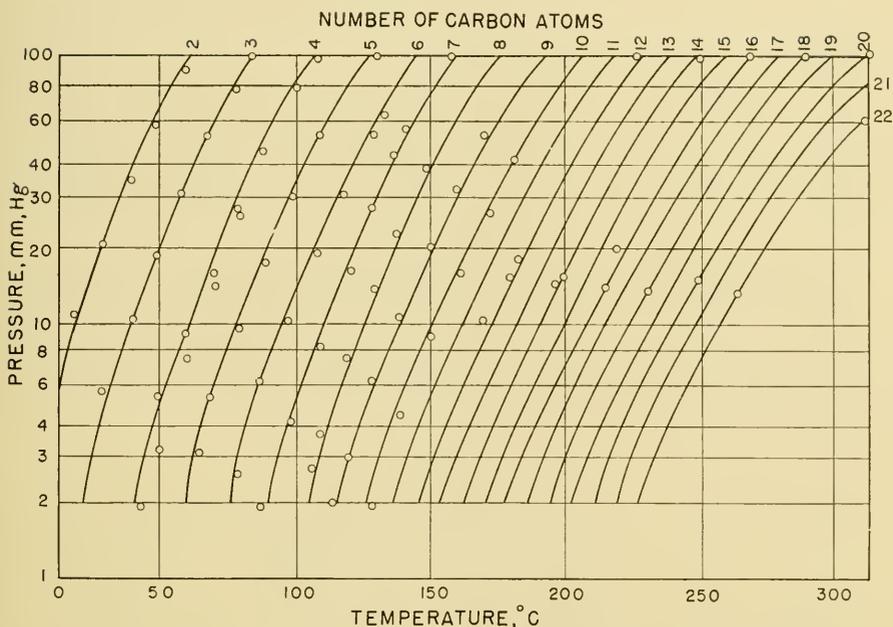


Fig. 1. Boiling points of the fatty acids under conditions of vacuum-steam distillation.²¹³

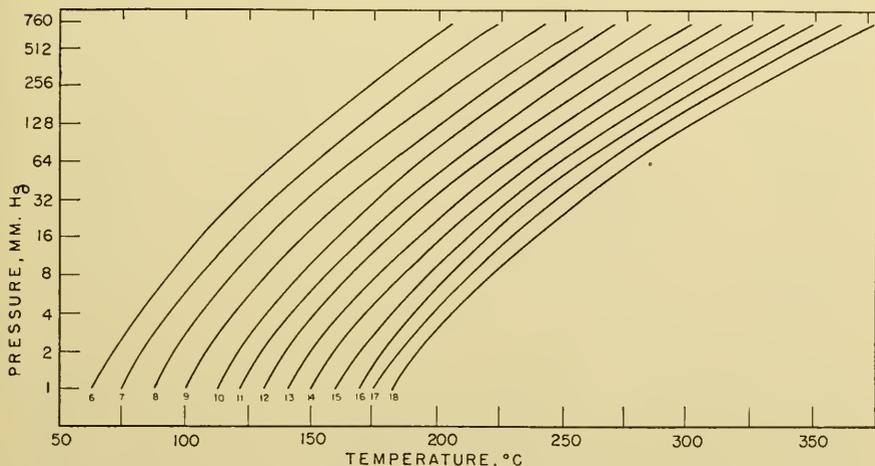


Fig. 2. Vapor pressure curves of the *n*-alkyl acids containing 6–18 carbon atoms.²¹⁴

atom. However, starting with myristic acid, and continuing for the higher pairs, the melting points of the even- and odd-chain fatty acids are almost identical. The methyl and ethyl esters follow the same pattern but tend to have values considerably below those of the corresponding free fatty

acid. The melting points and freezing points, which usually approximate each other, are summarized in Table 12.

The irregularity in the melting points for the even- and odd-chain acids is not noted in the boiling points as indicated by the results of Genseke^{57,213} obtained under conditions of vacuum-steam distillation (Fig. 1) or of Pool and Ralston,²¹⁴ who determined the boiling points over a range of pressures from 1 to 760 mm. and computed the corresponding vapor pressure-temperature curves given in Figures 1 and 2.

b. Melting Point as Related to Number of Double Bonds. Generally the introduction of one unsaturated linkage results in a considerable decrease in the melting point of an acid, while the presence of additional double bonds further lowers the melting point. This is illustrated by data on the commonly occurring acids given in Table 13.

TABLE 13
EFFECT OF DEGREE OF UNSATURATION ON MELTING POINT OF SOME *cis* FORMS OF FATTY ACIDS^a

Fatty acid	Systematic name	Number of double bonds	Melting point, °C.
Palmitic	<i>n</i> -Hexadecanoic	0	63.1
Palmitoleic	9-Hexadecenoic	1	-0.5 to 0.5
Stearic	<i>n</i> -Octadecanoic	0	69.6
Oleic	9-Octadecenoic	1	13.4 or 16.3 ^b
Linoleic	9,12-Octadecadienoic	2	-5
Linolenic	9,12,15-Octadecatrienoic	3	-11
Arachidic	<i>n</i> -Eicosanoic	0	75.3
Arachidonic	5,8,11,14-Eicosatetraenoic	4	-49.5
Behenic	<i>n</i> -Docosanoic	0	79.9
Erucic	13-Docosenoic	1	33.5
Lignoceric	<i>n</i> -Tetracosanoic	0	84.2
Selacholeic	15-Tetracosenoic	1	39

^a Adapted from K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 37 ff.

^b α and β forms, respectively.

c. Melting Point as Related to the Position of the Double Bonds. There is a progressive decrease in the melting points of the *cis*-octadecenoic acids as the distance between the double bond and the carboxyl group is increased. Thus, when the unsaturated linkage is present in the 2 position, the melting point is 59°C., while in the acid in which the double bond is at the 12 position, the substance is liquid at room temperature, with a melting point of about 10°C. However, in the case of the *trans*

²¹³ W. Genseke, in G. Hefter and H. Schönfeld, eds., *Chemie und Technologie der Fette und Fettprodukte*, Vol. II, Springer, Vienna, 1937, p. 508.

²¹⁴ W. O. Pool and A. W. Ralston, *Ind. Eng. Chem.*, *34*, 1104-1105 (1942).

forms, no such variations are to be found. These values are summarized in Table 14.

TABLE 14
MELTING POINTS OF ISOMERIC *cis*- AND *trans*-OCTADECENOIC ACIDS (C₁₈H₃₄O₂)

Double bond position	Common name	Structural formula	M.p., °C.	Ref.
<i>cis</i> -Forms				
2	—	CH ₃ (CH ₂) ₁₄ CH:CHCOOH	59	<i>b,c</i>
3	—	CH ₃ (CH ₂) ₁₃ CH:CHCH ₂ COOH	56–57	<i>d</i>
4	—	CH ₃ (CH ₂) ₁₂ CH:CH(CH ₂) ₂ COOH	52–53	<i>d</i>
6	Petroselinic	CH ₃ (CH ₂) ₁₀ CH:CH(CH ₂) ₄ COOH	30	<i>e</i>
9	Oleic	CH ₃ (CH ₂) ₇ CH:CH(CH ₂) ₇ COOH	13.4 or 16.3 ^a	<i>f</i>
12	—	CH ₃ (CH ₂) ₄ CH:CH(CH ₂) ₁₀ COOH	9.8 to 10.4	<i>g</i>
<i>trans</i> -Forms				
5	—	CH ₃ (CH ₂) ₁₁ CH:CH(CH ₂) ₃ COOH	47.5	<i>h</i>
6	Petroselaiddic	CH ₃ (CH ₂) ₁₀ CH:CH(CH ₂) ₄ COOH	53	<i>i,j</i>
7	—	CH ₃ (CH ₂) ₉ CH:CH(CH ₂) ₅ COOH	45.5	<i>h</i>
8	—	CH ₃ (CH ₂) ₈ CH:CH(CH ₂) ₆ COOH	53	<i>k</i>
9	Elaidic	CH ₃ (CH ₂) ₇ CH:CH(CH ₂) ₇ COOH	43.7	<i>k</i>
10	—	CH ₃ (CH ₂) ₆ CH:CH(CH ₂) ₈ COOH	42	<i>l</i>
11	Vaccenic	CH ₃ (CH ₂) ₅ CH:CH(CH ₂) ₉ COOH	39	<i>m</i>
12	—	CH ₃ (CH ₂) ₄ CH:CH(CH ₂) ₁₀ COOH	39.7 to 40.1	<i>g</i>

^a α - and β -forms, respectively.

^b H. R. Le Sueur, *J. Chem. Soc.*, 85, 1708–1713 (1904).

^c G. Ponzio, *Atti accad. sci. Torino*, 39, 552–560 (1903–1904); *Gazz. chim. ital.*, 34, 77–85 (1904).

^d A. Eckert and O. Halla, *Monatsh.*, 34, 1815–1824 (1913).

^e T. P. Hilditch and E. E. Jones, *J. Soc. Chem. Ind.*, 46, 174–177 (1927).

^f J. C. Smith, *J. Chem. Soc.*, 1939, I, 974–980.

^g A. Grün and W. Czerny, *Ber.*, 59, 54–63 (1926).

^h S. Pasternak, *Compt. rend.*, 162, 944–946 (1916).

ⁱ H. N. Griffiths and T. P. Hilditch, *J. Chem. Soc.*, 1932, 2315–2324.

^j A. Arnaud and S. Pasternak, *Compt. rend.*, 150, 1130–1132 (1910).

^k A. Arnaud and S. Pasternak, *Compt. rend.*, 150, 1525–1528 (1910).

^l J. Boeseken, and R. Hoovers, *Rec. trav. chim.*, 49, 1161–1174 (1930).

^m S. H. Bertram, *Biochem. Z.*, 197, 433–441 (1928).

TABLE 15
EFFECT OF CONJUGATION ON MELTING POINTS OF POLYETHENOIC ACIDS

Double bond position	Common name	Systematic name	Form of bonds ^a	M.p., °C.
9,12	Linoleic	9,12-Octadecadienoic	N-C	– 5
9,11 ^b	—	9,11-Octadecadienoic	C	57
9,12,15	Linolenic	9,12,15-Octadecatrienoic	N-C	– 11
9,11,13	α -Elaeostearic	9,11,13-Octadecatrienoic	C	48–49
9,11,13	β -Elaeostearic	9,11,13-Octadecatrienoic	C	71
9,11,13	Punicic	9,11,13-Octadecatrienoic	C	44
9,11,13	Trichosanic	9,11,13-Octadecatrienoic	C	35

^a N-C, non-conjugated; C, conjugated.

^b J. D. von Mikusch, *J. Am. Chem. Soc.*, 64, 1580–1582 (1942).

Not only does the position of a single double bond influence the melting point, but variations in the melting points of triethenoid acids occur with alterations in the relative positions of the double bonds. Whenever the bonds are in conjugate position, *i.e.*, on alternating carbon atoms, the resulting compounds melt at a considerably higher temperature than do the corresponding non-conjugated acids. This is illustrated by several well-known examples in Table 15.

d. Melting Point as Related to Geometrical Configuration. In establishing the melting point of unsaturated fatty acids, in addition to the number of bonds and their position in the molecule, a standard of equal importance is the factor of geometrical form. The change from a *cis* to a *trans* form is always accompanied by a marked rise in melting point, provided other features of the structure remain unchanged. This alteration occurs not only in the case of the acids themselves but also with their hydroxy-derivatives.

TABLE 16
MELTING POINTS OF *cis* AND *trans* ISOMERS OF MONO- AND POLYETHENOID ACIDS^a

Name of acid		Number of carbons	Double bond position	Melting point, °C.	
<i>cis</i> form	<i>trans</i> form			<i>cis</i> -acid	<i>trans</i> -acid
Petroselinic	Petroselaidic	18	6	30	53
Oleic	Elaidic	18	9	13.4 or 16.3 ^b	43.7
12-Octadecenoic	12-Octadecenoic	18	12	9.8-10.4	39.7-40.1
Erucic	13-Docosenoic	22	13	33.5	60
Selacholeic	15-Tetracosenoic	24	15	39	69
Linoleic	Linolelaidic	18	9,12	- 5	28-29
Linolenic	Elaidolinolenic	18	9,12,15	-11.0	29-30
Ricinoleic	12-Hydroxy-9-octadecenoic	18	9 ^c	5.5	53

^a Most of the data are from K. S. Markley, *Fatty Acids*, Interscience, New York, 1947.

^b α - and β -forms, respectively.

^c Contains hydroxyl group at position 12.

e. Melting Point as Related to Position and Number of Methyl Side Chains. The introduction of methyl side chains is always accompanied by the lowering of the melting point (Table 17). The extent of the decrease in melting point reaches a maximum when the methyl group is present near the center of the molecule. Thus, the 10-methyl-substituted octadecenoic acid has a markedly lower melting point than does the normal acid, while in the case of the 2-methylated acid the melting point is altered much less (Table 18). The introduction of two methyl groups into the position adjacent to the carboxyl (position 2) has an augmented effect in lowering the melting point over that of the monomethyl-substituted derivative (Table 19).

TABLE 17. EFFECT OF INTRODUCTION OF METHYL GROUP ON MELTING POINT OF SATURATED FATTY ACIDS

Systematic name of acid	Formula	Melting point, °C.		
		10-Methylated	2-Methylated	Comparable nonmethylated
<i>n</i> -Octadecanoic ^b	CH ₃ (CH ₂) ₁₆ COOH	—	—	69.6 ^{a,b}
10-Methyloctadecanoic ^c	CH ₃ (CH ₂) ₇ CH(CH ₃)(CH ₂) ₈ COOH	10-11 ^d	—	—
2-Methyloctadecanoic	CH ₃ (CH ₂) ₁₅ CH(CH ₃)COOH	—	54.5 ^e	—
<i>n</i> -Eicosanoic	CH ₃ (CH ₂) ₁₈ COOH	—	—	75.4
2-Methyleicosanoic	CH ₃ (CH ₂) ₁₇ CH(CH ₃)COOH	—	61.5-62.0	—
<i>n</i> -Docosanoic	CH ₃ (CH ₂) ₂₀ COOH	—	—	79.95 ^a
10-Methyldocosanoic	CH ₃ (CH ₂) ₁₁ CH(CH ₃)(CH ₂) ₈ COOH	45.5-46.0 ^e	—	—
2-Methyldocosanoic	CH ₃ (CH ₂) ₁₉ CH(CH ₃)COOH	—	67.0-67.5	—
<i>n</i> -Tetracosanoic	CH ₃ (CH ₂) ₂₂ COOH	—	—	84.15 ^a
10-Methyltetracosanoic	CH ₃ (CH ₂) ₁₃ CH(CH ₃)(CH ₂) ₈ COOH	51 ^e	—	—
2-Methyltetracosanoic	CH ₃ (CH ₂) ₂₁ CH(CH ₃)COOH	—	72.0-72.5	—
<i>n</i> -Hexacosanoic	CH ₃ (CH ₂) ₂₄ COOH	—	—	87.7 ^{a,f}
10-Methylhexacosanoic	CH ₃ (CH ₂) ₁₅ CH(CH ₃)(CH ₂) ₈ COOH	54.0-55.0 ^e	—	—
2-Methylhexacosanoic	CH ₃ (CH ₂) ₂₃ CH(CH ₃)COOH	—	75.5-76.0 ^e	—

^a F. Francis and S. H. Piper, *J. Am. Chem. Soc.*, 61, 577-581 (1939).

^b A. Gascard, *Ann. chim.* [9], 15, 332-382 (1921).

^c Tuberculostearic acid.

^d Isolated by R. J. Anderson and E. Chargaff, *J. Biol. Chem.*, 85, 77-88 (1929). M. A. Spielman, *J. Biol. Chem.*, 106, 87-96 (1934).

^e A. K. Schneider and M. A. Spielman, *J. Biol. Chem.*, 142, 345-354 (1942).

^f W. Bleyberg and H. Ulrich, *Ber.* 64, 2504-2513 (1931).

TABLE 18

EFFECT OF NUMBER OF METHYL GROUPS ON MELTING POINT OF SATURATED ACIDS^a

Systematic name of acid	Formula	Melting point, °C.		
		2-Me	2,2-diMe	Non-methylated
<i>n</i> -Dodecanoic	CH ₃ (CH ₂) ₁₀ COOH	—	—	44.2 ^d
2,2-Dimethyldodecanoic	CH ₃ (CH ₂) ₉ C(CH ₃) ₂ COOH	—	4 ^b	—
<i>n</i> -Octadecanoic	CH ₃ (CH ₂) ₁₆ COOH	—	—	69.6 ^{a,d}
2-Methyloctadecanoic	CH ₃ (CH ₂) ₁₅ CH(CH ₃)COOH	54.5 ^e	—	—
2,2-Dimethyloctadecanoic	CH ₃ (CH ₂) ₁₅ C(CH ₃) ₂ COOH	—	42 ^b	—

^a K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 114.

^b A. J. Birch and R. Robinson, *J. Chem. Soc.*, 1942, 488-497.

^c F. Francis and S. H. Piper, *J. Am. Chem. Soc.*, 61, 577-581 (1939).

^d A. Gascard, *Ann. chim.* [9], 15, 332-382 (1921).

^e A. K. Schneider and M. A. Spielman, *J. Biol. Chem.*, 142, 345-354 (1942).

TABLE 19

EFFECT OF POSITION OF SIDE CHAIN METHYL GROUP ON MELTING POINTS OF SOME OCTADECANOIC ACIDS

Systematic name of acid	Formula	Melting point, °C.	
		Methylated	Comparable non-methylated
<i>n</i> -Octadecanoic ^a	CH ₃ (CH ₂) ₁₆ COOH	—	69.6 ^{b,c}
18-Methyloctadecanoic ^d	CH ₃ (CH ₂) ₁₇ COOH	—	68.6 ^b
17-Methyloctadecanoic	CH ₃ CH(CH ₃)(CH ₂) ₁₅ COOH	67.3–67.8 ^{e,f}	—
16-Methyloctadecanoic	CH ₃ CH ₂ CH(CH ₃)(CH ₂) ₁₄ COOH	49.9–50.6 ^f	—
15-Methyloctadecanoic	CH ₃ (CH ₂) ₂ CH(CH ₃)(CH ₂) ₁₃ COOH	41.0–43.5 ^g	—
10-Methyloctadecanoic	CH ₃ (CH ₂) ₇ CH(CH ₃)(CH ₂) ₈ COOH	10–11 ^{h,i}	—
2-Methyloctadecanoic	CH ₃ (CH ₂) ₁₅ CH(CH ₃)COOH	54.5 ^j	—

^a Stearic acid.^b F. Francis and S. H. Piper, *J. Am. Chem. Soc.*, **61**, 577–581 (1939).^c A. Gascard, *Ann. chim.* [9], **15**, 332–382 (1921).^d Nonadecanoic acid.^e J. Cason, *J. Am. Chem. Soc.*, **64**, 1106–1110 (1942).^f J. Cason and F. S. Prout, *J. Am. Chem. Soc.*, **66**, 46–50 (1944).^g J. Cason, C. E. Adams, L. L. Bennett, and U. D. Register, *J. Am. Chem. Soc.*, **66**, 1764–1767 (1944).^h H. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 43.ⁱ M. A. Spielman, *J. Biol. Chem.*, **106**, 87–96 (1934).^j A. K. Schneider and M. A. Spielman, *J. Biol. Chem.*, **142**, 345–354 (1942).

The least effect of substitution obtains when it occurs on the penultimate carbon (next to the last carbon). The melting point of 17-methyloctadecanoic acid is practically identical with that of the straight-chain C₁₉ acid or with that of the parent non-methylated C₁₈ (stearic) acid. Similar results are reported for a series of iso-acids synthesized by Fordyce and Johnson.²¹⁵ These authors found that isomyristic (12-methyltridecanoic) acid melted at 50.5–51°C. compared with a value of 54.4°C. for myristic acid²¹⁶ or 41.5°C. for the *n*-C₁₃ acid⁴; isopalmitic (14-methylpentadecanoic) acid melted at 61.8–62.4°C., compared with a melting point of 62.8°C. for palmitic acid,²¹⁷ or one of 52.1°C. for pentadecanoic acid²¹⁸; isostearic (16-methylheptadecanoic) acid was found to melt at 67.6–68.2°C., while stearic acid^{216,219} melted at 69.6°C. and heptadecanoic acid²¹⁶ melted at 61.3°C.

Thus a number of factors influence the melting point of fatty acids. A shortening of the chain length and the introduction of one or more double bonds (especially at a distance from the carboxyl group) are factors which tend to lower the melting points of the acids. On the other hand, the ar-

²¹⁵ C. R. Fordyce and J. R. Johnson, *J. Am. Chem. Soc.*, **55**, 3368–3372 (1933).²¹⁶ F. Francis and S. H. Piper, *J. Am. Chem. Soc.*, **61**, 577–581 (1939).²¹⁷ F. Francis, F. J. E. Collins, and S. H. Piper, *Proc. Roy. Soc. London*, **A158**, 691–718 (1937).²¹⁸ F. Francis, S. H. Piper, and T. Malkin, *Proc. Roy. Soc. London*, **A128**, 214–252 (1930).²¹⁹ A. Gascard, *Ann. chim.* [9], **15**, 332–382 (1921).

rangement of the several double bonds in a conjugate position, or the change of a *cis* to a *trans* linkage, tends to bring about a marked elevation in the temperature at which such fatty acids melt. No less important than the several factors already mentioned is the presence of a methyl group as a side chain. The extent to which the resultant melting point is depressed is related to the position of substitution; it is greater when two methyl groups are introduced than when a single one is present.

The melting points of the triglycerides closely approximate those of the fatty acids (see page 251). Since the digestibility of a fat is decreased markedly when the melting points exceed 50°C., it is evident that some types of isomerism may be related to an alteration of the nutritional value of the fat.

(3) *Solubility of Fatty Acids*

In order for biochemical changes to occur, it is usually necessary for the reacting substances to be in solution in the same medium. Most biological reactions occur in aqueous media, since carbohydrates, proteins, amino acids, their decomposition products, and most inorganic constituents are soluble in water. Although the sodium and potassium soaps of the fatty acids are on the whole quite soluble in water, the fatty acids, as well as their monatomic and polyhydric esters, are quite immiscible with this solvent. Nevertheless, the short-chain fatty acids are very soluble in water. Even the higher fatty acids dissolve to some extent in water, and this solubility may be sufficient to enable biochemical changes to take place. Precise data on the solubility of many of the saturated fatty acids in water and in a large number of other solvents have been obtained through the recent comprehensive studies of Ralston, Hoerr, and their collaborators. Although space is not available to include the results on all of the solvents employed by these workers, it would seem to be of sufficient interest and importance to list the solubilities of the fatty acids in several of the specific lipid solvents.

a. Solubility of Saturated Fatty Acids in Water and of Water in Fatty Acids. The solubility of the saturated fatty acids from caproic (C_6) to stearic (C_{18}) has been reported by Ralston and Hoerr²²⁰ for temperatures from 0° to 60°C. These results are included in Table 20. No data are given for acetic, propionic, or butyric acids, but these are known to be miscible with water in all proportions. Valeric acid is soluble to the extent of 3.7 g. per 100 grams of water. However, with the exception of butyric acid, which occurs as a constituent of cow milk fat, none of these other short-chain acids is important, inasmuch as the acids are not present in naturally occurring fats.

²²⁰ A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, 7, 546-555 (1942).

TABLE 20. SOLUBILITIES OF FATTY ACIDS IN WATER^a

Name of acid	No. of carbon atoms	Grams acid per 100 grams water at				
		0.0°	20.0°	30.0°	45.0°	60.0°C.
Caproic.....	6	0.864	0.968	1.019	1.095	1.171
Heptanoic.....	7	0.190	0.244	0.271	0.311	0.353
Caprylic.....	8	0.044	0.068	0.079	0.095	0.113
Nonanoic.....	9	0.014	0.026	0.032	0.041	0.051
Capric.....	10	0.0095	0.015	0.018	0.023	0.027
Undecanoic.....	11	0.0063	0.0093	0.011	0.013	0.015
Lauric.....	12	0.0037	0.0055	0.0063	0.0075	0.0087
Tridecanoic.....	13	0.0021	0.0033	0.0038	0.0044	0.0054
Myristic.....	14	0.0013	0.0020	0.0024	0.0029	0.0034
Pentadecanoic.....	15	0.00076	0.0012	0.0014	0.0017	0.0020
Palmitic.....	16	0.00046	0.00072	0.00083	0.0010	0.0012
Margaric.....	17	0.00028	0.00042	0.00055	0.00069	0.00081
Stearic.....	18	0.00018	0.00029	0.00034	0.00042	0.00050

^a A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, 7, 546-555 (1942), p. 547.

TABLE 21. APPROXIMATE SOLUBILITY OF WATER IN SATURATED FATTY ACIDS AT SEVERAL TEMPERATURES^a

Fatty acid	Per cent of water dissolved in fatty acids at temperatures (°C.) given in parentheses					
Caproic.....	2.21	4.73	7.57	9.70	—	—
	(-5.4)	(12.3)	(31.7)	(46.3)	—	—
Heptanoic.....	2.98	—	—	9.98 ^b	—	—
	(-8.3)	—	—	(42.5)	—	—
Caprylic.....	—	3.88	—	—	—	—
	—	(14.4)	—	—	—	—
Pelargonic.....	—	3.45	—	—	—	—
	—	(10.5)	—	—	—	—
Capric.....	—	—	3.12	—	—	—
	—	—	(29.4)	—	—	—
Undecanoic.....	—	—	2.72	—	4.21	—
	—	—	(26.8)	—	(57.5)	—
Lauric.....	—	—	—	2.35	—	2.70
	—	—	—	(42.5)	—	(75.0)
Tridecanoic.....	—	—	—	2.00	—	—
	—	—	—	(40.8)	—	—
Myristic.....	—	—	—	—	1.70	—
	—	—	—	—	(53.2)	—
Pentadecanoic.....	—	—	—	—	1.46	—
	—	—	—	—	(51.8)	1.62
	—	—	—	—	—	(90.0)
Palmitic.....	—	—	—	—	—	1.25
	—	—	—	—	—	(61.8)
Margaric.....	—	—	—	—	—	1.06
	—	—	—	—	—	(60.4)
Stearic.....	—	—	—	—	—	0.92
	—	—	—	—	—	(68.7)
	—	—	—	—	—	(92.4)

^a Adapted from C. W. Hoerr, W. O. Pool, and A. W. Ralston, *Oil & Soap*, 19, 126-128 (1942).

^b Value reported erroneously^a later corrected in A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948.

When one considers the solubility of water in the fatty acids, the variations between caproic and stearic acid are much less marked and the temperature effects are considerably less pronounced than are noted in regard to solubilities of the fatty acids in water. In all cases the amount of water retained in the fatty acid is markedly greater than the quantity of fatty acid dissolved in water. Data on these studies of Hoerr *et al.*²²¹ are recorded in Table 21.

b. Solubility of Saturated Fatty Acids in Non-aqueous Solvents.

The solubilities of the saturated fatty acids in such non-aqueous solvents as benzene, cyclohexane, ethanol, and isopropanol are summarized in Tables 22 to 25, as well as in Figures 3 to 6. Ralston and Hoerr reported

TABLE 22
SOLUBILITY OF SATURATED FATTY ACIDS IN BENZENE^a

Name of acid	No. of C atoms	Grams acid dissolved per 100 grams benzene at					
		10°	20°	30°	40°	50°	60°C.
Caprylic.....	8	770	∞	∞	∞	∞	∞
Pelargonic.....	9	2680	∞	∞	∞	∞	∞
Capric.....	10	145	398	8230	∞	∞	∞
Undecanoic.....	11	208	663	∞	∞	∞	∞
Lauric.....	12	32.3	93.6	260	1390	∞	∞
Tridecanoic.....	13	42.4	117	354	7600	∞	∞
Myristic.....	14	6.95	29.2	87.4	239	1290	∞
Pentadecanoic.....	15	8.84	36.2	103.0	295	2280	∞
Palmitic.....	16	1.04	7.30	34.8	105	306	2170
Margaric.....	17	1.52	9.23	42.1	121	369	5450
Stearic.....	18	0.24	2.46	12.4	51	145	468

^a A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, 7, 546-555 (1942), p. 552.

TABLE 23
SOLUBILITY OF SATURATED FATTY ACIDS IN CYCLOHEXANE^a

Name of acid	No. of C atoms	Grams acid dissolved per 100 grams cyclohexane at					
		10°	20°	30°	40°	50°	60°C.
Caprylic.....	8	670	∞	∞	∞	∞	∞
Pelargonic.....	9	2340	∞	∞	∞	∞	∞
Capric.....	10	103	342	7600	∞	∞	∞
Undecanoic.....	11	150	525	∞	∞	∞	∞
Lauric.....	12	19.8	68	215	1310	∞	∞
Tridecanoic.....	13	31.0	100	330	8200	∞	∞
Myristic.....	14	5.3	21.5	72.0	217	1310	∞
Pentadecanoic.....	15	6.8	27.1	88.0	277	2460	∞
Palmitic.....	16	0.9	6.5	27.4	92.0	285	2530
Margaric.....	17	1.5	8.4	34.0	108	365	7600
Stearic.....	18	0.2	2.4	10.5	43.8	133	450

^a C. W. Hoerr and A. W. Ralston, *J. Org. Chem.*, 9, 329-337 (1944), p. 330.

²²¹ C. W. Hoerr, W. O. Pool, and A. W. Ralston, *Oil & Soap*, 19, 126-128 (1942).

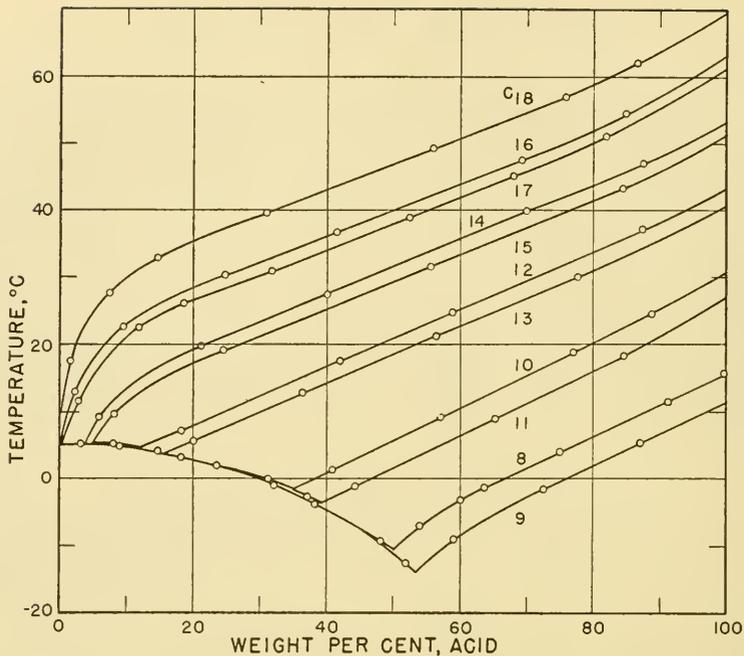


Fig. 3. Solubility of saturated fatty acids in anhydrous benzene.²²⁰

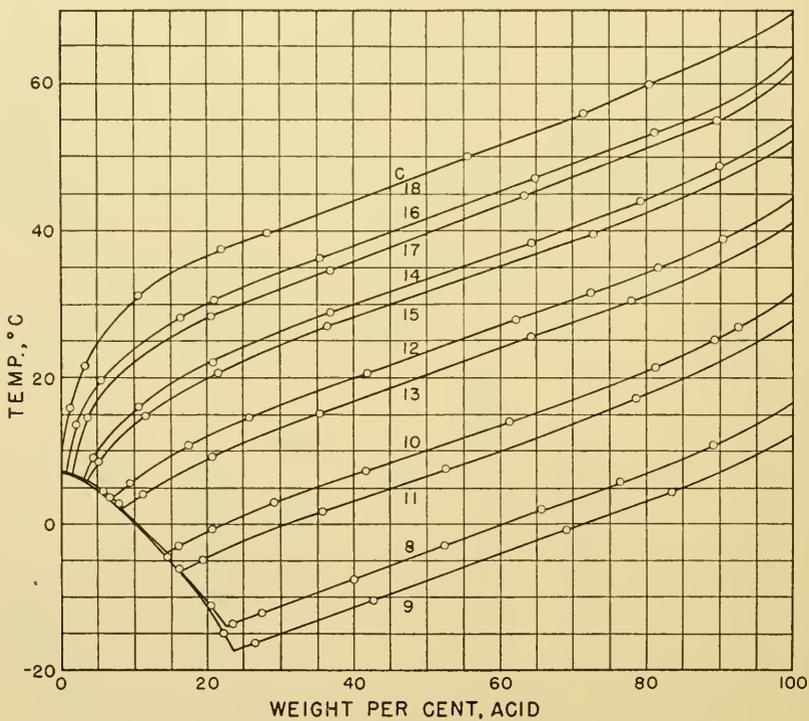


Fig. 4. Solubility of saturated fatty acids in cyclohexane.²²²

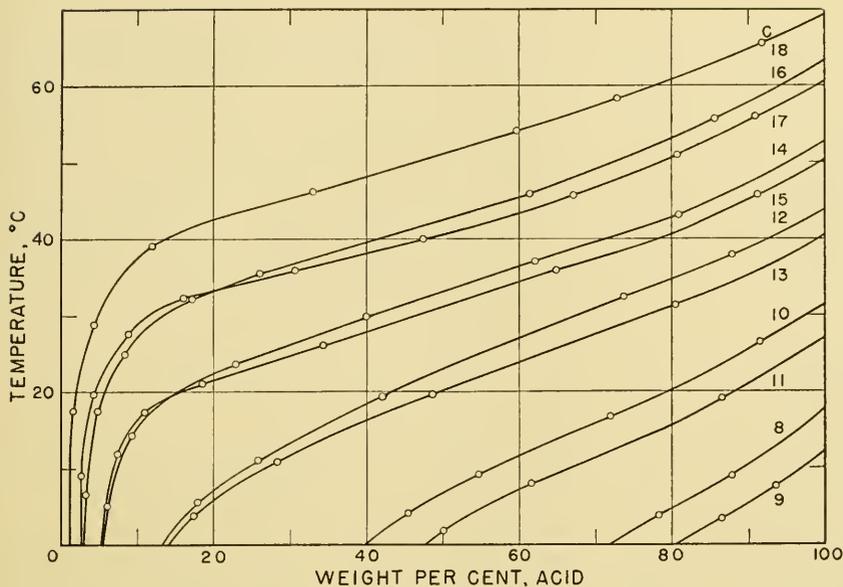


Fig. 5. Solubilities of saturated fatty acids in ethanol (95.0% by weight).²²⁰

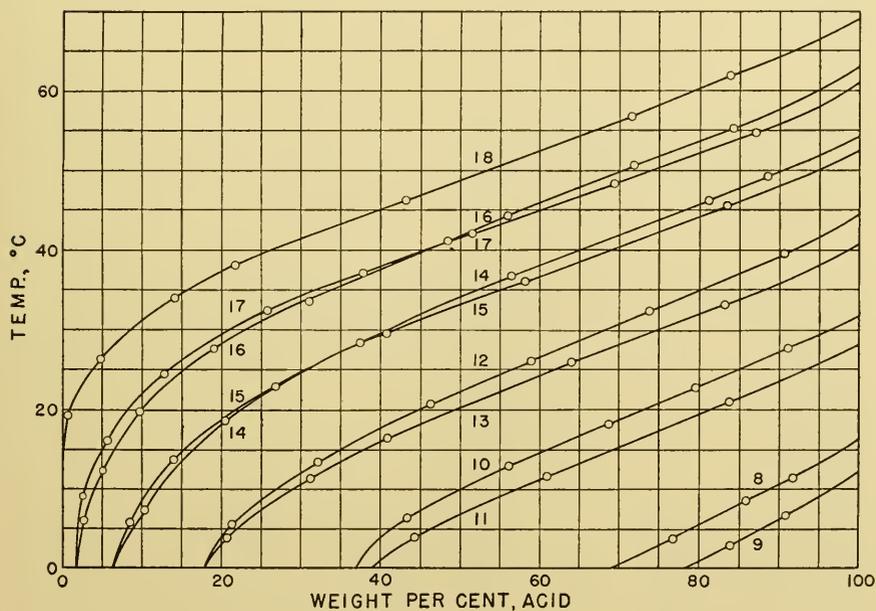


Fig. 6. Solubilities of saturated fatty acids in *n*-butanol.²²²

²²² C. W. Hoerr and A. W. Ralston, *J. Org. Chem.*, 9, 329-337 (1944).

TABLE 24. SOLUBILITY OF SATURATED FATTY ACIDS IN ETHANOL^a

Name of acid	No. of C atoms	Grams acid dissolved per 100 grams solvent at						
		0°	10°	20°	30°	40°	50°	60°C.
99.4% Ethanol by Weight								
Lauric.....	12	20.4	41.6	105	292	1540	∞	∞
Myristic.....	14	7.07	9.77	23.9	84.7	263	1560	∞
Palmitic.....	16	1.89	3.20	7.21	23.9	94.2	320	2600
Margaric.....	17	2.04	2.98	6.62	22.2	110	388	8230
Stearic.....	18	0.42	1.09	2.25	5.42	22.7	105	400
95.0% Ethanol by Weight								
Caprylic.....	8	262	1035	∞	∞	∞	∞	∞
Pelargonic.....	9	393	3230	∞	∞	∞	∞	∞
Capric.....	10	60.6	93.5	440	890	∞	∞	∞
Undecanoic.....	11	85.2	190	706	∞	∞	∞	∞
Lauric.....	12	15.2	34.0	91.2	260	1410	∞	∞
Tridecanoic.....	13	15.5	34.5	104	336	6560	∞	∞
Myristic.....	14	3.86	7.64	18.9	68.7	238	1485	∞
Pentadecanoic.....	15	3.82	7.18	19.5	78.5	295	2460	∞
Palmitic.....	16	0.85	2.10	4.93	16.7	73.4	287	2280
Margaric.....	17	1.03	1.68	4.17	15.3	84.2	344	6560
Stearic.....	18	0.24	0.65	1.13	3.42	17.1	83.9	365
91.1% Ethanol by Weight								
Palmitic.....	16	0.76	1.94	4.60	15.3	—	—	—
Stearic.....	18	0.13	0.35	0.66	2.30	13.5	68.7	—
80.8% Ethanol by Weight								
Stearic.....	18	ca. 0.06	0.10	0.20	0.81	3.20	50.8	238

^a A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, 7, 546-555 (1942), p. 549.

TABLE 25. SOLUBILITY OF SATURATED FATTY ACIDS IN ISOPROPANOL^a

Name of acid	No. of C atoms	Grams acid dissolved per 100 grams solvent at						
		0°	10°	20°	30°	40°	50°	60°C.
Caprylic.....	8	280	900	∞	∞	∞	∞	∞
Pelargonic.....	9	422	2920	∞	∞	∞	∞	∞
Capric.....	10	67	140	360	5750	∞	∞	∞
Undecanoic.....	11	82	182	540	∞	∞	∞	∞
Lauric.....	12	21.5	44.1	100	253	1270	∞	∞
Tridecanoic.....	13	22.1	52	125	340	6550	∞	∞
Myristic.....	14	7.2	13.6	31.6	82	230	1210	∞
Pentadecanoic.....	15	6.2	13.3	34.4	95	272	2070	∞
Palmitic.....	16	2.4	4.6	10.9	32.3	94	270	2460
Margaric.....	17	1.2	3.0	10.8	37.9	108	345	6550
Stearic.....	18	0.1	0.4	2.0	10.0	38.1	118	422

^a C. W. Hoerr and A. W. Ralston, *J. Org. Chem.*, 9, 329-337 (1944), p. 334.

similar data for a large number of other solvents which were studied; these include acetone, 2-butanone, and glacial acetic acid.²²⁰ In a later publication,²²² the results were extended to nitromethane, methanol, trichloromethane, tetrachloromethane, *n*-butanol, butyl acetate, ethyl acetate, nitroethane, and acetonitrile. Most recently, Hoerr, Sedgwick, and Ralston²²³ have added to their studies results on the solubilities of saturated acids in chlorobenzene, 1,2-dichloroethane, 1,4-dioxane, furfural, nitrobenzene, toluene, and *o*-xylene. The reader is referred to the original papers for the specific data. The comparative solubilities of the acids in all of the different solvents at 20° and 60°C. are summarized in Tables 26 and 27.

The most impressive fact that stands out from an examination of the data in Tables 23 to 28 is the marked increase in solubilities of the saturated acids, in all of the solvents studied, with a rise in temperature. The increases are far greater than have been observed with inorganic salts, carbohydrates, or amino acids in aqueous solutions under similar circumstances. Infinite solubility for the individual acids is reached at corresponding temperatures in all solvents. These temperatures are 20°C. for caprylic (C₈) and pelargonic (C₉), 30°C. for undecanoic (C₁₁), 40°C. for capric (C₁₀), 50°C. for lauric (C₁₂) and tridecanoic (C₁₃), and 60°C. for myristic (C₁₄) and pentadecanoic (C₁₅) acids. Had studies been made at higher temperatures (70°C. or 80°C.), it is probable that infinite solubility would have been observed for palmitic (C₁₆), margaric (C₁₇), and stearic (C₁₈) acids.

Whereas the solubility throughout varies inversely with the length of the carbon chain, marked discrepancies are observed between the even- and odd-chain acids. In practically every case the solubility of the odd-chain acid exceeds that of the next lower even-chain acid. This reminds one of the similar discrepancy in relation to melting point.

There is a considerable difference in the effectiveness of the several solvents, but this varies with temperature and also with the acids tested. Thus, in the case of palmitic acid, the three best solvents at 20°C. are trichloromethane, isopropanol, and 2-butanol, respectively. At 60°C., methanol is the best solvent for palmitic acid, although at 20°C. it has been found to be one of the poorest. The second and third best at 60°C. for this acid are 99.4% ethanol and cyclohexane, both of which are inferior solvents at 20°C. Similar variations are to be noted with stearic acid. The best solvents at 20°C. are trichloromethane, *n*-butanone, and benzene, while at 60°C., methanol, acetic acid, and benzene are the three solvents in which stearic acid is most readily soluble. Of these, methanol and acetic

²²³ C. W. Hoerr, R. S. Sedgwick, and A. W. Ralston, *J. Org. Chem.*, 11, 603-609 (1946).

TABLE 26. SUMMARY TABLE OF SOLUBILITIES OF VARIOUS SATURATED FATTY ACIDS IN SEVERAL SOLVENTS AT 20°C.

 (Grams of acid dissolved by 100 grams of solvent)

Solvent	C ₈	C ₉	C ₁₀	C ₁₁	C ₁₂	C ₁₂	C ₁₂	C ₁₂	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈
Water ^a	0.068	0.026	0.015	0.0093	0.0055	0.0033	0.0020	0.0012	0.00072	0.00042	0.00029	0.00029	0.00029
Benzene ^a	∞	∞	398	663	93.6	117	29.2	36.2	7.30	9.23	2.46	2.46	2.46
Cyclohexane ^b	∞	∞	342	525	68	100	21.5	27.1	6.5	8.4	2.4	2.4	2.4
Methanol ^b	∞	∞	510	740	120	148	17.3	16.4	3.7	2.5	0.1	0.1	0.1
Ethanol ^b 99.4%	—	—	—	—	105	—	23.9	—	7.21	6.62	2.25	2.25	2.25
95.0%	∞	∞	440	706	91.2	104	18.9	19.5	4.93	4.17	1.13	1.13	1.13
91.1%	—	—	—	—	—	—	—	—	4.60	—	0.66	—	0.66
80.8%	—	—	—	—	—	—	—	—	—	—	—	—	0.20
Isopropanol ^b	∞	∞	360	540	100	125	31.6	34.4	10.9	10.8	2.0	2.0	2.0
n-Butanol ^b	∞	∞	280	415	83	100	28.7	28.4	10.5	9.5	1.6	1.6	1.6
Acetone ^a	∞	∞	407	706	60.5	78.6	15.9	13.8	5.38	4.28	1.54	1.54	1.54
2-Butanone ^a	—	—	318	521	64.7	95.0	18.5	20.2	8.57	7.41	2.99	2.99	2.99
Glacial acetic acid ^a	—	—	567	800	81.8	96.8	10.2	8.76	2.14	1.27	0.12	0.12	0.12
Ethyl acetate ^b	∞	∞	289	425	52	70	15.3	15.4	6.1	5.3	0.5	0.5	0.5
Butyl acetate ^b	∞	∞	330	515	68	95	21.6	22.3	8.9	8.7	1.6	1.6	1.6
Trichloromethane ^b	∞	∞	326	485	83	116	32.5	38.1	15.1	17.8	6.0	6.0	6.0
Tetrachloromethane ^b	∞	∞	210	318	53	75	17.6	22.2	5.8	6.8	2.4	2.4	2.4
Nitroethane ^b	∞	∞	55	131	5.4	4.5	1.2	0.7	<0.1	—	—	—	—
1,2-Dichloroethane ^c	—	—	260	185	36.5	—	5.0	—	0.6	—	—	—	—
Acetonitrile ^b	∞	∞	66	185	7.6	5.8	1.8	1.1	0.4	0.2	<0.1	<0.1	<0.1
Furfural ^c	—	—	42.5	—	3.7	—	—	—	—	—	—	—	—
1,4-Dioxane ^c	—	—	356	—	101	—	32.6	—	10.9	—	—	—	4.3
Nitrobenzene ^c	—	—	131	—	8.8	—	3.0	—	0.1	—	—	—	—
Chlorobenzene ^c	—	—	305	—	87.0	—	23.6	—	7.8	—	—	—	2.2
Toluene ^c	—	—	323	—	97	—	30.4	—	8.7	—	—	—	2.0
o-Xylene ^c	—	—	316	—	92	—	26.1	—	7.9	—	—	—	1.7

^a A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, **7**, 546-555 (1942).

^b C. W. Hoerr and A. W. Ralston, *J. Org. Chem.*, **9**, 329-337 (1944).

^c C. W. Hoerr, R. S. Sedgwick, and A. W. Ralston, *J. Org. Chem.*, **11**, 603-609 (1946).

TABLE 27. SUMMARY TABLE OF SOLUBILITIES OF VARIOUS SATURATED FATTY ACIDS IN SEVERAL SOLVENTS AT 60°C.

Solvent	Grams of acid dissolved by 100 grams of solvent												
	C ₈	C ₉	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈		
Water ^a	0.113	0.051	0.027	0.015	0.0087	0.0054	0.0034	0.0020	0.0012	0.00081	0.00050		
Benzene ^a	∞	∞	∞	∞	∞	∞	∞	∞	2170	5450	468		
Cyclohexane ^b	∞	∞	∞	∞	∞	∞	∞	∞	2530	7600	450		
Methanol ^b	∞	∞	∞	∞	∞	∞	∞	∞	4650	12000	520		
Ethanol ^c 99.4%	—	—	—	—	—	—	—	—	2600	8230	400		
95.0%	∞	∞	∞	∞	∞	∞	∞	∞	2280	6560	365		
80.8%	—	—	—	—	—	—	—	—	—	—	238		
Isopropanol ^b	∞	∞	∞	∞	∞	∞	∞	∞	2460	6550	422		
n-Butanol ^b	∞	∞	∞	∞	∞	∞	∞	∞	1960	4900	370		
Acetone ^c	∞	∞	∞	∞	∞	∞	∞	∞	880 ^d	1330 ^d	220 ^d		
2-Butanone ^a	—	—	—	—	—	—	—	—	2390	6560	344		
Glacial acetic acid ^a	—	—	—	—	—	—	—	—	2280	6560	485		
Ethyl acetate ^b	∞	∞	∞	∞	∞	∞	∞	∞	2340	6000	348		
Butyl acetate ^b	∞	∞	∞	∞	∞	∞	∞	∞	2330	6350	350		
Trichloromethane ^b	∞	∞	∞	∞	∞	∞	∞	∞	1820	5000	365		
Tetrachloromethane ^b	∞	∞	∞	∞	∞	∞	∞	∞	1590	4650	325		
Nitroethane ^b	∞	∞	∞	∞	∞	∞	∞	∞	1650	4250	14.0		
1,2-Dichloroethane ^c	—	—	—	—	—	—	—	—	1650	—	280		
Acetonitrile ^b	∞	∞	∞	∞	∞	∞	∞	∞	1200	3600	10.3		
Furfural ^c	∞	∞	∞	∞	∞	∞	∞	∞	1800	—	28.5		
1,4-Dioxane ^c	—	—	—	—	—	—	—	—	1730	—	415		
Nitrobenzene ^c	∞	∞	∞	∞	∞	∞	∞	∞	1215	—	112		
Chlorobenzene ^c	∞	∞	∞	∞	∞	∞	∞	∞	230 ^e	—	102 ^e		
Toluene ^c	∞	∞	∞	∞	∞	∞	∞	∞	240 ^e	—	103 ^e		
o-Xylene ^c	∞	∞	∞	∞	∞	∞	∞	∞	235 ^e	—	102 ^e		

^a A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, **7**, 546-555 (1942).^b C. W. Hoerr and A. W. Ralston, *J. Org. Chem.*, **9**, 329-337 (1944).^c C. W. Hoerr, R. S. Sedgwick, and A. W. Ralston, *J. Org. Chem.*, **11**, 603-609 (1946).^d At 56.5°C.^e At 50°C.

acid rank as third and fourth from the bottom as the poorest solvents at 20°C.

Another point which is of interest is that the ranking of the solvents for the different acids varies with the temperature. Thus, glacial acetic acid and methanol are among the poorest for stearic acid at 20°C. On the other hand, at 60°C., as has been noted earlier, methanol and glacial acetic acid have been found to be the two best solvents for stearic acid. Acetonitrile and nitroethane are considered to be the poorest solvents throughout for all acids at all temperatures. It is therefore evident that, in order to specify the most satisfactory solvent for use, one must indicate not only the fatty acid for which it is to be used but also the temperature at which the extractions are to be made.

c. Solubility of Saturated and Unsaturated Fatty Acids in Non-aqueous Solvents at Low Temperatures. The comparative solubilities of saturated and unsaturated acids in a number of solvents at temperatures as low as -70°C. have been studied extensively by Foreman and Brown.²²⁴ The results indicate that the solubility of linoleic acid exceeds that of oleic acid, which in turn exceeds that of the next higher monoethenoid

TABLE 28. SOLUBILITY RATIOS OF OLEIC TO PALMITIC ACID OR LINOLEIC TO OLEIC ACID IN SEVERAL SOLVENTS AT LOW TEMPERATURES^a

Solvent	Temp., °C.	Grams acid dissolved per 100 grams solution			Ratio: (2) to (3) or (1) to (2)
		Linoleic (1)	Oleic (2)	Palmitic (3)	
Ethylidene dichloride.....	-25		26.8	3.24	82.7:1
Methyl acetate.....	-25		10.0	0.74	14.7:1
Acetone.....	-30		14.2	0.48	30.0:1
Methanol.....	-30		7.08	0.20	35.4:1
Butanol.....	-25		62.8	1.32	47.6:1
Skellysolve B.....	-30		11.8	0.09	131.1:1
Carbon disulfide.....	-30		15.7	<0.1	>157.0:1
Toluene.....	-30		50.2	<0.1	>500.0:1
Ethyl ether.....	-40		43.7	<0.1	>450.0:1
Skellysolve B.....	-70	0.60	0.24		2.5:1
Carbon disulfide.....	-62	4.12	0.398		10.3:1
Methanol.....	-70	3.94	0.32		12.3:1
Acetone.....	-70	5.19	0.40		13.0:1

^a H. D. Foreman and J. B. Brown, *Oil & Soap*, 21, 183-187 (1944).

acid, eicosenoic acid. The solubility of the latter acid exceeds that of the saturated C₁₄ acid, myristic acid, and in some cases of the C₁₂ saturated acid (lauric acid). However, these solubility ratios vary somewhat according to the solvent employed, as indicated in Table 28. The results on Skellysolve B, methanol, and acetone are illustrated in Figures 7 to 9.

²²⁴ H. D. Foreman and J. B. Brown, *Oil & Soap*, 21, 183-187 (1944).

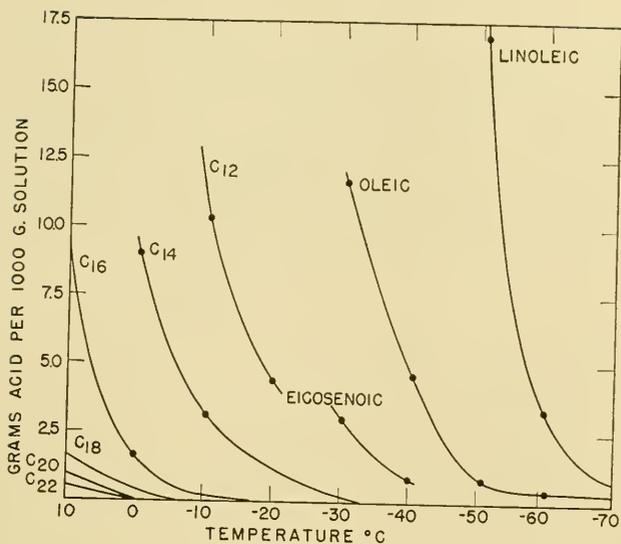


Fig. 7. Solubilities of fatty acids in Skellysolve B.²²⁴

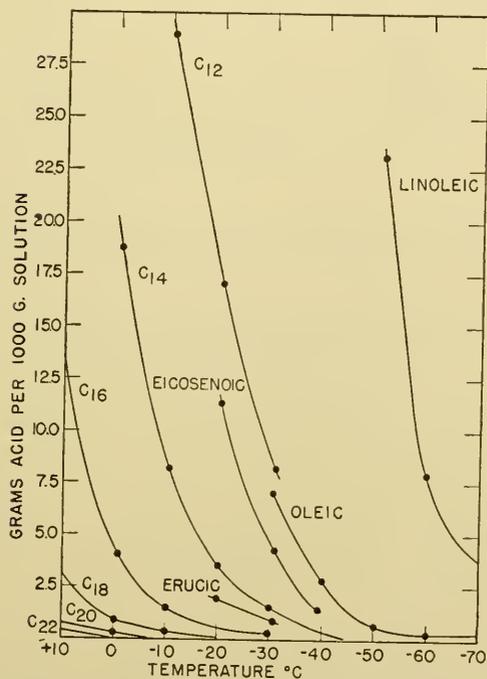


Fig. 8. Solubilities of fatty acids in methanol.²²⁴

Although data are not available for the solubility of linoleic acid at a temperature above -50°C ., or for oleic acid above -30°C ., it is evident that these acids are rapidly approaching infinite solubility. The solubility of the common fatty acids is thus shown to be influenced first by the degree of unsaturation, while the length of the carbon chain plays a secondary role. This finding is in line with the corresponding effects on the melting point. With monoethenoid acids the solubility is inversely proportional

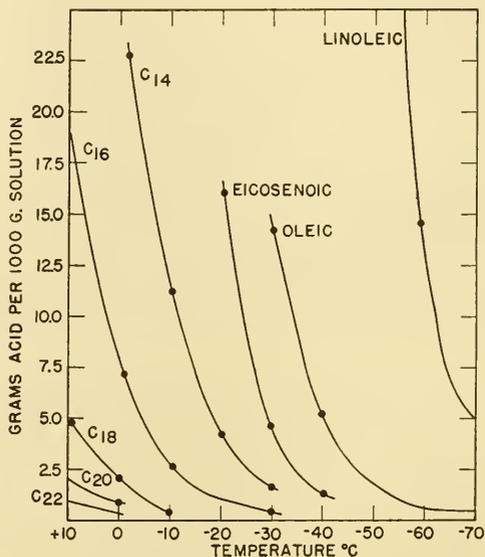


Fig. 9. Solubilities of fatty acids in acetone.²²⁴

to the chain length, as illustrated by the progressively lower rate of solubility of oleic (C_{18}), eicosenoic (C_{20}), and erucic (C_{22}) acids in methanol (Figure 8).

d. Effect of Substitution on the Solubility of Fatty Acids. (a) *Solubility of Hydroxy-Acids.* In the case of the fatty acids, the solubility may be markedly altered by the introduction of hydroxyl groups into the molecule. Such hydroxy-acids dissolve in water to a greater degree than do the unsubstituted acids. On the other hand, they may become entirely insoluble in petroleum ether; in fact, their solubility may even be depressed in such an excellent solvent as ethanol. Octahydroxyarachidic acid is readily soluble in water, hexahydroxystearic acid somewhat less so, while tetrahydroxystearic acid dissolves only to the extent of 50 milligram per cent in boiling water.⁶⁶

(b) *Solubility of Bromo-Acids.* Since bromine dissolved in chloroform or diethyl ether adds itself to most of the unsaturated fatty acids, even in the cold state, one has an excellent method for the characterization of un-

saturated acids. This reaction may be employed quite satisfactorily for the determination of the so-called *polybromide number*. Although the reaction of the various acids with bromine is not quantitative, certain empirical formulas are available from which the type or amount of the specific unsaturated acid may be deduced.

However, the bromo-derivatives vary considerably in solubility and they can be separated from each other by choosing the appropriate solvent. The solubility of hexabromostearic acid in 28 different solvents has recently been reported by Seidell.²²⁵

The monoethenoid acids such as oleic, erucic, and ricinoleic, form dibromo-acids on bromination which are soluble in most of the fat solvents. The tetrabromide of linoleic acid is soluble in ethyl ether, but is largely insoluble in petroleum ether. The hexabromide formed from linolenic acid is insoluble in diethyl ether and in a number of the other fat solvents. The octabromide of arachidonic acid is also insoluble in cold ethyl ether and benzene, while the polybromide prepared from clupanodonic acid is relatively insoluble in all organic solvents.

e. Variations in Solubility of Metallic Salts (Soaps) of Fatty Acids in Different Solvents. Both saturated and unsaturated fatty acids form combinations with a variety of metallic cations. Considerable data are available on the solubility, in a number of solvents, of the salts of the alkali metals (lithium, potassium, and sodium), of the alkali earth metals (barium, calcium, magnesium, and strontium), and of the heavy metals (cobalt, copper, gold, iron, lead, mercury, nickel, and silver). The variation in solubility of the salts of different acids in specific solvents has been an important basis for separation.

(a) *Lead Salts in Ether and Alcohol.* One of the oldest and most widely used methods for the separation of saturated and unsaturated fatty acids is based upon the observation first reported by Gusserow,²²⁶ that the lead salts of the liquid (unsaturated) acids are soluble in ether, while the lead salts of those fatty acids which are solid (saturated) are insoluble in ether. The procedure has been modified by Twitchell²²⁷ for the determination of saturated and unsaturated fatty acids by the use of 95% ethanol in place of diethyl ether. The Twitchell method is the basis of the official procedure adopted by the Association of Official Agricultural Chemists²²⁸ and

²²⁵ A. Seidell, *Solubilities of Organic Compounds*, Vol. II, 3rd ed., Van Nostrand, New York, 1941.

²²⁶ C. A. Gusserow, *Arch. Pharm.*, 27, 153-244 (1828). Cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 596. A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 282.

²²⁷ E. Twitchell, *J. Ind. Eng. Chem.*, 13, 806-807 (1921).

²²⁸ *Official and Tentative Methods of Analysis*, 5th ed., Assoc. Official Agr. Chem., Washington, D. C., 1940, p. 434-436.

²²⁹ *Official and Tentative Methods of Analysis*, 2nd ed., *Am. Oil Chem. Soc.*, 1946, V. G. Mehlenbacher, ed., Cd6-38.

later by the American Oil Chemists' Society.²²⁹ A slightly different method is recommended by Hilditch¹ for larger quantities of solid acids.

However, neither the lead-salt-ether method nor the lead-salt-alcohol procedure gives an exact separation of saturated and unsaturated acids. Jamieson²³⁰ states that the results are particularly unsatisfactory when used with butter fat, coconut, palm kernel, or similar oils in which saturated fatty acids such as myristic (C₁₄) or those with shorter chains are present in an appreciable amount. The lead salts of such short-chain fatty acids are quite soluble in ether or in alcohol, and would thereby be confused with the unsaturated acid fraction. Difficulties are also encountered with rapeseed or mustardseed oils because of the poor solubility of the lead salt of erucic acid, and its subsequent inclusion in the saturated fatty acid fraction. Similar difficulties are encountered with fats containing appreciable amounts of elaeostearic acids, such as China wood (tung) oil (*Aleurites fordii*) or hydnocarpic or chaulmoogric acids, which are present in chaulmoogra oil. Finally, the lead salts of some of the solid iso-oleic acids, formed during the hydrogenation of fats, are likewise largely insoluble, so that the Twitchell method must be modified when applied to hydrogenated fats. When such limitations of the lead-salt method are realized, they can be corrected by certain modifications.

(b) *Barium Salts in Benzene.* A somewhat less satisfactory procedure for the separation of the saturated from the unsaturated acids depends upon the difference in the solubility of the barium salts in benzene.²³¹ The barium-salt procedure¹⁰⁴ has been employed also for the concentration and isolation of hydroxypalmitic acid from butter fat, as well as for the preparation of oleic acid of high purity from refined and commercial olive oils^{232,233} and for the separation of oleic and linoleic acids from linseed oil.²³⁴ Smith and Chibnall²³⁵ used as a solvent benzene containing 5% of 95% ethanol for separating the fatty acids of forage grasses as their barium soaps.

(c) *Lithium Salts in Acetone.* Another useful procedure for the further fractionation of the unsaturated acids was introduced by Tsujimoto.^{32,236,237} This author showed that the lithium soaps of tetraethenoid and pentaethenoid acids are soluble in 95% acetone while those of the less highly unsaturated acids are much less soluble in this solvent. The procedure has

²³⁰ G. S. Jamieson, *J. Assoc. Official Agr. Chem.*, **11**, 301-310 (1928); *Vegetable Fats and Oils*, 2nd ed., Reinhold, New York, 1943, p. 407.

²³¹ K. Farnsteiner, *Z. Untersuch. Nahr. Genussm.*, **2**, 1-27 (1899); **4**, 63-65 (1901); **6**, 161-166 (1903).

²³² A. Lapworth, L. K. Pearson, and E. N. Mottram, *Biochem. J.*, **19**, 7-18 (1925).

²³³ J. H. Skellon, *J. Soc. Chem. Ind.*, **50**, 131-134T (1931).

²³⁴ E. Erdmann and F. Bedford, *Ber.*, **42**, 1324-1333 (1909).

²³⁵ J. A. B. Smith and A. C. Chibnall, *Biochem. J.*, **26**, 218-234 (1932).

²³⁶ M. Tsujimoto, *Chem. Umschau*, **33**, 285-291 (1926); *Chem. Abst.*, **21**, 661 (1927).

²³⁷ M. Tsujimoto and K. Kimura, *J. Soc. Chem. Ind. Japan*, **26**, 891-893 (1923); *Chem. Abst.*, **18**, 2259 (1924).

been used by several workers in isolating and purifying clupanodonic acid from fish oils.²³⁸⁻²⁴⁰ Lovern²⁴¹ combined the lead-salt-alcohol and the lithium-salt-acetone method for the examination of a number of oils of aquatic origin. The procedure has been found to agree excellently with the hexabromide method for the determination of arachidonic acid in suprarenal phospholipids.²⁴² However, the lithium salts of the monoethenoid acid, erucic acid,²⁴³ were found by Dorée and Pepper²⁴⁴ to be quite soluble in acetone, which precludes the use of this procedure with the fatty acids of rapeseed or mustardseed oils.

(d) *Magnesium Salts in Alcohol.* Still another soap-solvent combination which has proved useful has been the magnesium-salt-alcohol procedure. This method was used by Kerr²⁴⁵ and also by Thomas and Yu²⁴⁶ to separate the arachidic acid from peanut oil, although it has subsequently been shown to be somewhat unsatisfactory from a quantitative standpoint.²⁴⁷ The magnesium-salt-alcohol method has been used also by Chibnall *et al.*,¹¹⁰ for the purification of the hydroxy-acids in the cerebrosides, phrenosine, and cerasine.

f. Anomalous Solubility and Association. Although solubility values for the various fatty acids in the different solvents under standardized conditions can readily be duplicated, the results are quite unpredictable when two or more fatty acids are present in the same mixture. Waentig and Pescheck²⁴⁸ have reported that the solubility of palmitic acid in carbon tetrachloride is increased as much as 250% when lauric acid is present. The solubility of the first component is decreased when the concentration of the second component is increased until a limiting concentration is reached. Many instances are known in which anomalous results occur due to the effect of association on solubility. The failure to obtain crystallization from a mixture of acids (especially unsaturated acids), the inability to obtain hexabromostearic acid from a large amount of tetra- or dibromostearic acid, or of tetrabromostearic acid from an excess of dibromostearic acid, are all well-recognized examples of this phenomenon. Likewise, the separation of a crystalline mixture having the properties of margarinic acid

²³⁸ M. Tsujimoto, *Bull. Chem. Soc. Japan*, **3**, 299-307 (1928).

²³⁹ M. Tsujimoto and H. Koyanagi, *J. Soc. Chem. Ind. Japan*, suppl., **38**, 271-272B (1935).

²⁴⁰ Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, **10**, 441-453 (1935).

²⁴¹ J. A. Lovern, "The Composition of the Depot Fats of Aquatic Animals," *Dept. Sci. Ind. Research*, Brit. Food Investigation, Special Report, No. 51, H. M. Stationery Office, London, 1942; *Chem. Abst.*, **36**, 4727 (1942).

²⁴² W. C. Ault and J. B. Brown, *J. Biol. Chem.*, **107**, 615-622 (1934).

²⁴³ D. Holde and C. Wilke, *Z. angew. Chem.*, **35**, 105; 289-291 (1922).

²⁴⁴ C. Dorée and A. C. Pepper, *J. Chem. Soc.*, **1942**, 477-483.

²⁴⁵ R. H. Kerr, *J. Ind. Eng. Chem.*, **8**, 904 (1916).

²⁴⁶ A. W. Thomas and C. L. Yu, *J. Am. Chem. Soc.*, **45**, 113-128 (1923).

²⁴⁷ S. T. Voorhies and S. T. Bauer, *Oil & Soap*, **20**, 175-178 (1943).

²⁴⁸ P. Waentig and G. Pescheck, *Z. physiol. Chem.*, **93**, 529-569 (1919).

from a solution containing palmitic and stearic acid is ascribable to the same cause. It is claimed by Waentig and Pescheck²⁴⁸ that the mutual solubility effect occurs only in solvents such as benzene, carbon tetrachloride, chloroform, nitrobenzene, and toluene, where an association between the two fatty acids may take place, while no variation is to be noted in benzaldehyde, diethyl ether, ethyl acetate, and ethyl alcohol, in which the acids are known to be monomolecular. Ralston and Hoerr,²⁴⁹ on the other hand, believe that the melting point of a given mixture rather than the proportion of bimolecular complex present in a mixture is the determining factor which is related to solubility.

(4) *Fatty Acid Films*

The fatty acids having a chain length of 8 or more carbon atoms are only slightly soluble in water. The solubility which they do possess is largely the result of the carboxyl group, while the hydrocarbon chain has the opposite effect. The longer the hydrocarbon chain is extended, the less the carboxyl group will be able to promote solubility. However, even if the depressing effect on solubility of the hydrocarbon portion of the fatty acid is sufficiently marked as to result in almost complete immiscibility, the carboxyl group will still effect the water-acid interface so that oriented mono- or polymolecular films will be produced.

a. Monomolecular Films. (a) *Nature of Monomolecular Films.* The slightly soluble fatty acids have the property of spreading to form a thin and uniform layer when they come in contact with water. This property of forming a film also occurs with immiscible animal or vegetable oils, oxidized paraffin oils, fatty acid esters, higher alcohols, other lipids, and related compounds. On the other hand, pure hydrocarbons do not spread when they come in contact with distilled water and so do not possess the ability to form monomolecular films.

According to Langmuir,²⁵⁰ the liquids which do not possess the potentiality of film formation are substances which contain only nonpolar or *hydrophobic* groups. On the other hand, compounds which possess chiefly polar or *hydrophilic* groups usually enjoy a high solubility and therefore do not form films. It is only those compounds, like fatty acids, which possess both the hydrophilic carboxyl group and the hydrophobic hydrocarbon chain which have the ability to spread over water to form a fine film.

Most of such films are only one molecule thick. They appear to be oriented in such a manner that the hydrophilic carboxyl groups are dissolved in the water while the hydrocarbon side chains form a layer on the surface. The molecules are thus aligned parallel to each other and perpendicular to the surface of the water.

²⁴⁸ A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, **10**, 170-174 (1945).

²⁵⁰ I. Langmuir, *J. Am. Chem. Soc.*, **39**, 1848-1906 (1917).

Such oriented films have several characteristic properties. Most important of these is their response to compression, which in turn varies with pH , temperature, and with the particular fatty acid. The extent of such compression of an oxidized mineral oil can be qualitatively determined by the color which it produces. As the pressure is increased, the color progressively changes through yellow, gold, red-purple, blue, and green, and this may be repeated until the film again becomes colorless. Quantitative measurements may be made by a film balance⁴ or by a device introduced by N. K. Adam.²⁵¹

(b) *Factors Altering Monomolecular Films.* The type of monomolecular film was found to be similar for all fatty acids from C_{12} to C_{26} , including the even- and odd-chain acids. Adam²⁵²⁻²⁵⁴ observed that when the several fatty acids were spread on distilled water at a uniform temperature of $20^\circ C.$, all acids failed to display measurable resistance to compressibility until

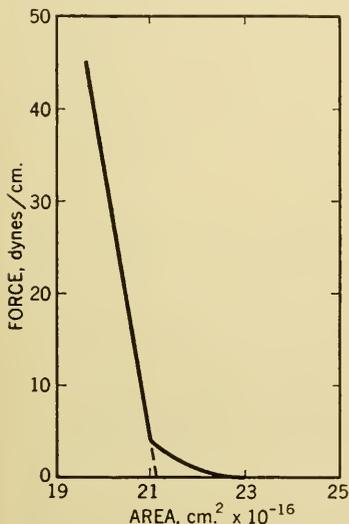


Fig. 10. Force-area curve for monomolecular films of saturated fatty acids on distilled water at $20^\circ C.$ ²⁵⁵

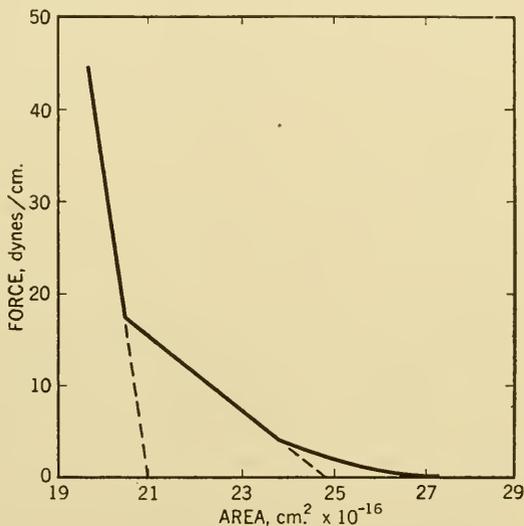


Fig. 11. Force-area curve for monomolecular films of saturated fatty acids on 0.01 N hydrochloric acid solution.²⁵⁵

the area reached a value of 21×10^{-16} sq. cm. per molecule. When they were compressed beyond this point, there was a sudden opposition to

²⁵¹ N. K. Adam, *Proc. Roy. Soc. London*, A101, 452-472 (1922).

²⁵² N. K. Adam, *Proc. Roy. Soc. London*, A99, 336-351 (1921).

²⁵³ N. K. Adam, *Proc. Roy. Soc. London*, A101, 516-531 (1922).

²⁵⁴ N. K. Adam, *Proc. Roy. Soc. London*, A103, 676-687, 687-695 (1923).

²⁵⁵ Data from H. S. Taylor, ed., *A Treatise on Physical Chemistry*, Vol. I, Van Nostrand, New York, 1925, p. 137, Fig. 6; p. 139, Fig. 7.

further concentration. The compressing force increased linearly until the film collapsed. Since the area occupied by each fatty acid molecule,⁴ irrespective of chain length, is 21×10^{-16} sq. cm., it is apparent that they are in all cases arranged perpendicularly to the surface. The limiting area is that occupied by a CH_2 group, which obviously is the diameter of the short axis. The thickness of any such monomolecular film will vary with the different fatty acids according to the length of the fatty acid chain. Since the surface tension of water is not decreased until the whole surface is covered with a monomolecular film, the area per molecule can be calculated by determining the number of molecules which must be added to a given area before an abrupt change in surface tension can take place. The force-area curve of saturated acids is given in Figure 10²⁵⁵ on page 73.

The fact that the carboxyl groups are projected into the water would seem also to be indicated by the effect of an acid solution on the compressibility of the film. The force-area curves obtained with fatty acid films in contact with a solution of hydrochloric acid as dilute as 0.01 *N* are entirely different from those observed when pure water is employed. It is not possible to compress such films to an area of 21×10^{-16} sq. cm. per molecule before resistance obtains, but only to an area of 25×10^{-16} , in the presence of hydrochloric acid. This alteration in compressibility is evident in Figure 11 as compared with Figure 10.

The explanation for the increase in the area (from 21×10^{-16} sq. cm. to 25×10^{-16} sq. cm.) occupied per molecule of fatty acid when dilute acid is used in place of distilled water is the fact that the attraction for the carboxyl group is decreased at the lower *pH*, and the fatty acid is forced to lie flat on the surface rather than being projected into the aqueous medium. The larger area occupied by each molecule of fatty acid in contact with a dilute solution of acid is therefore controlled by the diameter of the head of the molecule, *i.e.*, the carboxyl group, rather than by the hydrocarbon chain, in which case the smaller limiting diameter is that of the methylene group.

Temperature, also, has been shown by Adam²⁵²⁻²⁵⁴ to play an important role in relation to film compressibility. When a constant pressure of 1.4 dynes was applied to such an oil film in contact with 0.01 *N* HCl solution, and the temperature was gradually raised from 0°C., no change in area occupied occurred until a temperature of 28°C. was reached. Between this temperature and 35°C., a marked increase in area was noted to about 42×10^{-16} sq. cm. As the temperature was further increased, a slow progressive expansion in area occurred. This behavior is explained by the fact that, at the elevated temperature, the thermal agitation of the film becomes so violent that the lateral attraction of the molecules is overcome. Such films are known as *expanded* films, in contradistinction to the

condensed films which occur when the molecules are in close contact at low temperatures. The results of Adam²⁵³ are given in Figure 12.

Adam and Jessop²⁵⁶ have made a study of dibasic acids having 18, 22, 26, and 34 carbons and found that these form films in which only one carboxyl group is oriented into the water. Diethyl esters of dicarboxylic acids having 12, 13, 18, 22, and 34 carbon atoms also were shown to produce monomolecular films, as was the monoethyl ester, $\text{HOOC}(\text{CH}_2)_{16}\text{COOC}_2\text{H}_5$. All of the films were rather unstable, and readily collapsed when pressure was applied.

The formation of such surface films is influenced also by unsaturation of the fatty acid. Such acids produce films of the expanded type. When the double bond is in the middle of the molecule, the film expands more readily

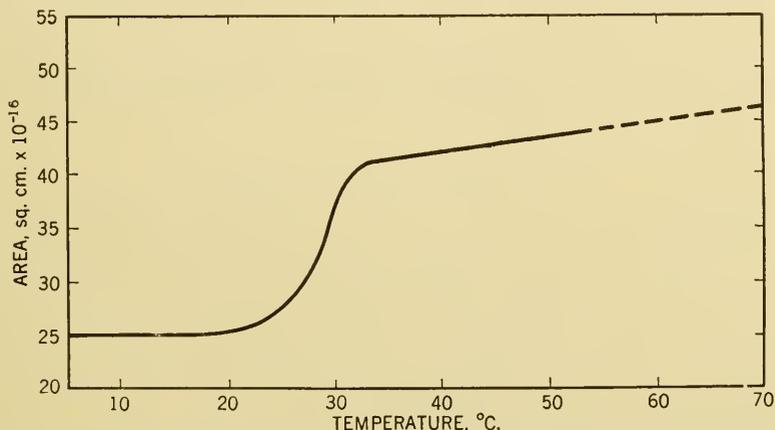


Fig. 12. Effect of temperature on the compressibility of a monomolecular film of palmitic acid on 0.01 *N* hydrochloric acid under a constant force of 1.4 dynes per centimeter.²⁵³

than in the case of the corresponding saturated fatty acid. When the unsaturated linkage is near the carboxyl end, as for example in 2-octadecenoic acid, the expansion of the film is considerably restricted as compared with that observed when the double bond is near the center of the molecule. Extensive studies on film formation of polyethenoid acids, stearolic acid, and triricinolein⁴ have been made by Rideal and his co-workers²⁵⁷⁻²⁵⁹ and by Adam.²⁶⁰

²⁵⁶ N. K. Adam and G. Jessop, *Proc. Roy. Soc. London*, *A112*, 362-375, 376-380 (1926).

²⁵⁷ A. H. Hughes and E. K. Rideal, *Proc. Roy. Soc. London*, *A140*, 253-269 (1933).

²⁵⁸ G. Gee and E. K. Rideal, *Proc. Roy. Soc. London*, *A153*, 116-128, 129-141 (1935).

²⁵⁹ G. Gee, *Trans. Faraday Soc.*, *32*, 187-195 (1936).

²⁶⁰ N. K. Adam, *Proc. Roy. Soc. London*, *A140*, 223-226 (1933).

Long *et al.*²⁶¹ studied the monomolecular films produced by stearic and oleic acids as well as by their mono-, di-, and triglycerides. The corresponding areas occupied per molecule were as follows: stearic, 22.5×10^{-16} sq. cm.; oleic, 52; triolein, 127; and tristearin, 72. These are in close harmony with the measurements of Adam and of Langmuir.

b. Polymolecular Films. Polymolecular films have been prepared by transferring the monomolecular film on water to a glass or metallic surface by a procedure described by Blodgett.²⁶² A highly polished metallic surface or foils of transparent Resoglaz have been used successfully. Even or odd numbers of layers can be deposited, of any desired thickness. Different polymorphic forms of the same substance can also be incorporated on such films. The arrangement of the atoms can be determined by obtaining x-ray diffraction patterns²⁶³ or electron diffraction patterns of the polymorphic films.²⁶⁴

The subject of films, particularly monomolecular ones, is discussed in the monograph of Adam²⁶⁵ while Harkins²⁶⁶ has reviewed the subject from the thermodynamic standpoint.

(5) *Spectral Behavior of Fatty Acids*

a. Introduction. One of the most useful methods for establishing the structure of the unsaturated fatty acids is absorption spectroscopy. Another related procedure which has yielded information of considerable importance not obtainable by other means is the Raman spectrum. The application of absorption spectra to the identification of many compounds of biological interest has been one of the features in the development of biochemistry during the past decade.

To date, spectroscopy has been of little value in the identification or quantitative estimation of the saturated fatty acids. However, it has played an important role in establishing the configuration of a number of the unsaturated acids. Absorption spectra have been very useful in indicating the position of double bonds and determining their number. The absorption of specific wave lengths is governed by certain characteristic groupings of the molecule, which possess the same electronic, vibrational, or rotational properties as are associated with energy of a given frequency.

²⁶¹ J. S. Long, W. W. Kittelberger, L. K. Scott, and W. S. Egge, *Ind. Eng. Chem.*, **21**, 950-955 (1929).

²⁶² K. B. Blodgett, *J. Am. Chem. Soc.*, **57**, 1007-1022 (1935).

²⁶³ L. H. Germer, "Arrangement of Molecules in Unimolecular and Multimolecular Layers," in *Recent Advances in Surface Chemistry and Chemical Physics*, F. R. Moulton, ed., Am. Assoc. Advancement Sci., Pub. No. 7, Science Press, Lancaster, Pa., 1939, pp. 47-53.

²⁶⁴ L. H. Germer and K. H. Storck, *J. Chem. Phys.*, **6**, 280-293 (1938).

²⁶⁵ N. K. Adam, *The Physics and Chemistry of Surfaces*, 3rd ed., Oxford Univ. Press, London, 1941.

²⁶⁶ W. D. Harkins, *Chem. Revs.*, **29**, 385-417 (1941).

For a further theoretical discussion of the application of spectroscopy to chemical and biological work, the reader is referred to the monographs of Brode²⁶⁷ and of Miller.²⁶⁸ A useful treatise dealing with spectrophotometric instruments was published by Burk and Grummitt.²⁶⁹ Hibben²⁷⁰ has written a monograph on the chemical applications of the Raman effect, and also several excellent reviews on this subject,^{271,272} which may be of interest to those to whom the monograph is not available.

b. Infrared Absorption. The infrared region is divided into several areas for convenience. That portion immediately adjacent to the visible end of the spectrum is called the near or short infrared. This includes the part between the wave lengths of 750 and 1000 $m\mu$ (7500 to 10,000 \AA).²⁷³ The middle region extends from 1000 to 6000 $m\mu$ (10,000 to 60,000 \AA), while the lower or far infrared region is that portion of the spectrum above 6000 $m\mu$ extending to an upper limit not exactly defined but probably overlapping the lower Hertzian waves. R. B. Barnes *et al.*²⁷⁴ reported on the characteristic absorption of a large number of natural and synthetic organic compounds (including fats and their derivatives) in the infrared. They²⁷⁵ recently published an excellent monograph entitled *Infrared Spectroscopy*.

The curves showing the absorption of the ethyl esters of oleic, linoleic, linolenic, elaidic, and linolelaidic acids in relation to that of stearic acid as determined by McCutcheon *et al.*⁵² is given in Figure 13, while the results of Gamble and Barnett²⁷⁶ on the absorption of the triglycerides of several unsaturated acids in the infrared region are reproduced in Figures 14 and 15.

A marked absorption maximum is noted for the ethyl esters of oleic, linoleic, and linolenic acids at 6.0 μ (6000 $m\mu$), while elaidic and linolelaidic esters show only a slight inflection at this wave length. On the basis of theoretical considerations, it is believed that the absorption in this area is associated with a *cis* arrangement, which would explain the absence of absorption by the *trans* isomers, elaidic and linolelaidic acids. McCutcheon *et al.*⁵² suggest also that the absorption curves prove that linoleic

²⁶⁷ W. R. Brode, *Chemical Spectroscopy*, 2nd ed., Wiley, New York, 1943.

²⁶⁸ E. S. Miller, *Quantitative Biological Spectroscopy*, Burgess, Minneapolis, 1939.

²⁶⁹ R. E. Burk and O. Grummitt, eds., *Major Instruments of Science and Their Application to Chemistry*, Interscience, New York, 1945.

²⁷⁰ J. H. Hibben, *The Raman Effect and Its Chemical Applications*, Reinhold, New York, 1939.

²⁷¹ J. H. Hibben, *Chem. Revs.*, *13*, 345-478 (1933).

²⁷² J. H. Hibben, *Chem. Revs.*, *18*, 1-232 (1936).

²⁷³ One micron (μ) is equal to 0.001 mm. One millimicron ($m\mu$) equals 0.001 μ , or 0.000,001 mm. One angstrom unit (\AA) equals 0.1 $m\mu$ or 0.000,000,1 mm.

²⁷⁴ R. B. Barnes, U. Liddel, and V. Z. Williams, *Ind. Eng. Chem., Anal. Ed.*, *15*, 659-709 (1943).

²⁷⁵ R. B. Barnes, R. C. Gore, U. Liddel, and V. Z. Williams, *Infrared Spectroscopy*, Reinhold, New York, 1944.

²⁷⁶ D. L. Gamble and C. E. Barnett, *Ind. Eng. Chem.*, *32*, 375-378 (1940).

acid possesses a *cis-cis* linkage while linolelaidic acid has a *trans-trans* configuration. The same authors conclude further that these data, in conjunction with results on the Raman spectra, indicate that linolenic acid possesses *cis-cis-cis* linkages. The glyceryl esters of oleic, linoleic, linolenic, and elaeostearic acids also show absorption maxima at $6\ \mu$, but a much more marked peak occurs at $8.4\ \mu$ which is attributed to the oxygen linkage. With glyceryltrielaostearate an additional inflection at $10\ \mu$ is ascribed by Gamble and Barnett²⁷⁶ to the effect of conjugation of the unsaturated bonds.

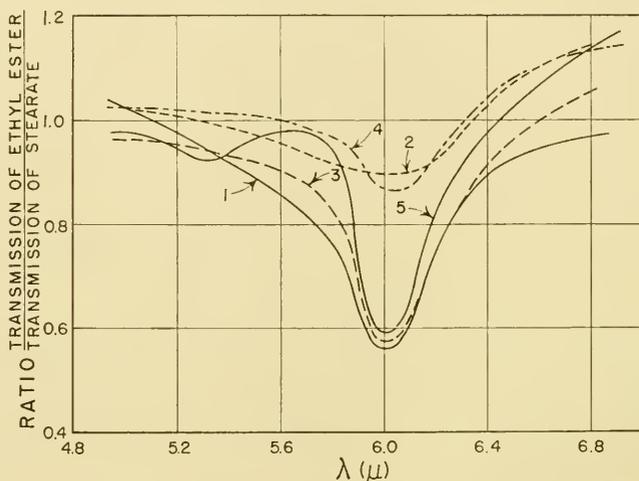


Fig. 13. A comparison of the infrared absorption curves for the ethyl esters of oleic (1), elaidic (2), linoleic (3), linolelaidic (4), and linolenic (5) acids with that of stearic acid.⁵²

c. Ultraviolet Absorption. Although the saturated fatty acids have no absorption in the region between 400 and $200\ m\mu$, characteristic curves are exhibited by most of the unsaturated acids in this area. There is some indication that the far ultraviolet may be a more satisfactory region for the study of the saturated acids. The investigation in the region of the far ultraviolet is hampered by the difficulty of obtaining simple instruments for such measurements. Most spectrophotometers and other instruments for measuring transmission in the ultraviolet record satisfactorily to about $300\ m\mu$, and hence the bulk of our information applies to the upper portion of the ultraviolet spectrum. The Beckman spectrophotometer, when used with the hydrogen emission tubes, and in conjunction with quartz cells, can be employed to $210\ m\mu$. However, a spectrograph devised by Schumann, having highly sensitized photographic plates and an evacuated chamber, allows accurate measurements to wave lengths as short as $120\ m\mu$. By the use of a fluorite optical system, estimations are possible in the far ultraviolet to wave lengths of only $50\ m\mu$. At this short

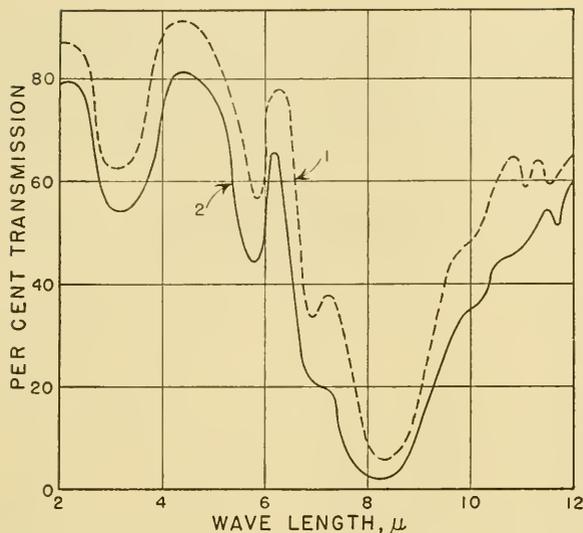


Fig. 14. The infrared absorption spectra of glyceryl trioleate (1) and glyceryl trilinoleate (2).⁵²

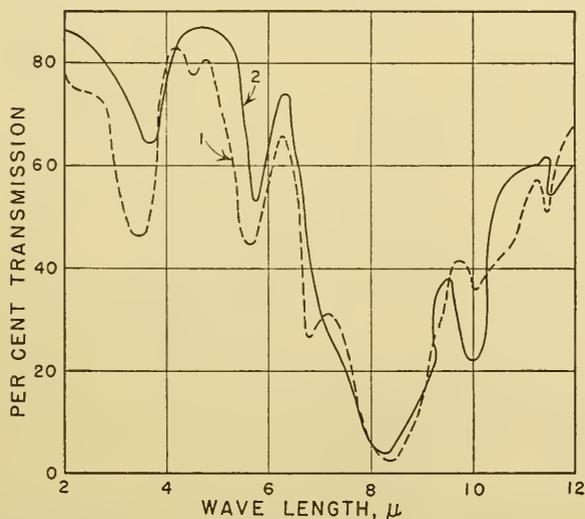


Fig. 15. The infrared absorption spectra of glyceryl trilinolenate (1) and glyceryl trielaeostearate (2).⁵²

wave length, it is apparent that there may be an overlapping with the x-rays. An extensive discussion of the application of ultraviolet spectroscopy to drying oils is given by Kass.²⁷⁷

²⁷⁷ J. P. Kass, "Ultraviolet Absorption Studies of Drying Oils," in *Protective and Decorative Coatings*, Vol. IV, J. J. Mattiello, ed., Wiley, New York, 1944, pp. 362-405.

The absorption of the fatty acids in the ultraviolet results both from the effect of the carboxyl group and from the unsaturated linkages. That which can be ascribed to the effect of the carboxyl is apparent only at wave lengths of approximately 200 $m\mu$ or shorter, whereas that due to the double bonds shows various maxima in the area between 200 and 350 $m\mu$. The

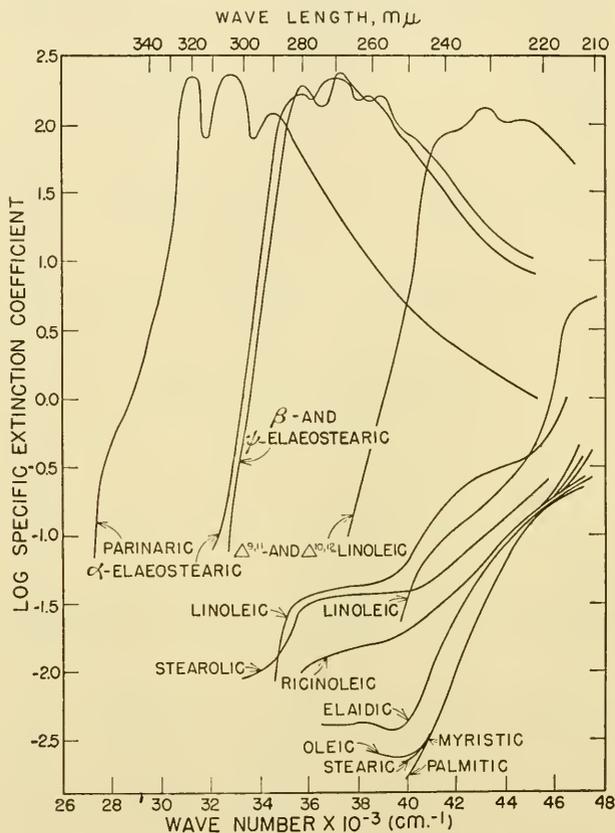


Fig. 16. The ultraviolet absorption spectra of saturated and unsaturated fatty acids.²⁷⁷

absorption curves of a number of saturated and unsaturated acids are included in Figure 16.

Several features of the absorption curves seem to be quite consistent. Maximum absorption of the *trans* isomer as compared with the *cis* isomer in general is shifted about 2.5 to 5 $m\mu$ (25 to 50 Å.) toward the visible spectrum.

An increase in the number of double bonds increases the absorption, particularly in the shorter wave lengths. The relative intensity of ab-

sorption at 210 $m\mu$ has been shown by R. H. Barnes *et al.*²⁷⁸ to be as follows: stearic acid (0 double bonds) 60; oleic acid (1), 180; methyl linoleate (2), 2500; methyl linolenate (3), 10,000; and methyl arachidonate (4), 14,500. The comparative absorption curves for these acids are given in Figure 17.

Another pronounced effect on absorption in the ultraviolet area of the spectrum is caused by conjugation. The intensity of the absorption is markedly increased, as is demonstrated by the higher values obtained for

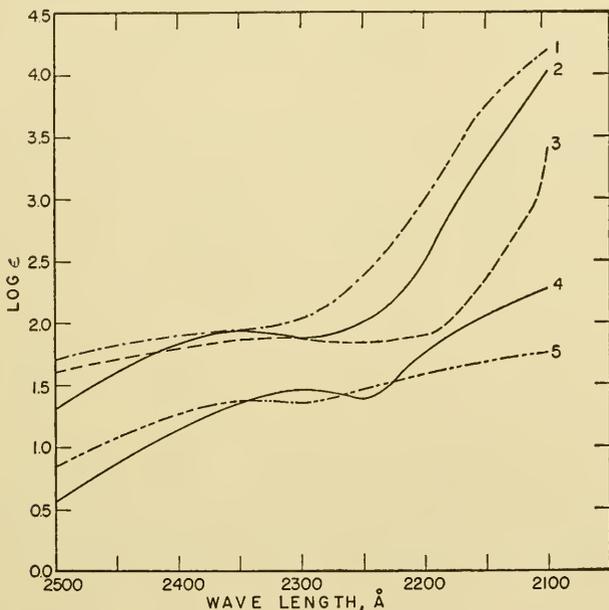


Fig. 17. The log of molecular extinction coefficients (ϵ) plotted against wave length for methyl arachidonate (1), methyl linolenate (2), methyl linoleate (3), oleic acid (4), and stearic acid (5).²⁷⁸

the two conjugated forms of C_{18} diethenoid acids studied (9,11- and 10,12-octadecadienoic acids) as compared with ordinary linoleic acid (9,12-octadecadienoic acid) where the bonds are not conjugated. Similar variations are to be noted between the non-conjugated trienoic acid, linolenic (9,12,15-octadecatrienoic acid), as compared with the conjugated form, elaeostearic acid (9,11,13-octadecatrienoic acid). The maximum absorption for the conjugated diene acids was found to be at 230, for triene acids at 270, and for the tetraene conjugated acid (parinaric) at 308 to 320 $m\mu$.

The absorption in the Schumann region is intense for the saturated acids; acetic, caprylic, and myristic acids have maxima at 205 $m\mu$ and below

²⁷⁸ R. H. Barnes, I. I. Rusoff, E. S. Miller, and G. O. Burr, *Ind. Eng. Chem., Anal. Ed.*, 16, 385-386 (1944).

173 $m\mu$, while peaks for oleic and linoleic acids were found also at 183 and 190 $m\mu$, respectively.²⁷⁹ The corresponding *trans* isomers had higher absorption maxima, which were 4 and 2.5 $m\mu$ toward the visible spectrum; the intensity of the absorption was increased 15% in the first case (elaidic *vs.* oleic), while it was decreased 11% with the second *trans* isomer (linolenelaidic *vs.* linoleic).

d. Raman Spectrum. The Raman spectrum has been of considerable importance in helping to establish the structural relationships of isomeric compounds, especially when used in connection with the results from infrared spectroscopy. The Raman effect, discovered as recently as 1928 by Sir C. V. Raman, is produced by irradiating the substance to be investigated with rays as nearly monochromatic as possible; under such conditions, the material upon which the light beam is focussed becomes a site of emission of light of the same or different wave length from that of the incident light. The emitted rays can best be observed at an angle of 90°C. to the direction of the applied light. The nature of such emitted light can be analyzed by the use of a spectrograph by which the intensity and position of the light waves can be registered on a photographic plate.

The emergent light usually owes its origin to several factors. The most important of these is, of course, the true Raman scattering. However, when homogeneous material is irradiated, considerable emitted light may be the result of the so-called *Rayleigh scattering* from particles of colloidal size, or even from the molecules themselves. Other causes for the production of emergent rays may be direct reflection when the material is heterogeneous, or a fluorescent emission in cases in which this phenomenon is possible. All of these factors may operate simultaneously.

The Raman effect has been ascribed to changes which occur when a quantum of light collides with a molecule of the irradiated substance. The latter substance is usually decomposed. The consequent loss of energy in the molecule is similar to that which takes place whenever an inelastic collision occurs in a reaction involving a quantum process. The energy released is absorbed as increased vibrational or rotational energy. The resulting emitted or scattered light is usually of greater wave length than that of the impinging light, by a fixed amount which depends upon the quantum process involved. In some cases in which the molecule is in a higher energy state than the normal, it may emit light of greater frequency, *i.e.*, of a lower wave length, than that of the incident light. Such spectral bands are known as "anti-Stokes" lines.

Characteristic Raman spectra are produced by ethylenic compounds at approximately 1.650 cm.^{-1} and 3.010 cm.^{-1} frequency units. The former band is less intense for the *cis* than for the *trans* compound, while the re-

²⁷⁹ I. I. Rusoff, J. R. Platt, H. B. Klevens, and G. O. Burr, *J. Am. Chem. Soc.*, 67, 673-678 (1945).

verse holds for the higher frequency band. In the case of monoethenoid acids, the *cis* forms show a strong infrared absorption, as noted earlier, at 6.0 μ . This corresponds to the characteristic ethylenic band frequency of 1.670 cm.^{-1} , while a similar absorption band does not occur with the *trans* form.

The Raman spectra of oleic, linoleic, and linolenic acids, as well as of their *trans* forms, were determined by McCutcheon, Crawford, and Welch⁵²; these data are summarized in Table 29. Kohlrausch and his associates^{280,281} have investigated the Raman spectra of several of the saturated acids.

TABLE 29

RAMAN LINES CHARACTERISTIC OF ETHYL ESTERS OF UNSATURATED FATTY ACIDS^a

<i>cis</i>		<i>trans</i>		<i>cis</i>		<i>cis</i>		<i>trans</i>			
Oleate	Elaidate	Linoleate		β -Linoleate		Linolelaidate		Linolenate			
—	—	953	1	952	1	—	—	954	0		
—	962	1	—	—	—	—	—	—	—		
974	3 <i>d</i>	—	972	5 <i>d</i>	973	4 <i>d</i>	977	1	971	2	
—	—	—	—	—	—	—	—	—	1250	1	
1267	7 <i>d</i>	1269	1	1264	7 <i>d</i>	1264	7 <i>d</i>	1261	2 <i>d</i>	1265	6 <i>d</i>
—	—	—	1643	6 <i>d</i>	1643	5 <i>d</i>	—	—	—	—	
1655	8 <i>p</i>	—	1658	10 <i>p</i>	1657	10 <i>p</i>	1656	10 <i>p</i>	1656	9 <i>p</i>	
—	1669	8 <i>p</i>	—	—	—	1668	5 <i>d</i>	—	—	—	
3009	6 <i>p</i>	3008	3 <i>p</i>	3012	5 <i>p</i>	3012	5 <i>p</i>	3009	2 <i>p</i>	3013	8 <i>p</i>

^a J. W. McCutcheon, M. F. Crawford, and H. L. Welch, *Oil & Soap*, 18, 9–11 (1941). Frequency shifts $\Delta\bar{\nu}$ are figures at left in each column; relative intensities (scale 0–10) are middle figures in each column; polarizations are listed separately at right; *p* = polarized, *d* = depolarized.

Earlier studies have established the fact that the ethylenic bond of all substances of the type CHR:CHR' gives rise to a band in the frequency of $\Delta\bar{\nu}$ 1650 for *cis* compounds, while the corresponding band is about $\Delta\bar{\nu}$ 10 to 20 more for the *trans* isomer. Such variations are noted between the oleate and the elaidate, as well as between the linoleate and the linolelaidate. It is known also that a Raman line occurs in ethylenic compounds at about $\Delta\bar{\nu}$ 3010, which is approximately twice as intense in the *cis* compound as in the *trans* compound. The corresponding intensities of the oleate and elaidate at $\Delta\bar{\nu}$ 3008–3009 are 6 and 3, respectively; for linoleate and linolelaidate, the intensities are found to be 5 and 2, respectively. From these results, it is evident that the data accumulated earlier on *cis* and *trans* ethylenic compounds are applicable to the unsaturated fatty acids.

²⁸⁰ K. W. F. Kohlrausch, F. Köppl, and A. Pongratz, *Z. physik. Chem.*, B21, 242–255 (1933).

²⁸¹ K. W. F. Kohlrausch, A. Pongratz, and R. Seka, *Ber.*, B66, 1–12 (1933).

It is obvious that such information can be used to establish or confirm the configuration of unknown geometric isomers.

McCUTCHEON *et al.*⁵² concluded, on the basis of their data on the Raman spectra, that α - and β -linoleates are identical. This supports the earlier results of McCUTCHEON⁴¹ as well as those of RIEMENSCHNEIDER and co-workers,²⁸² and also the findings of KASS and BURR.⁴⁵ They are, however, at variance with the interpretation of FRANKEL and BROWN.²⁸³

(6) *Isomerism of Fatty Acids*

Although isomerism is relatively less important in the lipids than in most other organic compounds, there are some cases in which any one of the several types of isomerism may play an important role in establishing the physical and chemical properties, as well as the biological behavior of the particular type of compound. Isomerism does not occur to any great extent in the saturated fatty acid series except in the case of the branched-chain acids. On the other hand, a number of unsaturated acids are known which have the same empirical formula as oleic acid, namely, $C_{18}H_{34}O_2$. These vary widely from each other in distribution in nature, in chemical and in physical properties, and in metabolism, depending upon the position of the ethylenic linkage as well as upon the geometric form of the double bond. There is experimental evidence that such compounds may not be interconvertible in the animal. The types of isomerism found in the fatty acids are best understood by means of classification according to the following scheme of SHRINER, ADAMS, and MARVEL²⁸⁴.

a. Simple Structural Isomerism. This can be explained by the use of simple structural formulas.

(a) *Nucleus or Chain Isomerism.* This type is exemplified by differences in arrangement of the carbon atoms in the chain such as would be exhibited by valeric ($CH_3(CH_2)_3COOH$) and isovaleric acids ($((CH_3)_2CHCH_2COOH$). It is of only slight significance in the case of fatty acids of biochemical importance.

(b) *Positional Isomerism.* In this case the substituted group or the position of the double or triple bond varies in the different isomers. This is frequently observed and is probably of the greatest importance.

(c) *Functional or Group Isomerism.* Here the isomers have the same empirical formulas but have different functional groups.

b. Stereoisomerism or Space Isomerism. In this case, the isomers have identical molecular formulas but the variation results from the difference in the three-dimensional space arrangement.

²⁸² R. W. RIEMENSCHNEIDER, D. H. WHEELER, and C. E. SANDO, *J. Biol. Chem.*, **127**, 391-402 (1939).

²⁸³ J. S. FRANKEL and J. B. BROWN, *J. Am. Chem. Soc.*, **65**, 415-418 (1943).

²⁸⁴ R. L. SHRINER, R. ADAMS, and C. S. MARVEL, in H. GILMAN, *Organic Chemistry*, 2nd ed., Vol. I, Wiley, New York, 1943, pp. 214-488.

(a) *Optical Isomerism.* This type of isomerism is possible when at least one asymmetric carbon atom is present in the molecule. An asymmetric carbon atom is one in which each of four valences is satisfied by different atoms or groups. When more than one asymmetric atom occurs in the same molecule, the number of possible isomers is increased to a total of 2^n , where n is the number of such asymmetric carbon atoms. Optical isomers are characterized by differences in the rotation of the plane of polarized light as well as by variations in most other physical properties. Examples of the importance of optical isomerism are the methyl- or hydroxy-substituted acids. In such compounds, this property may be useful for their identification as well as for their quantitative estimation.

(b) *Geometric or Cis-Trans Isomerism.* Geometric isomerism is possible when a compound has two carbon atoms attached by double bonds. A restricted rotation of these carbon atoms occurs because of the double bonds, and so they are not able to develop a free rotation. The shape of the molecule will differ according to whether the combination is of such a nature that the molecule is partially folded back on itself (*cis*) or extended to the maximum length (*trans*). The best known examples of such isomers are oleic and elaidic acids, which represent, respectively, the *cis* and *trans* forms of 9-octadecenoic acid.

a'. Elaidinization: The process of isomerization of a *cis* to a *trans* isomer is usually referred to as *elaidinization*. The general reaction was discovered over a hundred years ago by Poutet,²⁸⁵ who reported that the treatment of olive oil with mercurous nitrate resulted in its change to a semisolid fat resembling pork fat. Poutet's reagent has had wide application over a number of years; apparently, it is superior to other catalysts since it does not result in the formation of addition products. Nitrous acid,²⁸⁶⁻²⁸⁹ as well as nitrous oxide,²⁹⁰⁻²⁹³ have been used successfully to bring about the isomerization of oleic acid, as well as in the transformation of the *cis* acid, erucic (13-docosenoic acid) to the *trans* acid, brassidic acid.²⁹⁴⁻⁶ Lidoff²⁹⁰ had discovered its application to linoleic acid, and this work

²⁸⁵ J. J. E. Poutet, *Ann. chim. phys.* [2] 12, 58-68 (1819).

²⁸⁶ F. Boudet, *Ann.*, 4, 1-33 (1832).

²⁸⁷ H. Meyer, *Ann.*, 35, 174-188 (1840).

²⁸⁸ J. Gottlieb, *Ann.*, 57, 33-67 (1846).

²⁸⁹ F. Varrentrapp, *Ann.*, 35, 196-215 (1840).

²⁹⁰ A. Lidoff, *J. Russ. Phys. Chem. Soc.*, 24, 515-524 (1892); cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 517.

²⁹¹ A. Lidoff, *J. Russ. Phys. Chem. Soc.*, 24, 524-526 (1892); cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 517.

²⁹² A. Lidoff, *J. Russ. Phys. Chem. Soc.*, 27, 177-182 (1895); cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 517.

²⁹³ J. Jegerow, *J. prakt. Chem.* [2], 86, 521-539 (1912).

²⁹⁴ A. Fitz, *Ber.*, 4, 442-446 (1871).

²⁹⁵ J. J. Sudborough and J. M. Gittins, *J. Chem. Soc.*, 95, 315-321 (1909).

²⁹⁶ G. Rankoff, *J. prakt. Chem.* [2], 131, 293-300 (1931).

has recently been confirmed.²⁹⁰⁻²⁹⁷ Ricinelaidic acid was prepared by this method by Playfair²⁹⁸ and others.²⁹⁹⁻³⁰⁰ Nitric acid, usually in dilute solution, has also been employed (Haussknecht,³⁰¹ Keffler and Maiden³⁰²) to bring about the isomerization.

In addition to the nitrogen oxides, sulfurous acid is an active catalyst. Saytzeff³⁰³ obtained a transformation of oleic to elaidic acid with sulfurous acid or sodium bisulfate. In the case of erucic acid, the transformation can be effected in 24 hours at 200°C. Phosphorous acid acts catalytically in a manner similar to that of sulfurous acid,³⁰⁴ while Rankoff^{305,306} has successfully used sulfur or phosphorus.

Bertram³⁰⁷⁻³⁰⁹ proposed the use of selenium, and this has turned out to be one of the best agents to effect such transformations. An equilibrium mixture of triolein and trielaidin can be produced in 8 hours with 0.3% selenium at 150-220°C. In addition to selenium itself, hydrogen selenide, selenium dioxide, and selenium dibromide are active. The closely related element, tellurium, has been tested by Bertram, and it is also potent.

The elaidinization reaction is a reversible one. In the case of the oleic acid \rightleftharpoons elaidic acid change, equilibrium is reached when the mixture contains 34% of oleic acid and 66% of elaidic acid. The point of equilibrium is independent of the catalyst, and results irrespective of whether one starts with the *cis* or the *trans* form.

c. Isomers of Oleic Acid. There are a number of positional isomers of oleic acid, as well as an additional geometric isomer for each compound. Not all of these potential compounds have been prepared.

Among the six known *cis* isomers, petroselinic acid, $\text{CH}_3(\text{CH}_2)_{10}\text{CH}:\text{CH}(\text{CH}_2)_4\text{COOH}$, and oleic acid, $\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_7\text{COOH}$, are best known. The other related acids are 2-, 3-, 4-, and 12-octadecenoic acids.

Eight *trans* isomers have been studied. These include three well-known compounds, petroselaidic acid (the *trans* form of petroselinic acid), elaidic acid (related to oleic acid), and vaccenic acid, $\text{CH}_3(\text{CH}_2)_5\text{CH}:\text{CH}(\text{CH}_2)_9-$

²⁹⁷ T. G. Green and T. P. Hilditch, *Biochem. J.*, **29**, 1552-1563 (1935).

²⁹⁸ L. Playfair, *Phil. Mag.* [3], **29**, 475-480 (1846).

²⁹⁹ F. Krafft, *Ber.*, **21**, 2730-2737 (1888).

³⁰⁰ C. Mangold, *Monatsh.*, **15**, 307-315 (1894).

³⁰¹ O. Haussknecht, *Ann.*, **143**, 40-57 (1867).

³⁰² L. Keffler and A. M. Maiden, *Bull. soc. chim. Belg.*, **44**, 467-472 (1935).

³⁰³ M. Saytzeff, C. Saytzeff, and A. Saytzeff, *J. prakt. Chem.* [2], **50**, 73-80 (1894).

³⁰⁴ S. Fokin, *J. Russ. Phys. Chem. Soc.*, **42**, 1068-1073 (1910); *Chem. Abst.*, **5**, 3923 (1911).

³⁰⁵ G. Rankoff, *Ber.*, **B62**, 2712-2717 (1929); **B64**, 619-621 (1931); **B69**, 1231-1238 (1936).

³⁰⁶ G. Rankoff, *Ber.*, **63**, 2139-2142 (1930).

³⁰⁷ S. H. Bertram, *Chem. Weekblad*, **33**, 3-5 (1936); *Chem. Abst.*, **30**, 3405-3406 (1936).

³⁰⁸ S. H. Bertram, *U. S. Patent* No. 2,165,530 (July 11, 1939).

³⁰⁹ S. H. Bertram, *Rec. trav. chim.*, **59**, 650-652 (1940).

COOH. Other *trans* octadecenoic acids which are known have the double bonds at positions 5-6, 7-8, 8-9, 10-11, or 12-13, respectively.

The properties of the fatty acids having a *trans* linkage are distinct from those with the *cis* form. Elaidic acid, which is the best known and typical *trans* representative, can be readily distinguished from oleic acid by its melting point (see Table 16), by the fact that it forms solid solutions with saturated fatty acids,⁵ in accordance with the findings of Mascarelli *et al.* that *trans* isomers such as brassidic acid form solid solutions with saturated compounds (behenic), and that *cis* forms, like erucic, do not,³¹⁰⁻³¹³ by its longer molecular length as determined by x-ray examination,³¹⁴⁻³¹⁵ by the difference in behavior of the monomolecular film,³¹⁶ and by other physical properties.

d. Isomerism of the Polyethenoid Acids. (*a*) *Linoleic Acid.* There is no clear-cut proof for position or geometric isomers of linoleic acid in nature. Hilditch¹ has cited considerable evidence to indicate that linoleic acid from a wide variety of plant oils conforms to the pattern of *cis*-9-*cis*-12-octadecadienoic acid. Although a large variety of positional isomers are theoretically possible, the 9,12-octadecadienoic acid is the only one which occurs naturally.

Four geometric isomers are theoretically possible in the case of the diethenoid acids. Thus, linoleic acid may have the following configuration of the double bonds at the 9 and 12 positions, respectively: *cis*-*cis*, *cis*-*trans*, *trans*-*cis*, and *trans*-*trans*. In addition to the natural form (*cis*-*cis*), the *trans*-*trans* isomer (linolelaidic acid) is well known also, since it can be produced by the elaidinization reaction. The structures of the above two acids have been established unequivocally.

There is some question whether the *cis*-*trans* or the *trans*-*cis* isomers have been demonstrated. The chief indication for their presence has been based upon failures to obtain a correlation between the tetrabromide value, the thiocyanogen number, and the iodine number. Inoue and Suzuki⁴² interpreted their results as evidence of a new linoleic acid isomer from silkworm pupae, since they were unable to obtain the tetrabromide in the usual solid form. Similarly, Smith and Chibnall²³⁵ could not separate the crystalline tetrabromide from the linoleic acid fraction of cocksfoot and perennial rye grass. Moreover, Frankel *et al.*³¹⁷ were unable to obtain a

³¹⁰ G. Bruni and F. Gorni, *Atti accad. Lincei* [5], 8, II, 181-190 (1899).

³¹¹ L. Mascarelli, *Atti accad. Lincei* [5], 23, II, 583-585 (1914).

³¹² L. Mascarelli and B. Toschi, *Atti accad. Lincei* [5], 23, II, 586-590 (1914).

³¹³ L. Mascarelli and G. Sanna, *Atti accad. Lincei* [5], 24, II, 30-37 (1915).

³¹⁴ A. Müller, *J. Chem. Soc.*, 123, 2043-2047 (1923).

³¹⁵ A. Müller and G. Shearer, *J. Chem. Soc.*, 123, 3156-3164 (1923).

³¹⁶ J. Marsden and E. K. Rideal, *J. Chem. Soc.*, 1938, 1163-1171.

³¹⁷ J. S. Frankel, W. Stoneburner, and J. B. Brown, *J. Am. Chem. Soc.*, 65, 259-262 (1943).

correlation of the tetrabromide value and the iodine number with olive oil, although an excellent agreement obtained with corn, sesame, cottonseed, grapeseed, and poppyseed oils. These investigators believed that olive oil contains isomeric linoleic acid. On the other hand, Kass *et al.*³¹⁸ concluded that only one octadecadienoic acid is present in olive oil, as well as in almond, coconut, corn, cottonseed, peanut, poppyseed and sunflowerseed oils, and cacao butter, in spite of irregularities in the yield of tetrabromostearic acid. These workers believe that the tetrabromide method gives unreliable results. However, in spite of this debatable circumstantial evidence for the presence of linoleic acid isomers, no isomer has been isolated. In any event, the amounts and the distribution of the disputed products are quite insignificant, and one must conclude that practically all of the octadecadienoic acid found naturally is linoleic acid.

(b) *Triethenoid Acids.* Linolenic acid and elaeostearic acids are the most important triethenoid acids. While linolenic acid is 9,12,15-octadecatrienoic acid, elaeostearic acid has the structure of 9,11,13-octadecatrienoic acid. These are the only two positional isomers known, although the possibilities of such compounds are far greater with three double bonds as compared with two unsaturated linkages.

The differences in arrangement of the double bonds in linolenic and elaeostearic acid are responsible for considerable variation in their properties. While the unsaturated bonds are separated in linolenic acid by a methylene group, they occupy positions between alternate carbon atoms in the case of elaeostearic acid. This latter arrangement is spoken of as conjugation. Acids in which such conjugate double bonds occur do not add halogens to complete saturation, in contradistinction to the quantitative response of acids where the unsaturated linkages are not conjugated. However, acids with conjugate double bonds add oxygen more readily and polymerize more easily than do their non-conjugate isomers. This property renders them especially satisfactory for use in quick-drying paints. Other properties of acids with double bonds in alternating positions also differentiate them from their non-conjugated compounds. This is particularly noticeable as regards the melting point. Whereas linolenic acid melts at -11°C ., α -elaestearic acid melts at $48-49^{\circ}\text{C}$., and the β -form at $71-72^{\circ}\text{C}$.

The opportunities for geometric isomers increase exponentially with the rise in the number of double bonds. Eight such isomers are theoretically possible in the case of the triethenoid acids, including *cis-cis-cis* and *trans-trans-trans*, as well as all intermediate configurations. The geometrical structure of α -linolenic acid is not known with certainty, although it is assumed to be the *cis-cis-cis* form. Kass, Nichols, and Burr³¹⁹ have pre-

³¹⁸ J. P. Kass, W. O. Lundberg, and G. O. Burr, *Oil & Soap*, 17, 50-53 (1940).

³¹⁹ J. P. Kass, J. Nichols, and G. O. Burr, *J. Am. Chem. Soc.*, 63, 1060-1063 (1941).

pared the solid elaidinized form, elaidolinolenic acid, which melts at 29–30°C. This is probably the all-*trans* isomer, but there is some question as to whether all the *cis* linkages have been changed to *trans* forms in the latter acid, or whether the change is only a partial one.

(7) Crystalline Structure

Although it is generally considered that solid matter exists in only two states, crystalline and amorphous, it is well known that the same molecular structures are able to arrange themselves in different patterns, with the result that they may occur in several crystalline forms. Because of the close relationship of such structures to the melting point, it is of especial importance to consider at some length the nature of the variations. Other physical properties related to crystalline form are refractive index, compressibility, coefficient of thermal expansion, electrical conductivity, and heat conductivity. While some idea of crystal structure may be obtained by utilizing the laws of symmetry and by observing the external forms of natural crystals, such methods of approach have not proved fruitful in the case of the fatty acids.

a. X-Ray Diffraction Patterns and Crystal Structure. Although the application of x-ray technic to the determination of crystalline structure was first applied to such inorganic materials as calcite and sodium chloride, it was soon found that it could equally well be employed with organic compounds such as long-chain hydrocarbons, fatty acids, esters, alcohols, glycerides, ketones, and many other compounds.

Two methods are available for the application of this technic. It can be employed by making use of a single crystal sufficiently large to allow the x-rays to be focussed through it. This technic is exceedingly difficult and cumbersome, but the maximum information can be obtained as to the structure of the single crystal which is being studied. The commoner and simpler procedure involves the use of an oriented layer or film of the substance under investigation. This is especially satisfactory for determining the purity of a fatty acid, an ester, or other compound.

While a space lattice occurs in the case of the organic as well as of the inorganic crystals, the unit cells differ. In the case of the inorganic compound, the individual points of the lattice are occupied by atoms; in the fatty acids, the units represent a group of molecules which contain a representation of the lattice; each of these unit cells is the smallest parallelepiped which, on repetition, eventually forms a microscopic crystal.

Through the pioneer work of Müller,^{320–322} who determined the x-ray

³²⁰ A. Müller, *Proc. Roy. Soc. London*, A114, 542–561 (1927).

³²¹ A. Müller, *Proc. Roy. Soc. London*, A120, 437–459 (1928).

³²² A. Müller, *Trans. Faraday Soc.*, 25, 347–348 (1929).

diffraction of single stearic acid crystals, it was found that a unit cell of stearic acid is an elongated monoclinic prism which contains four molecules. Three characteristic spacings were found in the x-ray diffraction patterns which Müller and co-workers,^{314,315} in earlier work, had designated the d_1 axis (long spacing or c axis), d_2 , and d_3 axes (b and a , respectively). The axial dimensions of such an elementary parallelepiped are 5.546 Å. (a axis), 7.381 Å. (b axis), and 48.84 Å. (c axis). The b axis is perpendicular to the ac plane, but the c axis is inclined to produce an angle (β) of $63^\circ 38'$ between the a and c axes.⁴ These relationships are pictured in the diagrammatic representation of a stearic acid unit crystal given in Figure 18.

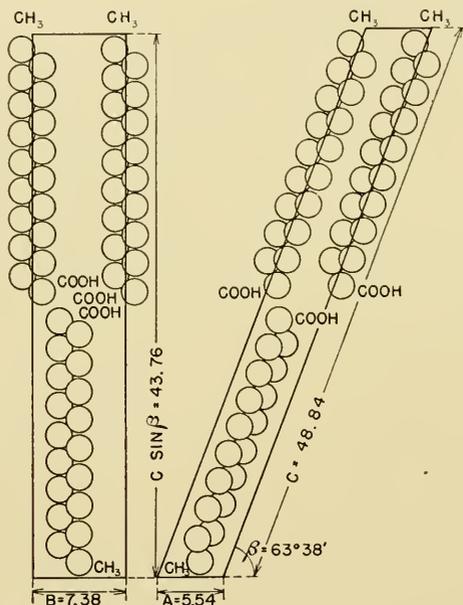


Fig. 18. A diagrammatic representation of a unit cell of a stearic acid crystal.⁴

The fatty acids all form monoclinic prisms and, in all cases, there are four molecules in a unit. In the case of hydrocarbons, although the unit cell contains four molecules, the crystal is orthorhombic. In the dicarboxylic acids having an even number of carbons, only two molecules exist in each unit, although when they are composed of an odd number of carbons the unit cell contains four molecules.

Numerous variations exist in the measurements of the different axes in the various fatty acid crystals. The c axis is directly proportional to the length of the carbon chain. Variations also occur in the a and b axes, al-

though the product of these measurements multiplied by the sine β (angle of inclination) does give a constant. The relationship of these values in lauric and stearic acids, as well as in unsaturated and substituted fatty acids and in a hydrocarbon,³²³ is shown in Table 30.

The chains of stearic acid molecules are so oriented that the carboxyl groups are in juxtaposition. This causes each layer or sheet to consist of two layers of molecules. This causes each layer or sheet to consist of two layers of molecules. The thickness of the sheets is correspondingly increased over that of the aliphatic hydrocarbons, which are packed regularly with the chain axes parallel in flat sheets a single molecule in thickness. The methyl esters of the fatty acids behave in a manner similar to that of the fatty acids; however, in the case of the ethyl esters, the layer is only a single molecule in thickness.

The values measured by the x-ray photograph are the perpendicular distances between the planes. In the case of the fatty acids, this space approximates that of two fatty acid molecules, although it is somewhat less than this value.³²⁴ The plane length in the case of the stearic acid molecule is readily calculated from the expression $2l \sin \beta$, where l is the length of one stearic acid molecule and β is the angle between the c axis and the ac axis (Fig. 18).

Another interesting fact on the arrangement of the carbon atoms in a single fatty acid molecule can be gleaned from the x-ray measurements. If one assumes that the distance between adjacent carbon atoms is 1.54 Å., as will occur when the carbon atoms are arranged in a zigzag chain, and also that the corresponding tetrahedral angles on the carbon valences are $109^\circ 28'$, then the distance between the alternate carbon atoms is calculated as 2.52 Å. This is in excellent agreement with the figure arrived at from the measurement of the c axis of n -nonacosane; the latter has been shown to be at 2.54 Å. These relationships are illustrated in Figure 19.

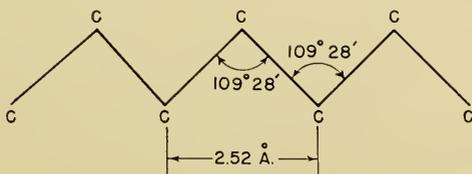


Fig. 19. A diagrammatic representation of the zigzag arrangement of the carbon atoms of aliphatic acids.⁴

Malkin³²⁵ has suggested that this zigzag arrangement of the carbon atoms accounts for the phenomenon of alternation of properties in long-chain aliphatic compounds which possess odd- and even-numbered carbon atoms. In the even-numbered carbon molecules, the end groups are

³²³ S. B. Hendricks, *Chem. Revs.*, 7, 431-477 (1930).

³²⁴ S. H. Piper, *J. Soc. Chem. Ind.*, 56, 61-66T (1937).

³²⁵ T. Malkin, *Nature*, 127, 126-127 (1931).

TABLE 30
AXIAL DIMENSIONS (ANGSTRÖM UNITS) AND ANGLES OF INCLINATION OF LONG-CHAIN FATTY ACIDS AND RELATED COMPOUNDS^a

Name of compound	Formula	a	b	c	β	ab	$\frac{ab \times \beta}{\sin \beta}$	$\frac{c \times \beta}{\sin \beta}$	$\frac{a \times \beta}{\sin \beta}$	ϵ
Lauric acid	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	9.76	4.98	36.9	48°6'	48.6	36.5	27.6	7.32	0.681
Stearic acid	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	5.55	7.38	48.84	63°16'	40.94	36.0	43.76	4.95	0.671
Behenic acid	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$	9.55	4.69	59.10	53°30'	44.76	36.0	47.51	7.67	0.612
Stearolic acid	$\text{CH}_3(\text{CH}_2)_{17}\text{C}(\text{CH}_2)_7\text{COOH}$	9.55	4.69	49.18	53°4'	44.76	35.8	39.28	7.63	0.614
Bromostearic acid	$\text{CH}_3(\text{CH}_2)_{15}\text{CHBrCOOH}$	11.04	4.90	52.88	43°15'	54.13	37.1	36.23	7.56	0.648
Nonacosane	$\text{CH}_3(\text{CH}_2)_{27}\text{CH}_3$	7.45	4.97	77.2	90°	37.0	37.0	77.2	7.45	0.667

^a K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 84 (from S. B. Hendricks, *Chem. Revs.*, 7, 431-477 (1930), p. 456).

parallel, while in the odd-numbered carbon chains the end groups are at an angle to each other. This requires a difference in the inclination of the chains in the layers which, in turn, will influence many of the physical properties. In all cases of long-chain aliphatic compounds, irrespective of whether they contain an odd or an even number of carbons, the addition of another carbon atom is always accompanied by an increase in the c axis by 4.6 Å.

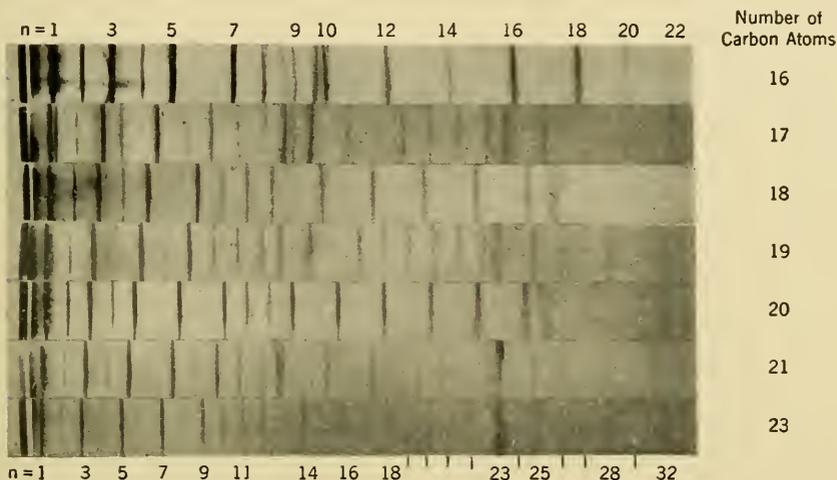


Fig. 20. The effect of chain length on the diffraction pattern of n -aliphatic acids.⁴

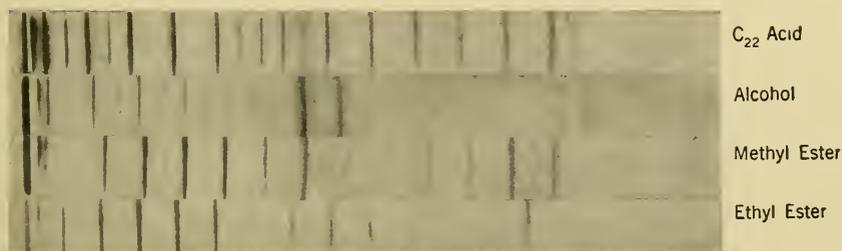


Fig. 21. The x-ray patterns of the C_{22} acid and corresponding derivatives.⁴

As has been indicated by Figure 18, the four molecules of stearic acid in a unit cell are so arranged that two molecules are packed side by side in the upper and lower half of the cell, respectively. The second one in the lower half in Figure 18 lies directly behind it and in the same plane. The "packing distance" between these individual chains has been shown to be between 3.7 and 4.0 Å., compared to a distance of 1.54 Å. between the ad-

jacent carbon molecules in the individual chain. The effect of chain length on the diffraction patterns of the *n*-aliphatic acids is illustrated in the photographs in Figure 20. Figure 21 illustrates the variations in the pattern of a C₂₂ acid caused by conversion to the methyl or ethyl ester or by a reduction to the corresponding alcohol.

(8) Polymorphism

a. Introduction. Fatty acids crystallize in more than one solid or polymorphic form. Such polymorphic forms are solid phases which have the same chemical composition but differ from each other in crystalline structure, free energy, melting point, as well as in other physical and chemical properties. However, upon fusion or evaporation, they yield identical liquid or gaseous phases. This property differentiates the polymorphic forms from isomeric forms, which do not lose their identity on melting or on vaporization.

There are two types of polymorphism. These are known as *enantiotropism* and *monotropism*. In the case of enantiotropic polymorphs, the two forms have different vapor pressure curves which approach each other with a change in temperature; the vapor pressure curves intersect at the so-called *transition point*. At this temperature, both solid phases exist in equilibrium as stable compounds. As heat is added to the system, a transformation may take place to the form stable at the higher temperature, without increase in temperature (isothermal transition). When heat is removed, the reverse change may obtain at a constant temperature, namely, the form stable at the lower temperature will result. This form of polymorphism is completely reversible without changing the solid state of the compound.

In the case of monotropism, the vapor pressures of the two components do not meet at any temperature, and there is consequently no transition point. If both polymorphs are present, the one with the higher vapor pressure is always the metastable form, while the one possessing the lower vapor pressure is the stable modification. On distillation or by some similar process, the metastable type can be changed to the stable type. No reversibility exists between such polymorphic forms in the solid state.

Polymorphism occurs in the case of practically all long-chain aliphatic compounds, including not only the saturated fatty acids but also the unsaturated acids, their esters, triglycerides, alcohols, and hydrocarbons. The phenomenon is of very great importance in the fat industry, since the consistency of samples of lard, butter, and hydrogenated fats, including oleomargarines, is dependent upon the crystalline form. The fatty acids exhibit only monotropism (irreversible polymorphism), although both

enantiotropism (reversible polymorphism) and monotropism are known to occur with the monoesters of the fatty acids.

b. Polymorphism in the *n*-Aliphatic Fatty Acid Series. Three polymorphic forms have been reported for the even-chain fatty acids, while an additional type of polymorph has been noted in the case of the odd-chain fatty acids. The usual designation employed at present for these modifications is by the Greek letters, α , β , and γ ; the fourth form, found only with the odd-chain fatty acids, is referred to as the α' -modification. The polymorphic modifications have different x-ray diffraction patterns, which Piper³²⁶ has termed *A*, *B*, and *C* for the even-carbon series and *A'*, *B'*, *C'*, and *D'* for the odd-chain series of fatty acids. Confusion has arisen concerning the two terminologies employed. The α -, β -, and γ -modifications produce the x-ray diffraction patterns of the *C*, *B*, and *A* types, respectively (also *C'*, *B'*, and *A'* types). The *D'* diffraction pattern is given by the α' -modification. The different terminologies are summarized in Table 31.

TABLE 31

X-RAY DIFFRACTION PATTERNS ASSOCIATED WITH SEVERAL POLYMORPHIC FORMS OF FATTY ACIDS

Even-chain fatty acids		Odd-chain fatty acids	
Polymorphic forms	X-ray diffraction patterns ^a	Polymorphic forms	X-ray diffraction patterns ^a
α	<i>C</i>	α	<i>C'</i>
β	<i>B</i>	β	<i>B'</i>
γ	<i>A</i>	γ	<i>A'</i>
—	—	α'	<i>D'</i>

^a S. H. Piper, *Trans. Faraday Soc.*, 25, 348-351 (1929).

An extended discussion of polymorphism has been given by Francis and Piper^{216,217} and by J. C. Smith,³²⁷ which can be only briefly reviewed below. All three modifications are indistinguishable on the basis of the melting point. This is presumably due to the fact that on heating they are rapidly changed to one form which has a constant melting point. On the other hand, all of the polymorphs can be readily differentiated from each other by variations in the x-ray spacings on the long axis.

The different polymorphic forms are usually interconvertible. They tend to revert to the stable modification, *i.e.*, the type which is most stable at ordinary temperature. However, in many instances it is possible to isolate the metastable form, although in some cases the presence of such substances is manifested only by a thermal effect when a transition occurs.

³²⁶ S. H. Piper, *Trans. Faraday Soc.*, 25, 348-351 (1929).

³²⁷ J. C. Smith, "Fatty Acids and Other Long-Chain Compounds," in *Ann. Repts. Prog. Chem.*, 1933, 35, Chemical Society, London, 1939, pp. 251-268.

The ease with which polymorphic forms may be changed is markedly influenced by the presence of extremely small amounts of impurities, as has been recently pointed out by Ralston.⁵ Thus, when small amounts of octadecane are present in hexadecane, two crystalline forms of the latter regularly occur.³²⁸ On the other hand, the presence of a homologue stabilizes the metastable modifications of the higher alcohols³²⁹ and of the nitriles.³³⁰ In some cases the conversion of one modification into the higher melting and more stable form cannot be realized if impurities are present.³³⁰⁻³³² However, a small amount of impurity is apparently necessary in the case of such secondary amines as dihexylamine, dioctylamine, and didecylamine.⁵

The α -modification of the fatty acids is a metastable form which is generally obtained on cooling the melted acid or when a solution of the acid is rapidly evaporated. In the case of the even-chain fatty acids, the α -form is converted to the more stable β -modification at a certain temperature which is specific for the fatty acid involved. Dupré la Tour³³³ found this transition temperature to be the following: lauric acid, 6.5°C.; myristic acid, 24°C.; palmitic acid, 40°C.; stearic acid, 54°C.; and cerotic acid, 80°C. The reverse change does not occur when the β -form is cooled.³³⁴ In the case of the odd-chain fatty acids, the α -modification formed on rapid solidification of the melted acid changes to the β -form without change in temperature. In this case the α -form of the odd-carbon acids can be retained only when very rapid cooling is employed.³³⁵ When fatty acids are crystallized from highly polar solvents such as hot glacial acetic acid, even at a slow rate, the α -polymorph results. The x-ray diffraction patterns in the case of the α -form have been shown to be of the *C* type, in which the spacings on the long axis are shorter than in the *B* spacing⁵ (see Table 32).

The β -form of fatty acid crystals is usually produced immediately when solutions of the fatty acid in non-polar solvents are allowed to evaporate slowly. Benzene is an excellent solvent from which to prepare the β -polymorphic form. *n*-Eicosanoic acid is an exception to the above rule, since both α - and β -forms result when crystallization takes place from hot glacial acetic acid. The molecular arrangements of the β -forms show the *B* spacings. The long axis in this case has a considerably greater length than in the α -form.

³²⁸ J. C. Smith, *J. Chem. Soc.*, 1932, 737-741.

³²⁹ E. J. Hoffman, C. W. Hoerr, and A. W. Ralston, *J. Am. Chem. Soc.*, 67, 1542-1545 (1945).

³³⁰ C. W. Hoerr, H. J. Harwood, and A. W. Ralston, *J. Org. Chem.*, 11, 199-206 (1946).

³³¹ M. C. Bloom and M. J. Buerger, *Z. Krist.*, 96, 365-375 (1937).

³³² R. H. Ferguson and E. S. Lutton, *Chem. Revs.*, 29, 355-384 (1941).

³³³ F. Dupré la Tour, *Ann. phys.* [10], 18, 199-284 (1932).

³³⁴ J. Thibaud and F. Dupré la Tour, *J. chim. phys.*, 29, 153-167 (1932).

³³⁵ G. M. de Boer, *Nature*, 119, 634-635 (1927).

TABLE 32 LONG-SPACING VALUES FOR EVEN-NUMBERED AND ODD-NUMBERED NORMAL ALIPHATIC ACIDS^a

Name of acid	Number of carbon atoms	Crystal spacing, A.				Methyl esters	Ethyl esters (B)
		Acids					
		B form (β)	C form (α)	A form (γ)	D form (α')		
Even-Numbered Acids							
<i>n</i> -Tetradecanoic	14	34.9	31.60	—	—	—	—
<i>n</i> -Hexadecanoic	16	39.1	35.60	41.0	—	43.45	22.9
<i>n</i> -Octadecanoic	18	43.75	39.75	46.6	—	47.95	25.80
<i>n</i> -Eicosanoic	20	48.75	44.15	—	—	52.30	27.6
<i>n</i> -Docosanoic	22	52.95	48.3	—	—	57.02	30.1
<i>n</i> -Tetracosanoic	24	57.75	52.6	—	—	61.70	32.15
<i>n</i> -Hexacosanoic	26	62.2	56.25	—	—	66.15	34.45
<i>n</i> -Octacosanoic	28	67.15	61.05	—	—	70.80	36.65
<i>n</i> -Triacotanoic	30	71.4	65.2	—	—	75.25	38.75
<i>n</i> -Dotriacontanoic	32	76.3	69.25	—	—	79.95	41.1
<i>n</i> -Tetraatriacontanoic	34	80.5	73.3	—	—	84.15	43.45
<i>n</i> -Hexatriacontanoic	36	85.25	78.1	—	—	89.30	46.3
<i>n</i> -Octatriacontanoic	38	90.0	82.1	—	—	93.00	48.05
<i>n</i> -Hexatetracontanoic	40	108.2	99.05	—	—	121.7	57.45
Odd-Numbered Acids							
<i>n</i> -Tridecanoic	13	31.65	30.00	35.3	25.8	—	—
<i>n</i> -Pentadecanoic	15	35.8	34.2	40.0	29.9	—	—
<i>n</i> -Heptadecanoic	17	40.45	38.6	—	33.9	46.3	24.6
<i>n</i> -Nonadecanoic	19	44.50	43.15	—	—	50.8	26.95
<i>n</i> -Henetacosanoic	21	49.25	47.8	—	—	55.25	29.35
<i>n</i> -Tricosanoic	23	53.40	51.8	—	—	60.0	31.50
<i>n</i> -Pentacosanoic	25	57.65	56.2	—	—	64.55	33.60
<i>n</i> -Heptacosanoic	27	62.0	60.5	—	—	—	—
<i>n</i> -Nonacosanoic	29	66.35	64.8	—	—	73.75	38.1

^a Some of the data are adapted from H. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, pp. 335, 336, 345.

Although the α - and β -forms are the types of crystals most frequently encountered in both the odd and even type of fatty acids, Piper *et al.*,³³⁶ in 1926, were able to demonstrate a third form, the γ -modification (*A* spacing), which possesses an even longer spacing on the long axis than does the β -form. This variety of palmitic or of stearic acid was formed when the acid crystals were pressed on a glass plate. It was found that the γ -form can co-exist with the α -form but not with the β -form, although the α - and β -types can occur together. The γ -modification has not been demonstrated for any odd- or even-chain fatty acids with more than 18 carbon atoms.³²⁷

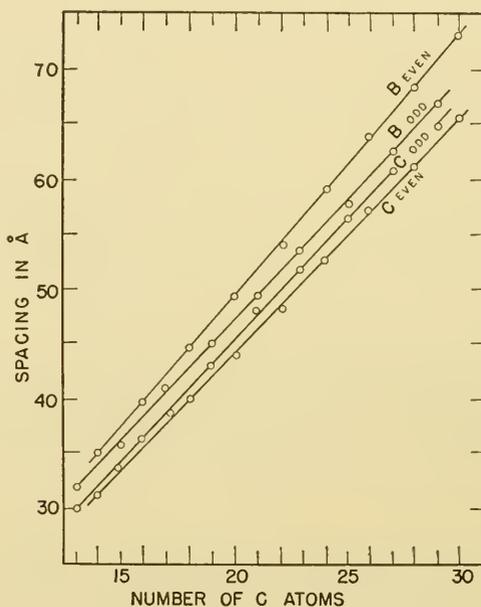


Fig. 22. Crystal spacings of saturated acids.⁵

The odd-chain acids exhibit certain peculiarities. One of these, to which reference has already been made, is the almost spontaneous transformation from the α - to the β -form after crystallization of the melted acid has taken place. Moreover, the β -form of crystal always results with the odd-chain acids when they are crystallized from solvents, even those of the highly polar type. The odd-carbon fatty acids, however, do exhibit a reversibility of the transition $\alpha \rightarrow \beta$ in the case of undecanoic, nonanoic, heptonic, and the lower acids, but not for acids containing 13 or more carbon atoms.³³⁷ Piper³²⁶ discovered the modification having the *D* pattern, but little is

³³⁶ S. H. Piper, T. Malkin, and H. E. Austin, *J. Chem. Soc.*, 1926, 2310-2318.

³³⁷ W. E. Garner and A. M. King, *J. Chem. Soc.*, 1929, 1849-1861.

known about the nature of this polymorphic form other than that the length of the long axis is the shortest in any of the four modifications of the fatty acid crystals.

The long-spacing values for the fatty acids are summarized in Table 32. When the spacings of the *B* (β) or *C* (α) types are plotted against the length of the chain, a straight-line curve is obtained for the even- and the odd-chain acids (Fig. 22).

c. Polymorphism in Unsaturated Fatty Acids. Although the evidence for polymorphism has not been demonstrated for many of the polyethenoid acids which are liquids under most conditions, evidence that oleic acid can exist in two forms was advanced over 30 years ago by Kirschner.³³⁸ This proof was largely based upon the fact that two distinct melting points exist for oleic acid in different preparations, one at about 13°C. and the second at about 16°C. Although Smith³³⁹ and Stewart and Wheeler³⁴⁰ were able to confirm Kirschner's results unequivocally, it has been demonstrated only recently, by Lutton,³⁴¹ that the polymorphic forms have a different crystalline structure as shown by x-ray diffraction patterns. The lower melting sample (13.3°C.) had a main short spacing at 4.19 Å. and its long spacing at 40.5 Å., which indicated similarities to the *B* and *C* forms of stearic acid. On the other hand, the higher melting polymorph (16.2°C.) had main short spacings at 4.65 Å. and 3.67 Å. and a long spacing at 84.4 Å. (or possibly 42.2 Å.). Lutton³⁴¹ suggests that it is probably unique in crystal structure among the long-chain monocarboxylic acids.

(9) *Physical Properties of Fatty Acids in Liquid State*

A number of the physical properties of fatty acids can be determined only in the liquid state. These properties are not only of theoretical importance but they are also of considerable practical value. They include density, molar volume, refractive index, surface tension, viscosity, boiling point, specific heat, specific conductance, and dielectric constant. These can be only briefly considered here, but are more extensively discussed in the monographs of Markley⁴ and Ralston.⁵

a. Density. The density of the fatty acids shows the greatest regularity when determined on the compounds in the liquid state. Density is ordinarily considered as the weight in grams per cubic centimeter and is frequently called the absolute density. This value varies only slightly from that of the specific gravity, which is the ratio of the weight of the substance compared with the weight of an equal volume of water at 4°C.

³³⁸ A. Kirschner, *Z. physik. Chem.*, 79, 759-761 (1912).

³³⁹ J. C. Smith, *J. Chem. Soc.*, 1939, 974-980.

³⁴⁰ H. W. Stewart and D. H. Wheeler, *Oil & Soap*, 18, 69-71 (1941).

³⁴¹ E. S. Lutton, *Oil & Soap*, 23, 265-266 (1946).

The comparative values for the specific gravities of the saturated fatty acids and of their methyl or ethyl esters at several temperatures are recorded in Table 33, together with data on densities of the acids obtained³⁴² at a uniform temperature of 80°C.

TABLE 33

SPECIFIC GRAVITIES OF SATURATED FATTY ACIDS OR THEIR ESTERS, USUALLY AT 20°C., AND DENSITIES OF FATTY ACIDS DETERMINED AT 80°C.

Name of acid	No. of C atoms	Acid		Specific gravity	
		Sp. gr. ^{a, b}	Density at 80°C	Methyl ester ^{a, b}	Ethyl ester ^{a, b}
Acetic	2	1.049	—	0.933	0.899
Propionic	3	0.992	—	0.917	0.891
Butyric	4	0.959	—	0.898	0.879
Valeric	5	0.942	—	0.910 ⁰	0.877
Caproic	6	0.929	0.8751	0.904 ⁰	0.875 ¹⁵ ₄
Heptanoic	7	0.922	0.8670	0.881 ¹ ₅	0.872 ¹⁵
Caprylic	8	0.910	0.8615	0.887	0.878 ¹⁷
Nonanoic	9	0.907	0.8570	0.877 ^{17.5}	0.866 ^{17.5}
Capric	10	0.895 ³⁰	0.8531	—	0.862
Undecanoic	11	—	0.8505	—	—
Lauric	12	0.883	0.8477	—	0.868 ¹³
Tridecanoic	13	—	0.8458	—	—
Myristic	14	0.858 ⁶⁰	0.8439	—	—
Pentadecanoic	15	—	0.8423	—	—
Palmitic	16	0.853 ⁴²	0.8414	—	—
Heptadecanoic	17	0.853 ⁶⁰	0.8396	—	—
Stearic	18	0.847 ^{69.3}	0.8390	—	—

^a Data from K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 211.

^b The superscripts refer to the temperature at which determinations were made and the subscripts give the temperatures of the comparison substances; where neither figure is given, the values are for 20°/4°C.

^c A. Dorinson, M. R. McCorkle, and A. W. Ralston, *J. Am. Chem. Soc.*, 64, 2739–2741 (1942).

There is a progressive decrease in density with the lengthening of the carbon chain. Since a volume expansion occurs on heating, the density is further decreased as the temperature is raised. The regularity of the drop in density with increased chain length is best demonstrated at a fixed temperature sufficiently high so that all members of the series will be liquid.

The density of unsaturated acids shows little variation from that of the saturated fatty acids of corresponding chain length when determined at the same temperature. Some of the values for specific gravity reported by Markley⁴ are as follows: petroselinic, 0.868⁴⁰; erucic, 0.860^{57.1}₄; linoleic, 0.903²⁰₃; and linolenic, 0.914²⁰. The values for oleic acid and some alkyl

³⁴² A. Dorinson, M. R. McCorkle, and A. W. Ralston, *J. Am. Chem. Soc.*, 64, 2739–2741 (1942).

oleates at several temperatures, reported by Keffler and McLean,³⁴³ are given in Table 34.

TABLE 34

DENSITIES OF OLEIC ACID AND ALKYL OLEATES AT DIFFERENT TEMPERATURES^a

Temp., °C.	Oleic acid	Methyl oleate	Ethyl oleate	Propyl oleate	Butyl oleate
15	0.8939	0.8774	0.8724	0.8708	0.8704
20	0.8905	0.8738	0.8687	0.8673	0.8669
25	0.8870	0.8702	0.8651	0.8637	0.8634
30	0.8835	0.8666	0.8613	0.8601	0.8599
60	0.8634	0.8450	0.8400	0.8389	0.8390
90	0.8429	0.8234	0.8183	0.8175	0.8178

^a L. Keffler and J. H. McLean, *J. Soc. Chem. Ind.*, 54, 178-185T (1935), p. 182.

(a) *Specific Volumes of Fatty Acids.* One of the constants which can be deduced from density measurements is the specific volume. This may be defined as the reciprocal of the density. The specific volumes of the saturated fatty acids from C₈ to C₁₂ were determined at different temperatures by Garner and Ryder,³⁴⁴ who employed the air-thermometer method. The values for the solid and liquid acids at their melting points were as follows: C₈, 0.9737 and 1.0925; C₉, 1.0104 and 1.0966; C₁₀, 0.9870 and 1.1169; C₁₁, 1.0121 and 1.1206; and C₁₂, 0.9971 and 1.1403. There were changes in molecular volume at the melting point in Δ cc. per gram mole as follows: C₈, 17.51; C₉, 13.95; C₁₀, 23.24; C₁₁, 20.68; and C₁₂, 29.07. As would be expected, the specific volume increases with the lengthening of the fatty acid chain and with a rise in temperature.

(b) *Molar Volumes of Fatty Acids.* The molar volume is defined as the volume occupied by one gram mole of the substance. It is readily calculated by dividing the molecular weight of a substance by its density. When used for the acids in the solid state, it furnishes information on the cross-section area of the unit cell of the crystal, especially when used in conjunction with x-ray measurements.

Pauly³⁴⁵ found that the molar volume of the lower fatty acids increases by an average of 22.3 cc. per gram mole in the liquid state for each CH₂ group added, while Garner and Ryder³⁴⁴ have given the figure as 16.8 cc. Alternation in the values occurs in the solid but not in the liquid state. Since a change in cross section was shown to be a non-alternating property, it has been concluded that variations in molecular volume are to be ascribed exclusively to alterations in chain length. The regularity of the increment in the molar volumes as the fatty acid chain is lengthened is in-

³⁴³ L. Keffler and J. H. McLean, *J. Soc. Chem. Ind.*, 54, 178-185T (1935).

³⁴⁴ W. E. Garner and E. A. Ryder, *J. Chem. Soc.*, 127, 720-730 (1925).

³⁴⁵ H. Pauly, *Z. anorg. allgem. Chem.*, 119, 271-291 (1921).

licated by the close correspondence of calculated and determined values in the studies of Dorinson *et al.*³⁴² Molar volume was calculated by the following formulas:

$$\text{At } 20^{\circ}\text{C., } V_m = 16.89 n + 23.62$$

$$\text{At } 80^{\circ}\text{C., } V_m = 17.25 n + 28.88$$

where V_m is molecular volume, and n is the number of carbon atoms in the chain.

Table 35 gives a comparison of the values calculated by the above formulas with those actually determined.

TABLE 35
COMPARISON OF CALCULATED AND EXPERIMENTALLY DETERMINED VALUES FOR MOLAR VOLUMES OF SATURATED FATTY ACIDS^a

Name of acid	No. of C Atoms	V_m at 20°C., cc.		V_m at 80°C., cc.	
		Calculated	Found	Calculated	Found
Formic	1	40.51	37.71	—	—
Acetic	2	57.40	57.21	63.68	61.11
Propionic	3	74.29	74.55	80.63	79.68
Butyric	4	91.18	91.93	97.88	97.95
Valeric	5	108.07	108.69	115.13	115.33
Caproic	6	124.96	125.04	132.38	132.67
Heptanoic	7	141.85	141.89	149.63	150.07
Caprylic	8	158.74	158.57	166.88	167.30
Nonanoic	9	175.63	174.53	184.13	184.50
Capric	10	—	—	201.38	201.80
Undecanoic	11	—	—	218.63	218.90
Lauric	12	—	—	235.88	236.29
Tridecanoic	13	—	—	253.13	253.27
Myristic	14	—	—	270.38	270.41
Pentadecanoic	15	—	—	287.63	287.61
Palmitic	16	—	—	304.88	304.56
Heptadecanoic	17	—	—	322.13	321.90
Stearic	18	—	—	339.38	338.85

^a A. Dorinson, M. R. McCorkle, and A. W. Ralston, *J. Am. Chem. Soc.*, 64, 2739–2741 (1942).

(c) *Dilation of Fatty Acids.* The expansion of fatty acids on heating, or dilation, is another property related to the density. Such changes in molar volume can be ascertained by determination of the density of the acid at a series of different temperatures. However, this relatively cumbersome procedure may be replaced by a much simpler method which involves direct readings on a dilatometer. The latter instrument has the advantage of permitting continuous measurements at all temperatures, and includes determinations of the values of the solid *vs.* the liquid state, as well as those

for the several polymorphic forms. Many different dilatometers have been suggested, including those of Normann,³⁴⁶ Coffey and Spannuth,³⁴⁷ and of Bailey and co-workers.³⁴⁸⁻³⁵¹

b. Refractive Index. The refractive index is another widely used constant, since it can be readily determined with great accuracy. It has been employed to ascertain the purity of the fatty acids.

The refractive index gives a measure of the deviation when a beam of light passes from air into a liquid. The law of refraction was established as early as 1621 by Willebrod Snell, who demonstrated that the ratio of the sines of the angles of incidence and of refraction is a constant at the boundary between two media.⁴ The refractive index, n , was shown to be the ratio of the velocity of light in a vacuum to that of the light in a liquid. It can be calculated by the equation:

$$n = \text{sine } i / \text{sine } r$$

The extent of refraction is governed by the interaction of electrostatic and electromagnetic forces set up by the atoms in the molecules of the liquid with the corresponding properties of the traversing light waves. Since it depends upon intermolecular attraction, it is related to molecular volume and internal pressure.

The importance of the refractive index was evident from the pioneer work of Eijkman³⁵² and of Scheij³⁵³ on the naturally occurring acids and of Falk³⁵⁴ on butyric acid. More complete studies have been made by Eisenlohr and Wöhlisch³⁵⁵ and by Waterman and Bertram.³⁵⁶ The former workers consider that the product of the refractive index and the molecular weight is additive, while the latter investigators believe that the increments of this value follow two distinct patterns, the signs for which change between the C₁₁ and C₁₂ acids. Verkade and Coops³⁵⁷ have challenged the latter view and ascribe the results to the experimental conditions under which the determinations were made. Data on the refractive indices of the common saturated fatty acids are summarized in Table 36. Wyman and Barkenbus³⁵⁸ have determined the refractive index on some methyl esters of the fatty acids, and this work has been extended by Mattil and

³⁴⁶ W. Normann, *Chem. Umschau*, **38**, 17-22 (1931); *Chem. Abst.*, **25**, 3858 (1931).

³⁴⁷ C. A. Coffey and H. T. Spannuth, *Oil & Soap*, **17**, 41-42 (1940).

³⁴⁸ A. E. Bailey and E. A. Kraemer, *Oil & Soap*, **21**, 251-253 (1944).

³⁴⁹ E. A. Kraemer and A. E. Bailey, *Oil & Soap*, **21**, 254-256 (1944).

³⁵⁰ A. E. Bailey and W. S. Singleton, *Oil & Soap*, **22**, 265-271 (1945).

³⁵¹ W. S. Singleton and A. E. Bailey, *Oil & Soap*, **22**, 295-299 (1945).

³⁵² J. F. Eijkman, *Rec. trav. chim.*, **12**, 157-197; 268-285 (1893).

³⁵³ L. T. C. Scheij, *Rec. trav. chim.*, **18**, 169-210 (1899).

³⁵⁴ K. G. Falk, *J. Am. Chem. Soc.*, **31**, 86-107 (1909).

³⁵⁵ F. Eisenlohr and E. Wöhlisch, *Ber.*, **53**, 1746-1766 (1920).

³⁵⁶ H. I. Waterman and S. H. Bertram, *Rec. trav. chim.*, **46**, 699-702 (1927).

³⁵⁷ P. E. Verkade and J. Coops, *Rec. trav. chim.*, **47**, 45-51 (1928).

³⁵⁸ F. W. Wyman and C. Barkenbus, *Ind. Eng. Chem., Anal. Ed.*, **12**, 658-661 (1940).

Longenecker.³⁵⁹ Few determinations are available on the unsaturated acids. Wood *et al.*³⁶⁰ have reported the following values (at 50°C.): oleic acid, 1.4487; elaidic acid, 1.4468; linoleic acid, 1.4588; and linolenic acid, 1.4678. Wan and Chen³⁶¹ have found a considerably higher value for α -elaeostearic acid, namely, 1.5112 at 50°C., while β -elaeostearic acid has a refractive index of 1.5022 at 75°C. Results on ethyl linoleate and ethyl linolenate over a wide range of temperatures are reported by McCutcheon.^{41,362}

TABLE 36

REFRACTIVE INDICES OF SATURATED FATTY ACIDS AS INFLUENCED BY TEMPERATURE AND MOLECULAR REFRACTIVITY AT 80°C.^a

Fatty acid	Refractive indices (n_D^t)						
	20°	30°	40°	50°	60°	70°	80°C.
Caproic	1.4170	1.4131	1.4095	1.4054	1.4012	1.3972	1.3931
Heptanoic	1.4230	1.4192	1.4155	1.4114	1.4073	1.4037	1.3939
Caprylic	1.4280	1.4243	1.4205	1.4167	1.4125	1.4089	1.4049
Nonanoic	1.4322	1.4287	1.4250	1.4210	1.4171	1.4132	1.4092
Capric	—	—	1.4288	1.4248	1.4210	1.4169	1.4130
Undecanoic	—	—	1.4319	1.4279	1.4240	1.4202	1.4164
Lauric	—	—	—	1.4304	1.4267	1.4230	1.4191
Tridecanoid	—	—	—	1.4328	1.4290	1.4252	1.4215
Myristic	—	—	—	—	1.4310	1.4273	1.3246
Pentadecanoic	—	—	—	—	1.4329	1.4292	1.4254
Palmitic	—	—	—	—	—	1.4309	1.4272
Heptadecanoic	—	—	—	—	—	1.4324	1.4287
Stearic	—	—	—	—	—	1.4337	1.4299

^a A. Dorinson, M. R. McCorkle, and A. W. Ralston, *J. Am. Chem. Soc.*, *64*, 2739-2741 (1942).

The refractive index is a straight-line function of temperature between 40° and 80°C., although the slope changes³⁶² below 40°C. The refractive index varies with the polymorphic form³⁶³; it becomes progressively greater with extension of chain length.

(a) *Molecular Refractivity.* The molecular refractivity is a value which relates refractive index to molecular weight and density. It is readily calculated from the Lorentz-Lorenz formula.^{364,365}

³⁵⁹ K. F. Mattil and H. E. Longenecker, *Oil & Soap*, *21*, 16-19 (1944).

³⁶⁰ T. R. Wood, F. L. Jackson, A. R. Baldwin, and H. E. Longenecker, *J. Am. Chem. Soc.*, *66*, 287-289 (1944).

³⁶¹ S. W. Wan and M. C. Chen, *J. Am. Chem. Soc.*, *61*, 2283-2284 (1939).

³⁶² J. W. McCutcheon, *Can. J. Research*, *B18*, 231-239 (1940).

³⁶³ J. Thibaud and F. Dupré la Tour, *Compt. rend.*, *190*, 945-947 (1930).

³⁶⁴ H. A. Lorentz, *Ann. physik. Chem.*, *9*, 641-665 (1880).

³⁶⁵ L. Lorenz, *Ann. physik. Chem.*, *11*, 70-103 (1880).

$$R_m = \frac{n^2 - 1}{n^2 + 1} \cdot \frac{M}{d}$$

in which R_m is the molecular refractivity, n is the refractive index, M is the molecular weight, and d is the density.

The relationship of chain length to molecular refractivity is illustrated by the fact that the values obtained by application of the Lorentz-Lorenz formula can be almost exactly duplicated by using the equation:

$$R_m = 4.654n + 3.83$$

where n represents the number of carbon atoms. These data are recorded in Table 37.

TABLE 37

VALUES FOR MOLECULAR REFRACTIVITY (R_m) AT 80°C. CALCULATED FROM REFRACTIVE INDEX OR THEORETICAL FORMULA BASED ON NUMBER OF CARBON ATOMS^a

Name of acid	No. of C Atoms	R_m calcd. from L.-L. formula	R_m calcd. from $4.654n + 3.83^b$
Caproic	6	31.70	31.75
Heptanoic	7	36.34	36.40
Caprylic	8	41.08	41.06
Pelargonic	9	45.66	45.71
Capric	10	50.36	50.37
Undecanoic	11	55.02	55.02
Lauric	12	59.73	59.68
Tridecanoic	13	64.35	64.33
Myristic	14	69.00	68.99
Pentadecanoic	15	73.65	73.64
Palmitic	16	78.30	78.30
Margaric	17	80.01	82.95
Stearic	18	87.59	87.61

^a Adapted from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 390.

^b n refers to number of carbon atoms.

Although the calculated and the measured values of the molecular refractivity of the saturated acids are practically identical, this agreement does not obtain in the case of some of the polyethenoid acids, especially the elaeostearic acids. This divergence, although also present in the refractive index and density, is magnified in the molecular refractivity. It is referred to as *molecular exaltation*, or *EM*, and is believed to be largely attributable to the effect of conjugation.^{4,361,366}

c. Surface Tension and Interfacial Tension. The surface tension is another property of the fatty acids which is related to the density and molecular weights by the equation:

$$P = M \gamma^{1/3} / (D - d)$$

³⁶⁶ W. C. Smit, *Rec. trav. chim.*, 49, 539-551 (1930).

in which M is the molecular weight, γ is the surface tension in dynes per square centimeter, D is the density in the liquid state, and d the density in the vapor state. This calculation gives the value of P , which is the parachor. Since the value of the parachor does not vary with temperature, it provides a simple means of expressing the relationships between surface tension, density, and molecular weight.

TABLE 38

SURFACE TENSION, INTERFACIAL TENSION AND PARACHOR OF SOME FATTY ACIDS AND THEIR ESTERS^a

Compound	Surface tension against air, dynes/cm. ^b	Interfacial tension against water, dynes/cm. ^b	Parachor ^c
Heptanoic acid.....	28.31 ²⁰	7.00 ²⁰	—
Caprylic acid.....	28.82 ^{18.1}	8.22 ^{18.1}	—
Undecanoic acid.....	30.64 ²⁵	10.14 ²⁵	—
Lauric acid.....	—	—	522.5
Palmitic acid.....	—	—	668
Stearic acid.....	26.99 ³⁰	—	777.79
	26.42 ³⁵	—	778.87
Behenic acid.....	37.77 ³⁰	—	950.07
	37.61 ³⁵	—	951.36
Oleic acid.....	32.50 ²⁰	15.59 ²⁰	—
	27.94 ³⁰	—	765.07
	27.52 ³⁵	—	766.93
Elaidic acid.....	26.56 ³⁰	—	760.44
	26.31 ³⁵	—	764.93
Erucic acid.....	28.56 ³⁰	—	932.40
	27.77 ³⁵	—	938.91
Brassicidic acid.....	27.40 ³⁰	—	929.20
	27.28 ³⁵	—	934.36
Ricinoleic acid.....	35.81 ¹⁶	14.25 ¹⁶	—
Ethyl caproate.....	25.81 ²⁰	19.80 ²⁰	—
Ethyl nonanoate.....	28.04 ²⁰	23.88 ²⁰	—
Ethyl oleate.....	—	21.34 ²⁰	—

^a Data are assembled from K. S. Markley, *Fatty Acids*, Interscience, New York, 1947. E. L. Lederer, *Seifensieder-Ztg.*, 57, 575-576 (1930); *Chem. Abst.*, 24, 5549 (1930). G. B. Semeria and G. Ribotti-Lissone, *Gazz. chim. ital.*, 60, II, 862-866 (1930); *Chem. Abst.*, 25, 1800 (1931).

^b The superscripts are the temperatures at which the determinations were made.

^c Although the parachor is independent of temperature, the calculated values relate to the temperatures at which surface tension was determined.

Surface tension may be defined as a measure of the attractive forces of molecular origin which tend to keep a liquid together. They are exerted only over very short distances. During evaporation of a liquid, these attractive forces must be overcome; the heat required to produce evaporation is considered to be a measure of such a force.

As a result of surface tension, drops of a liquid tend to contract to a form

which has the smallest volume. The drops therefore assume a spherical shape, which has the minimum surface area. Surface tension is thus responsible for the formation of raindrops, the production of round lead shot when molten lead is dropped through air, for the rounding of a glass tube on fusion, and for many similar phenomena.

The surface tension of liquids can be easily measured by such standard procedures as the capillary tube method, the determination of drop weight, and the method of maximum pull on a ring. The latter procedure can be carried out quite simply by the use of the du Nouy tensiometer, which permits the determination of surface tension at a liquid-air interface or of interfacial tension at a liquid-liquid junction. The surface tension, interfacial tension, and parachors of some acids and esters are reported in Table 38.

Surface tension decreases with increasing temperature, reaching a value of zero at the critical temperature. Through a large part of the temperature range, it has been found that the relation between surface tension and temperature is a linear one. The experimentally determined values of surface tension, and especially of interfacial tension, are markedly influenced by the presence of impurities. Data on the effect of fatty acids and soaps on surface tension have been assembled by McBain.³⁶⁷ The reader is referred to Sugden³⁶⁸ for a more detailed discussion of surface tension, especially with regard to the use of the parachor.

d. Viscosity. Viscosity is the resistance which a liquid offers to change in shape. While a liquid offers no permanent resistance to forces tending to change its shape, the rates at which the change is accomplished vary and are, in fact, measures of its internal friction or viscosity.

Some liquids, like glycerol or oleic acid, have a higher viscosity than water. They flow down an inclined surface or through a tube at a much slower rate than is the case with water. Such a movement consists in a continuous change in shape for each part of the liquid.

The unit for expressing viscosity is the *poise*. When one layer of a fluid exerts a tangential force upon another layer equal to one dyne, and causes a tangential velocity of one centimeter per second, the viscosity is one poise. In general, viscosity is determined by comparison with water. The absolute viscosity of water at 20.20°C. is 0.01 poise or 1.000 *centipoise*.⁴ The latter unit is more frequently used in expressing the viscosities of fatty acids.

The viscosities of the fatty acids have been determined by Dunstan³⁶⁹ and Deffet.³⁷⁰ Some of these values are recorded in Table 39.

³⁶⁷ J. W. McBain, "Properties of Soaps and Their Aqueous Solutions," in *International Critical Tables*, Vol. V, McGraw-Hill, New York, 1929, pp. 446-460.

³⁶⁸ S. Sugden, *The Parachor and Valency*, Routledge, London, 1930.

³⁶⁹ A. E. Dunstan, *J. Chem. Soc.*, 107, 667-672 (1915).

³⁷⁰ L. Deffet, *Bull. soc. chim. Belg.*, 40, 385-402 (1931).

TABLE 39
 VISCOSITIES OF SATURATED FATTY ACIDS AT SEVERAL TEMPERATURES^a

Acid	Viscosity, centipoises						
	15°	20°	25°	30°	50°	60°	70°C.
Formic	—	1.782	—	—	—	—	—
Acetic	—	1.219	—	—	—	—	—
Propionic	—	1.099	—	0.956	—	—	—
Butyric	—	1.538	—	1.301	—	—	—
Valeric	—	2.30	—	—	—	—	—
Caproic	—	3.23	2.84	—	—	—	—
Heptanoic	4.766	4.33	3.80	3.30	—	—	—
Caprylic	—	5.74	—	4.69	2.62	—	—
Nonanoic	9.66	8.08	7.00	6.11	3.79	—	—
Capric	—	—	—	—	4.34	—	2.88
Undecanoic	—	—	—	—	7.30	—	—
Lauric	—	—	—	—	7.3	—	4.43
Myristic	—	—	—	—	—	7.43	5.83
Palmitic	—	—	—	—	—	—	7.8
Stearic	—	—	—	—	—	—	9.87

^a Data from A. E. Dunstan, *J. Chem. Soc.*, 107, 667-672 (1915) and L. Deffet, *Bull. soc. chim. Belg.*, 40, 385-402 (1931).

The viscosity increases with prolongation of the fatty acid chain. In long-chain compounds, it greatly exceeds that of substances which possess a ring or a compact structure. Viscosity is decreased by increasing temperature, but the change is not a linear one. However, it has been de-

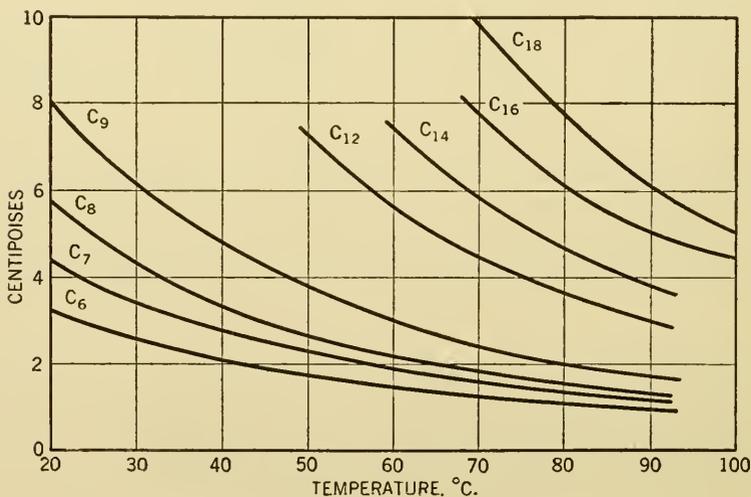


Fig. 23. Temperature-viscosity relationships for saturated acids from C₆ to C₁₈.

monstrated by Dunstan and co-workers^{369,371,372} that the curves of the logarithms of the viscosities and molecular weights are approximately linear for the normal fatty acids and for certain of the alkyl esters, including the methyl and ethyl esters. The values for formic and acetic acids, however, are atypical. The temperature-viscosity relationships are plotted in Figure 23.

e. Boiling Points of Fatty Acids. The analysis of mixtures of fatty acids by fractional distillation is only possible because of differences in vapor pressure and boiling point. Pool and Ralston²¹⁴ have determined the boiling points of the saturated fatty acids from C₆ to C₁₈ over a wide range of barometric pressures from 1 to 760 mm. The vapor pressure-temperature curves are given in Figure 2. Table 40, on page 110, gives the boiling points of the saturated fatty acids at varying pressures.

The boiling point of the saturated fatty acids increases with greater chain length. The increment declines slightly as the number of carbon atoms is increased. Boiling point is a non-alternating property in contradistinction to the alternation observed in the melting points. The higher members of the series decompose on boiling at ordinary pressures, but can be distilled at reduced pressures without destruction. Oleic acid has been observed to boil at the following temperatures (°C.): 1.2 mm., 200°; 5.0, 215°; 10, 225°; 15, 234°³⁷³; 29.5, 250°; and 49, 264°.³⁷⁴ The following are the boiling points (°C.) reported⁴ for some other unsaturated acids: 9-decenoic, 142° (4 mm.); erucic, 281° (30 mm.); chaulmoogric, 248° (20 mm.); and elaeostearic, 235° (12 mm.).

The methyl esters of the fatty acids are also readily distillable. In general, they boil at a considerably lower temperature than do the corresponding acids. Table 41 gives data on the boiling points of methyl esters of both saturated and unsaturated acids.

4. Chemical Properties of Fatty Acids and Related Compounds

The fatty acids are not the inactive compounds that they were formerly supposed to be, but undergo many reactions of considerable importance commercially. Many of these reactions involve the carboxyl group. They may be either ionic in nature or they may be concerned with ester formation. Any one of several types of interesterification also falls into the latter category. Other reactions of the carboxyl group result in the synthesis of various derivatives such as aldehydes, alcohols, amides, nitriles, amines, and acid chlorides.

³⁷¹ A. E. Dunstan, T. P. Hilditch, and F. B. Thole, *J. Chem. Soc.*, 103, 133-144 (1913)

³⁷² A. E. Dunstan, F. B. Thole, and P. Benson, *J. Chem. Soc.*, 105, 782-795 (1914).

³⁷³ J. B. Brown and G. Y. Shinowara, *J. Am. Chem. Soc.*, 59, 6-8 (1937).

³⁷⁴ E. L. Lederer, *Seifensieder-Ztg.*, 57, 67-71 (1930); cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 172.

TABLE 40
BOILING POINTS OF *n*-ALKYL FATTY ACIDS AT SEVERAL PRESSURES^a

No. of C atoms	Boiling points (°C.) at following pressures (mm. Hg.)										
	1	2	4	8	16	32	64	128	256	512	760
6	61.7	71.9	82.8	94.6	107.3	120.8	136.0	152.5	171.5	192.5	205.8
7	74.9	85.3	96.3	108.3	121.1	135.2	150.8	168.2	187.5	209.3	223.0
8	87.5	97.9	109.1	121.3	134.6	149.2	165.3	183.3	203.0	225.6	239.7
9	98.9	109.6	121.2	134.0	147.5	162.4	178.8	196.9	217.4	240.9	255.6
10	110.3	121.1	132.7	145.5	159.4	174.6	191.3	209.8	230.6	254.9	270.0
11	119.8	131.1	143.3	156.5	170.8	186.1	203.1	222.2	243.8	268.7	284.0
12	130.2	141.8	154.1	167.4	181.8	197.4	214.6	234.3	256.6	282.5	298.9
13	139.9	151.5	164.2	177.8	192.2	207.9	225.8	245.9	268.6	295.4	321.4
14	149.2	161.1	173.9	187.6	202.4	218.3	236.3	257.3	281.5	309.0	326.2 ^b
15	157.8	169.7	182.8	196.8	212.0	228.1	246.4	268.0	292.7	321.2	339.1 ^b
16	167.4	179.0	192.2	206.1	221.5	238.4	257.1	278.7	303.6	332.6 ^b	351.5 ^b
17	175.1	187.6	200.8	214.9	230.7	247.9	266.6	288.4	314.3	343.8 ^b	363.8 ^b
18	183.6	195.9	209.2	224.1	240.0	257.1	276.8	299.7	324.8	355.2 ^b	376.1 ^b

^a W. O. Pool and A. W. Ralston, *Ind. Eng. Chem.*, 24, 1104-1105 (1942).

^b By interpolation.

TABLE 41
 BOILING POINTS OF METHYL ESTERS OF SATURATED AND UNSATURATED ACIDS

Methyl	Boiling points (°C.) at following pressures (mm. Hg)									
	1	2	4	6	8	10	12	20	40	
Caproate	—	15 ^a	26 ^a	33 ^a	38 ^a	42 ^a	—	55 ^a	70 ^a	
Caprylate	—	45 ^a	58 ^a	65 ^a	71 ^a	76 ^a	—	89 ^a	106 ^a	
Caprate	—	77 ^a	89 ^a	97 ^a	103 ^a	108 ^a	—	123 ^a	139 ^a	
Laurate	—	100 ^a	113 ^a	121 ^a	128 ^a	134 ^a	—	149 ^a	166 ^a	
Myristate	114 ^b	127 ^a , 125 ^b	141 ^a	150 ^c	157 ^a	162 ^a , 157.5 ^b	—	177 ^a , 172.5 ^b	197 ^a	
Palmitate	136 ^b	148 ^a , 149 ^b	162 ^a	172 ^a	177 ^a	184 ^a , 180.5 ^b	—	202 ^a , 196.5 ^b	c	
Stearate	155.5 ^b	166 ^a , 170 ^b	181 ^a	191 ^a	199 ^a	204 ^a , 204.5 ^b	—	c, 222 ^b	c	
Arachidonate	160-165 ^d	—	—	—	—	—	—	—	—	
Oleate	152.5 ^b	166.5 ^b	182	192 ^a	201 ^b , 199.5 ^a	205.3 ^a	—	218.5 ^b	—	
Linoleate	149.5 ^b	166.5 ^a	182.4 ^a	193.0 ^a	199.9 ^a	206.0 ^a , 198 ^b	—	215 ^b	—	
Linolenate ^e	—	—	184	198	—	—	—	—	—	
9-Decenoic	—	—	—	—	—	—	115-116 ^d	—	—	
6-Tetradecenoic	110-111 ^d	—	—	—	—	—	—	—	—	
Myristoleic	108-109 ^d	—	—	—	—	—	—	—	—	
Palmitoleic	134-135 ^d	—	—	—	—	—	—	—	—	
Elaidic	150 ^d	—	—	—	—	—	—	—	—	
Erucic	169-170 ^d	—	—	—	—	—	—	—	—	
Ricinoleic	—	—	—	—	—	225 ^d	—	—	—	
Hydnocarpic	—	—	—	—	—	—	—	203 ^d	—	
Chaulmoogric	—	—	—	—	—	—	—	227 ^d	—	
Gorlic	—	—	—	—	—	—	219 ^d	—	—	
Clupanodonic	170-175 ^d	—	—	—	—	—	—	—	—	

^a P. M. Althouse and H. O. Triebold, *Ind. Eng. Chem., Anal. Ed.*, 16, 605-606 (1944).

^b F. A. Norris and D. E. Terry, *Oil & Soap*, 22, 41-46 (1945).

^c Decomposes.

^d K. S. Markley, *Fatty Acids*, Interscience, New York, 1948, p. 174.

^e Ethyl ester. J. W. McCutcheon, *Can. J. Research*, B18, 231-239 (1940).

In addition to the chemical reactions which are concerned only with the carboxyl group, the unsaturated acids readily add halogens or hydrogen to form saturated compounds. Furthermore, they are oxidizable at their unsaturated linkages to yield hydroxy-acids, oxides, or peroxides. Under conditions of strenuous oxidation, such oxidation products may be split between the carbons which constituted the original double bond, to form short-chain acids or aldehyde acids.

(1) *Reactions Involving Carboxyl Group*

a. Ionic Reaction with Production of Soaps. The fatty acids readily react to form salts wherein the hydrogen of the carboxyl is replaced by a metal. Such metallic salts of the higher fatty acids are referred to as soaps. All metallic salts of the fatty acid are included in this category, irrespective of whether the resulting compounds are soluble or insoluble in water. The soaps most widely known are the sodium and potassium forms; however, there is a wide variety of other metallic soaps which are of importance in industry and in our everyday life.

Soaps are prepared by any one of several methods. The simplest is by the reaction of a base on the free fatty acid. A second method involves double decomposition by mixing solutions of soluble soap and soluble metallic salt, with the resultant precipitation of the insoluble soap. The third procedure commonly employed involves the heating of the triglyceride or other ester with a suitable amount of the metallic oxide or hydroxide. Caustic soda (sodium hydroxide) or potash (potassium hydroxide) are the alkalis most frequently used; for the preparation of special products such as calcium or barium soaps, calcium or barium hydroxide is substituted for the commoner alkalis.

There are various special soaps which have specific uses. The soaps which are used for driers in paints, varnishes, enamels, and other protective coverings are chiefly the lead, manganese, cobalt, zinc, or calcium compounds. These can be made by the reaction of the oxides or salts on linseed oil with the application of heat or, preferably, by precipitation of the soluble soaps of linseed oil.

A second application of the special soaps is in the manufacture of greases. The usual greases consist of a liquid phase of mineral oil or vegetable oil which is mixed with a dispersed or gel phase, generally consisting of a soap. Calcium stearate or oleate is frequently used in cup greases, while aluminum oleate, lead salts of oleic, stearic, iso-oleic or erucic acids, zinc stearate or oleate, and barium, magnesium, chromium, iron, cobalt, or nickel soaps find special application in certain lubricants. Lithium stearate has found some use in lubricants for aircraft engines. An extensive treatise on the manufacture of greases has been written by Klemgard.³⁷⁵

³⁷⁵ E. N. Klemgard, *Lubricating Greases: Their Manufacture and Use*, Reinhold, New York, 1937.

Certain soaps are also important as catalysts in the manufacture of mono- and diglycerides. They are likewise used to accelerate interesterification reactions. Lead and chromium soaps serve as catalysts in the reduction of the fatty acids to alcohol. Sodium soaps have been used as emulsifiers in the manufacture of synthetic rubber. Stearate and oleate have been widely employed in the manufacture of pharmaceutical preparations for the skin, in which they act as antiseptics and astringents.

b. Reactions Involving Esterification. Esters are compounds which yield an alcohol and an acid on hydrolysis. The esters of chief importance to the fat chemist are those in which the acid component is an organic acid. The principal organic acids involved in the animal kingdom are the fatty acids. Conversely, the fatty acids occur in nature almost exclusively in the form of esters.

Until recently esters have been regarded as compounds analogous to soaps, where the hydrogen of the carboxyl is replaced by an alkyl group instead of by a metal. On this basis they have frequently been referred to as alkyl salts of the fatty acids. However, the newer views of esterification have postulated that the esters are formed by replacement of the hydroxyl radical of the carboxyl by an alkoxy group of the alcohol.

(a) *Classification of Esters.* There are a large number of theoretically possible esters, but the types of compounds fall into relatively few groups. All of the *n*-fatty acid series, the unsaturated acids, and the substituted fatty acids are usually found in nature in the form of esters. The more useful classification of these compounds is based upon their alcohol component. Thus, the following types of alcohols are present in ester combination:

1. *Monohydric or Monatomic* alcohols, which have an alkyl chain and a hydroxyl group. Since the aldehydes can be formed from the fatty acids in the animal body, it is probable that the corresponding alcohols may also originate here. It is not surprising that counterparts of most of the fatty acids are represented in the biologically distributed alcohols. The monohydric alcohols may be further subdivided: (1) *Primary* alcohols ($R \cdot CH_2OH$), in which the hydroxyl group is attached to the terminal carbon.

(2) *Secondary* alcohols $\left(\begin{array}{l} R' \\ \diagdown \\ C \\ \diagup \\ R'' \end{array} \right) CHO$, in which the carbon to which the hydroxyl is attached is joined to two

other carbon atoms. (3) *Tertiary* alcohols $\left(\begin{array}{l} R' \\ \diagdown \\ C \\ \diagup \\ R''' \end{array} \right) CHO$, in which the carbon to

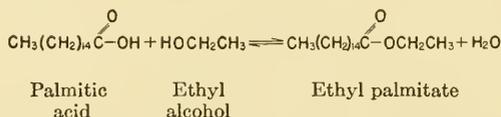
which the hydroxyl is attached is joined to three other carbon atoms.

2. *Polyhydric* alcohols having two or more alcohol groups in the chain: (1) *Esters of the dihydric alcohol, glycol* ($CH_2OH \cdot CH_2OH$) and its homologues. Such esters would contain two fatty acid residues. (2) *Esters of the trihydric alcohol, glycerol* ($CH_2OH \cdot CHOH \cdot CH_2OH$). Such esters normally contain three fatty acid residues, but monoglycerides (with one acid) and diglycerides (with two acids) are well known and are produced in large amounts commercially. The fatty acids in nature are largely found in the form of triglycerides (see Chapter III). (3) *Esters of the tetrahydric alcohol, eryth-*

ritol ($\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CH}_2\text{OH}$). When completely esterified, these esters will contain four fatty acids. (4) *Esters of polyhydric alcohols having more than four hydroxyl groups.* Esters of the hexitols, mannitol, and sorbitol, have come into prominence within recent years, especially since the commercial production of these hexitols has made it possible to supply them at low cost.³⁷⁶ The sorbitol esters have found wide application as emulsifying agents.

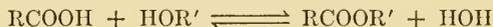
(b) *Preparation of Esters.* There are a number of general methods which may be used for the synthesis of the esters. These are listed below.

a'. *Reacting Acid and Alcohol in the Presence of a Catalyst:* This is exemplified in the reaction of ethanol and palmitic acid to form ethyl palmitate and water:



The reaction proceeds when the concentrated alcohol and the concentrated acid are heated together in the presence of an inorganic acid which acts as a catalyst. Anhydrous sulfuric or hydrochloric acid is generally employed in a concentration of 3 to 5%. Hilditch¹ has suggested a concentration of 2% of sulfuric acid and about twice the weight of methyl alcohol as of the fatty acids. This should give a yield of 97–98%. Dean³⁷⁷ has recommended 4% of sulfuric acid with 2 to 3 times the weight of methanol as of the fatty acids. Methyl alcohol saturated with dry hydrochloric acid gas before or after mixing with the acid but before refluxing has been employed by Cruz and West.³⁷⁸

The esterification reaction is a reversible one and is a classical example of the law of mass action. If one of the products of reaction is removed or allowed to escape, the same equilibrium is maintained, irrespective of whether one starts with a mixture of alcohol and fatty acid or with one of ester and water. The *equilibrium constant*, K , for the reversible reaction:



can be calculated from the proportions in gram moles per liter of the reactants at equilibrium, by the application of the following formula:

$$K = \frac{k}{k'} = \frac{[\text{RCOOR}'][\text{HOH}]}{[\text{RCOOH}][\text{HOR}]}$$

where k and k' represent the velocity constants for the esterification and hydrolysis reactions, respectively.

When K is large, the proportion of the ester (RCOOR') to water will be high for the synthetic reaction. Under such conditions the amount of

³⁷⁶ H. J. Creighton, *Trans. Electrochem. Soc.*, 75, 289–307 (1939).

³⁷⁷ H. K. Dean, *Utilization of Fats*, Chem. Pub. Co., Brooklyn, 1938, p. 75.

³⁷⁸ A. O. Cruz and A. P. West, *Philippine J. Sci.*, 48, 77–88 (1932).

hydrolysis which results when one starts with the ester is minor. On the other hand, when K is small, the reverse will be true.

A number of considerations alter the point of equilibrium. The first factor is the proportion of reactants. If one starts with equal molecular amounts of acetic acid and ethyl alcohol, equilibrium is reached when 66.7% of the potential yield of ester is obtained. This can be increased to 82% when 2 parts of alcohol are used to 1 part of acetic acid. Further excess of alcohol does not markedly increase the yield, and it cannot be rendered quantitative by this procedure. On the other hand, esterification can be made largely complete with an excess of alcohol in the acid-catalyzed reactions. Similarly, hydrolysis of the esters to alcohol and acid is rendered almost quantitative when they are acid-catalyzed and when an excess of water is employed.

A second factor which alters the extent of esterification is the prevention of accumulation of the reaction products. Thus, when high molecular weight alcohols are used, the water separates as a layer and can be removed, thus increasing the yield of ester. The addition of calcium chloride or other dehydrating agents will have the same effect in augmenting the yield of ester.

Another condition which might be expected to alter the speed and equilibrium constant is temperature. However, since the heat evolved in esterification reactions is relatively low, the resulting effect of temperature on the equilibrium constant is minor. The esterification reaction is, however, speeded up when agitation is employed.

Probably the most important consideration in the equilibrium and velocity constants is the nature of the reacting components.^{295, 379-392} Menschutkin³⁹³⁻³⁹⁷ and others have shown that primary alcohols are most

³⁷⁹ W. Kistiakowsky, *Z. physik. Chem.*, *27*, 250-266 (1898).

³⁸⁰ V. Meyer, *Ber.*, *27*, 510-512 (1894).

³⁸¹ V. Meyer and J. J. Sudborough, *Ber.*, *27*, 1580-1592, 3146-3153 (1894).

³⁸² J. J. Sudborough and L. L. Lloyd, *J. Chem. Soc.*, *75*, 467-483 (1899).

³⁸³ W. A. Bone, J. J. Sudborough, and G. H. G. Sprankling, *J. Chem. Soc.*, *85*, 534-555 (1904).

³⁸⁴ J. J. Sudborough and E. R. Thomas, *J. Chem. Soc.*, *91*, 1033-1036 (1907).

³⁸⁵ J. J. Sudborough and J. M. Gittins, *J. Chem. Soc.*, *93*, 210-217 (1908).

³⁸⁶ J. J. Sudborough and M. K. Turner, *J. Chem. Soc.*, *101*, 237-240 (1912).

³⁸⁷ E. R. Thomas and J. J. Sudborough, *J. Chem. Soc.*, *101*, 317-328 (1912).

³⁸⁸ H. Goldschmidt and O. Udby, *Z. physik. Chem.*, *60*, 728-755 (1907).

³⁸⁹ H. Goldschmidt and A. Thuesen, *Z. physik. Chem.*, *81*, 30-67 (1912).

³⁹⁰ C. N. Hinshelwood and A. R. Legard, *J. Chem. Soc.*, *1935*, 587-596.

³⁹¹ A. T. Williamson and C. N. Hinshelwood, *Trans. Faraday Soc.*, *30*, 1145-1149 (1934).

³⁹² R. A. Fairclough and C. N. Hinshelwood, *J. Chem. Soc.*, *1939*, 593-600.

³⁹³ N. Menschutkin, *Ann.*, *195*, 334-364 (1879).

³⁹⁴ N. Menschutkin, *Ann.*, *197*, 193-225 (1879).

³⁹⁵ N. Menschutkin, *Ann. chim. phys.* [5], *20*, 289-361 (1880).

³⁹⁶ N. Menschutkin, *Ann. chim. phys.* [5], *23*, 14-85 (1881).

³⁹⁷ N. Menschutkin, *Ann. chim. phys.* [5], *30*, 81-144 (1883).

reactive, secondary alcohols have less than 50% of the activity of the primary alcohols, while tertiary alcohols present almost no ester formation. In a standardized test in which the alcohol was treated with acetic acid at 155°C. for one hour, the following percentages of esterification were observed by Menshutkin³⁹³⁻³⁹⁷ with primary alcohols: methanol, 55.6; ethanol, 46.9; propanol, 46.9; *n*-butanol, 46.8; and *n*-octyl alcohol, 46.6. In the case of the secondary alcohols under identical conditions, the results were as follows: dimethylcarbinol, 26.5; methylethylcarbinol, 22.6; methylhexylcarbinol, 21.2; methylisopropylcarbinol, 18.9; and diethylcarbinol, 16.9. The values for ester formation obtained with the tertiary alcohols under the same conditions were: trimethylcarbinol, 1.4; dimethylethylcarbinol, 0.8; methyldiethylcarbinol, 1.0; dimethylpropylcarbinol, 2.1; and dimethylisopropylcarbinol, 0.9. In the absence of an added catalyst, equilibrium was reached with primary alcohols at 65-70% of ester formed, with secondary alcohols at 50-60%, and with tertiary alcohols at only 5% completion.

The nature of the acid component has a corresponding effect on the esterification reaction. However, in the tests with different fatty acids, the results are less clear-cut, since the rate of esterification without added catalyst is a function of the amount of the acid plus the effect of the fatty acid as a catalyst. In spite of this difficulty, marked variations between different acids have been noted. The rate of reaction is lowered as the length of the aliphatic chain increases, and especially in relation to the extent to which the fatty-acid chain is branched. Menshutkin,^{4,393-397} has reported that ester is formed in the following percentages when several acids are treated for one hour with isobutanol at 155°C.: formic, 61.7; acetic, 44.4; propionic, 41.2; butyric, 33.3; caprylic, 30.9; isobutyric, 29.0; α -methylacetic, 21.5; trimethylacetic, 8.3; and dimethylacetic, 3.5.

The speed of reaction between methanol or ethanol and a large number of fatty acids has been reported by the Sudborough group.³⁸¹⁻³⁸⁷ When these values were recalculated by Skrabal³⁹⁸ on the basis of $k_{\text{MeOH}}^{15^\circ}$ using HCl as one, the following were the results with the aliphatic acids: formic, 2568; acetic, 239; propionic, 211.7; butyric, 115.2; valeric, 123.2; caproic, 118.7; heptanoic, 120.9; caprylic, 125.8; nonanoic, 123.5; capric, 119.3; lauric, 121.9; myristic, 120.9; palmitic, 114.4; and stearic, 123.7. The value for k was shown to be reduced markedly in a series of monoenoid acids, particularly when the point of unsaturation was approximate to that of the carboxyl group. The values for k of the several mono-unsaturated acids investigated were as follows: undecenoic, 53.0; oleic,

³⁹⁸ A. Skrabal, "Chemical Kinetics," in *International Critical Tables*, Vol. VII, McGraw-Hill, pp. 113-152, New York, 1938, p. 138.

54.4; elaidic, 54.4; erucic, 51.2; brassidic, 51.8; and $\Delta^{2,3}$ -oleic, 1.3. It is apparent that the *cis* forms (oleic and erucic) give identical results with those of their *trans* forms (elaidic and brassidic).

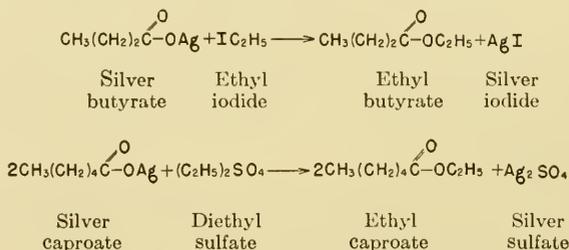
The extent to which esterification takes place is also influenced by the number of hydroxyl groups in the alcohol. When more than one alcohol group is present, there is the possibility of several types of ester, *i.e.*, one in which the alcohol groups are all esterified, and another in which only partial esterification is obtained. The number of such partially esterified compounds increases progressively with the increase in the number of alcohol groups. Menschutkin³⁹⁶ reported that, when the mole ratio of acid to alcohol groups was kept constant, the percentage of the ester at equilibrium became progressively lower as the number of alcohol groups was increased. Thus, the percentage values at equilibrium when acetic acid was allowed to react at 155°C., without an acid catalyst, with ethanol, or with the several polyhydric alcohols, were as follows: ethanol (1 OH group), 66.6; ethylene glycol (2), 53.9; glycerol (3), 46.0; erythritol (4), 40.1; and mannitol (6), 26.4. However, when one mole of acid and one mole of alcohol were reacted, the equilibrium values were all practically the same, falling between 62 and 70%. Esterification of the acids with the polyhydric alcohols is controlled by the same factors as is that with the monohydric alcohols. Thus, they are catalyzed by inorganic acids, and esterification is promoted by heat, by the removal of water, and by agitation. The extent of esterification of the polyhydric alcohols is adversely affected by isomerism (branched chains) as it is in the case of the alcohols having a single hydroxyl group.

On the other hand, the esterification reaction with the polyhydric alcohols differs from that between the acids and monohydric alcohols by the fact that it is not promoted by increase in the concentration of the alcohol. The net effect of having a greater proportion of polyhydric alcohols to the acids is to increase the number of partially esterified molecules rather than to augment the proportion of completely esterified molecules. This occurs because certain of the hydroxyl groups of the polyhydric alcohols are preferentially attacked; with a larger number of molecules available, only the most susceptible hydroxyl radicals react, and a larger proportion of partially esterified molecules is produced. Practical application of this procedure is made in the manufacture of mono- and diglycerides (see Chapter III).

b'. Alcoholysis as a Method for the Synthesis of Esters: Ester interchange with the triglycerides by the use of an excess of alcohol is a simple method for preparing esters, since it does not require the intermediate separation of the acids. However, it has the disadvantage that one seldom obtains pure esters but rather mixtures, inasmuch as the natural triglycerides are usually mixed triglycerides.

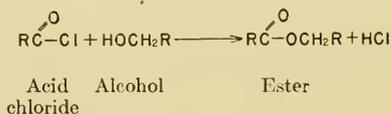
As early as 1906, Haller³⁹⁹ demonstrated that the methyl esters of fatty acids and glycerol are formed when the fats are heated with an excess of methanol to which 1–2% of hydrochloric acid has been added. The low molecular weight triglycerides such as are present in coconut oil⁴⁰⁰ react most readily, since they are more soluble in alcohol than the longer chain triglycerides; castor oil is easily reactive because ricinoleic acid dissolves in alcohol.⁴⁰¹ Although hydrochloric acid is generally used as a catalyst, phenylsulfonic acid is also satisfactory.⁴⁰² The reaction proceeds when alkali hydroxides are used as catalysts,⁴⁰³ or when the reaction is carried out under pressure.⁴⁰⁴ In the case of ethanolsis, the ester interchange has been shown to be a stepwise reaction, since it has been demonstrated that di- and monoglycerides are intermediate products.⁴⁰⁵ Thus, from the partial alcoholysis of 3000 g. of tristearin in the presence of hydrochloric acid, Grün *et al.*⁴⁰⁵ were able to separate 400 g. of unchanged triglyceride, 300 g. of distearin, 200 g. of monostearin, and 1200 g. of ethyl stearate. This process is closely related to interesterification, which is described more thoroughly in Chapter III.

c'. Reaction of Alkyl Halides (or Dialkyl Sulfates) with the Acids or Their Salts: Typical reactions are given below:



These reactions are classical methods for the preparation of all of the esters. They have also been used to establish the structure of the esters.

d'. Reaction of Acid Halides and Alcohol: The acid chlorides readily react with alcohols to form esters according to the following reaction:



³⁹⁹ A. Haller, *Compt. rend.*, 143, 657–661 (1906).

⁴⁰⁰ G. D. Elsdon, *Analyst*, 38, 8–11 (1913).

⁴⁰¹ A. Haller, *Compt. rend.*, 144, 462–466 (1907).

⁴⁰² A. Haller and Youssoufian, *Compt. rend.*, 143, 803–806 (1906).

⁴⁰³ Y. Toyama and T. Tsuchiya, *J. Soc. Chem. Ind. Japan*, 36, suppl., 232–233B (1933).

⁴⁰⁴ V. L. Hansley, *U. S. Patent* No. 2,177,407 (Oct. 24, 1939).

⁴⁰⁵ A. Grün, F. Wittka, and E. Kunze, *Chem. Umschau*, 24, 15–16, 31–34 (1917); *Chem. Abst.*, 13, 2768 (1919).

e'. Prelog and Piantanida Method:⁴⁰⁶ This procedure involves the decomposition by heat of the tetramethylammonium salt. It is most useful with the polyterpenoid acids and sapogenins, such as oleanolic and related acids, which are stable at the temperature required for the decomposition of the tetramethylammonium salt. It is not a satisfactory procedure for the esters of *n*-aliphatic acids.

Glycol esters were prepared as early as 1859 by Wurtz,⁴⁰⁷ while Berthelot⁴⁰⁸ synthesized some of the esters of erythritol, dulcitol, mannitol, and glucose 4 years earlier. The synthesis of triglycerides by esterification of fatty acids with glycerol antedated both of these discoveries by a decade.⁴⁰⁹ A description of esterification methods insofar as they are related to glycerol is given in Chapter III. A comprehensive review of the higher fatty acid esters of the polyhydric alcohols, which includes a discussion of preparation, properties, and industrial applications, was prepared by Goldsmith.⁴¹⁰

(c) *Properties of Esters.* a'. Monoesters: The esters are, in general, neutral substances. On standing, they may slowly hydrolyze to their acid and alcohol components. The rate of hydrolysis is greatly accelerated by an increase in temperature in the presence of moisture, by the presence of an inorganic catalyst, or an enzyme, or by the action of alkalis. The splitting of the esters of low molecular weight takes place much more readily than in the case of those composed of longer carbon chains.

The monoesters are relatively stable toward heat, and most of them can be distilled without decomposition. For this reason, fatty acid mixtures are usually converted to their methyl esters for separation and identification. The melting and boiling points of the methyl and ethyl esters are lower than those of the corresponding acids. However, when the length of the aliphatic chain in the alcohol is increased, the melting and boiling points of the esters are gradually increased until they exceed those of the free acids. For example, the several esters of palmitic acid have the following melting points in °C.: methyl, 30.6; ethyl, 25; butyl, 27.5; octyl, 22.5; decyl, 30; dodecyl, 41; tetradecyl, 48; pentadecyl, 55.5; hexadecyl, 56.5; and triacontyl, 72. Palmitic acid melts at 63.1°C.

The solubility of the esters corresponds, to some extent, to those of the fatty acids. However, the one exception is that the esters composed of the short-chain acids and alcohols are insoluble in water, in contradistinction to their component acids and alcohols, which are water-soluble. Esters readily dissolve in most organic solvents and, in fact, they are themselves

⁴⁰⁶ V. Prelog and M. Piantanida, *Z. physiol. Chem.*, **244**, 56-58 (1936).

⁴⁰⁷ A. Wurtz, *Ann. chim. phys.* [3], **55**, 400-478 (1859).

⁴⁰⁸ M. Berthelot, *Compt. rend.*, **41**, 452-456 (1855).

⁴⁰⁹ J. Pelouze and A. Gélis, *Ann. chim. phys.* [3], **10**, 434-456 (1844).

⁴¹⁰ H. A. Goldsmith, *Chem. Revs.*, **33**, 257-349 (1943).

TABLE 42
 MELTING POINTS, BOILING POINTS, DENSITIES, AND REFRACTIVE INDICES OF METHYL AND ETHYL ESTERS OF COMMON FATTY ACIDS^a

Name of acid	Melting pt., °C.			Boiling pt., °C. ^c			d_4^{20}			n_D^{20}		
	Me	Et		Me	Et		Me	Et		Me	Et	
	Valeric	-91.0 ^b	-91.2 ^b		127.3	144.6 ^{7,36}		0.9097 ⁰	0.8765 ²⁰		1.40699 ¹⁵	0.8890 ⁰
Caproic	-71.0 ^b	-67.5 ^b		151.5	166-167		0.90327 ⁰	0.8890 ⁰		1.41334 ¹⁵	0.88622 ⁰	1.41537 ¹⁵
Heptonic	-55.8 ^b	-66.3 ^b		173.8	188.6		0.89802 ⁰	0.88420		1.40669 ¹⁶	0.88420	1.41989 ¹⁵
Caprylic	-34	-43.2 ^b		193-194	208.5		0.89420	0.86920 ¹⁶		1.42415 ¹⁵	0.86920 ¹⁶	1.42415 ¹⁵
Nonanoic		-36.7 ^b		213-214 ⁵⁵	216-219		0.87654 ^{7,5}	0.85693 ³⁰		1.4161 ⁴⁵	0.85693 ³⁰	1.42771 ¹⁶
Capric	-18	-19.9 ^b		224	243-245							
Undecanoic		-14.7 ^b		123 ⁹ -10	140 ³⁰							
Lauric	5	-1.8 ^b		141 ¹⁵	163 ²⁵							
Tridecanoic		-4.8 ^b			163-165 ⁵							
Myristic	18.5	12.3		155-157 ⁷	139 ¹					1.4220 ¹⁵	0.8071 ¹⁹	1.43269 ^{12,9}
Pentadecanoic	18.5	14		199 ³⁰						1.4281 ⁴⁵	0.8573 ²⁵	1.4362 ²⁰
Palmitic	30.55	25.7		184 ¹²						1.4390 ²⁰		1.4278 ⁵⁰
Margaric	29.7	25.7								1.4317 ⁴⁵		
Stearic	39.1	33.9										
Nonadecanoic	39.3	36.1		215 ¹⁵								1.4346 ⁴⁵
Eicosanoic	46.6	41.6			152 ^{0,18}							
Heineicosanoic	47.6	44.5			166-168 ^{0,27}							
Docosanoic	53.3	48.7			177 ^{0,28}							
Tricosanoic	54.4	51.4			184-185 ^{0,20}							
Tetracosanoic	58.4	54.8			198-199 ^{0,27}							
Pentacosanoic	60.0	57.2			198-199 ^{0,24}							
Hexacosanoic	63.4	60.2										
Octacosanoic	67.5	64.6										
Nonacosanoic	68.8	66.6										
Triacosanoic	71.7	68.4										
Dotriacontanoic	74.9	72.5										
Tetratriacontanoic	77.9	75.4										
Hexatriacontanoic	80.9	78.6										
Octatriacontanoic	83.1	80.6										
Hexatriacontanoic	91.0	90.5										

^a Data adapted from A. W. Rabston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, pp. 502, 503.

^b Denotes freezing point.

^c Superior number indicates barometric pressure in millimeters of mercury at which boiling point was determined.

^d Superior number indicates temperature in °C. at which determination was made at *t*.

excellent solvents for various types of lipids, especially the cellulose-type lacquers.

The esters composed of the lower acids and alcohols are liquids which possess a fruity, aromatic odor. They are present as such in the fruits; the synthetic products are widely employed in the manufacture of artificial fruit essences. The esters composed of longer chain acids and alcohols are no longer liquid, but are hard, brittle, lustrous solids, which possess a crystalline structure. These higher molecular weight esters are usually mixtures which are not readily separable into pure compounds. The solid esters of higher molecular weight are known as waxes. They are discussed in Chapter IV. Some esters having an unsaturated acid or alcohol component have been reported to occur in natural plant or animal products.

Table 42 shows the physical constants of the common methyl and ethyl esters, while Table 43 lists the melting and boiling points of the mono- and dimethyl and -ethyl esters of some of the dicarboxylic acids. For more details the reader is referred to the monograph of Ralston.⁵

TABLE 43

MELTING POINTS AND BOILING POINTS OF MONO- AND DIMETHYL AND ETHYL ESTERS OF SOME DICARBOXYLIC ACIDS^a

Dicarboxylic acid	Melting point, °C.				Boiling point, °C. ^c			
	Methyl	Ethyl	Dimethyl	Diethyl	Methyl	Ethyl	Dimethyl	Diethyl
Adipic	3	28-29	8.5	-21.4	162 ¹⁰			130 ¹⁴
Pimelic		10	-20.6 ^b	-23.8 ^b		181-182 ¹⁸	121-122 ¹¹	139-141 ¹⁵
Suberic	10 ^b	21-22	-3.1 ^b	-5.9 ^b	146-150 ¹	186-188.5 ¹⁶	130-131 ⁹	251-253 ²⁰
Azelaic		28-29	-3.9	-18.5		178-179 ^{5,5}		291
Sebacic	40-41	35	26.4	5.1	208 ²⁰	202-203 ¹⁵	175 ²⁰	158-159 ^{7,5}

^a Adapted from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 512.

^b Freezing point.

^c Superior number indicates barometric pressure in millimeters of mercury at which boiling point was determined.

b'. Esters of Polyhydric Alcohols: Most of the esters of the polyhydric alcohols are solids which melt at higher temperatures than is the case with the corresponding monoesters. In the case of ethylene and propylene glycols, the diesters melt at appreciably higher temperatures than do the monoesters. On the other hand, this relationship is completely reversed with glycerol.

The sugars and the sugar alcohols form a wide variety of esters. Glucose esters having 5 fatty acid residues have been prepared, as well as the sucrose octa-ester. Many partially esterified products are known. Probably the most important of the carbohydrate esters are those of cellulose. Such an inorganic ester as cellulose nitrate has been recognized for a long while. This polysaccharide forms esters not only with acetic acid but also

with the higher fatty acids. These esters readily form films which find application in the waterproofing of paper⁴¹¹ or as a protective coating for metals.⁴¹² Mixed esters of cellulose have been prepared in which several different fatty acids make up the acid components.⁴¹² The monoesters of cellulose are insoluble in organic solvents; as the number of fatty acid residues is increased, they become increasingly soluble in organic solvents and

TABLE 44
MELTING POINTS (°C.) OF PALMITATE ESTERS OF VARIOUS POLYHYDRIC ALCOHOLS AND CARBOHYDRATES^a

Polyhydric alcohol	Monoester	Diester	Higher esters	
			No. of fatty acids	Melting pt., °C.
Methylene glycol	—	49.5	—	—
Ethylene glycol	52.5	72	—	—
1,2-Propylene glycol	55-56	69.5-70	—	—
1,3-Propylene glycol	42-43.5	56.5	—	—
1,3-Butylene glycol	—	39-40	—	—
1,4-Butylene glycol	—	63	—	—
Glycerol 1-; 1,3-	77	72.5	3	65
Glycerol 2-; 1,2-	69	64	3	65
Erythritol	—	99.5-101	3	81-88
<i>l</i> -Arabinose	—	—	4	69.5
α -Methyl-D-glucoside	—	—	3	77
“ “ “	—	—	4	69
α -Glucose	—	—	5	72-75
β -Glucose	—	—	5	68-72
Mannitol	—	—	6	64.5
Dulcitol	—	—	6	74
Sucrose	—	—	8	54-55
Raffinose	—	—	11	52-53
Cellulose ^b	180	100	3	80

^a Most of the data are adapted from K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 290.

^b H. Gault and P. Ehrmann, *Caoutchouc & Guttapercha*, 24, 13532-13533, 13603-13605 (1927); *Chem. Abstr.*, 21, 1350, 2794 (1927). Cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 569.

their properties become more similar to those of the fats. Most of the polyesters decompose before melting. On hydrolysis of the esters, cellulose and the fatty acids can be regenerated, indicating that esterification proceeds without injury to the cellulose molecule. The melting points of the cellulose esters are lowered as an increasing number of fatty acids are combined with them.

The amylose component of starch is believed to retain fatty acids by a

⁴¹¹ J. A. Mitchell, *U. S. Patent* No. 2,193,831 (Mar. 19, 1940).

⁴¹² C. R. Fordyce and G. D. Hiatt, *U. S. Patent* No. 2,170,016 (Aug. 22, 1939).

physical adsorption rather than in ester linkage.^{413,414} Since the so-called *fat-by-hydrolysis* represented lipid material in starch which could not be removed by ether or carbon tetrachloride extraction, it was formerly believed to be present in ester linkage. Schoch,⁴¹⁵ however, found that the fat could be removed almost completely by methanol, 80% dioxane, or other hydrophilic fat solvents. This investigator also demonstrated that it was possible to reintroduce fatty acids into defatted starches by treatment with fatty acid solutions; these reintroduced fatty acids were as tightly bound as the true fat-by-hydrolysis. Since it has recently been shown that the presence of fatty acids interferes with the amylose-iodine complex,⁴¹⁶ it is believed that the fatty acid-amylose reaction is similar to that of the iodine-amylose combination. Mikus *et al.*⁴¹⁷ have suggested that the fatty acid retention is not due to a surface phenomenon but rather to a retention in holes of the helices in the amylose structure.

Melting points of the palmitate esters of the polyhydric alcohols are included in Table 44. A description of cholesterol esters is included in Chapter IV, while a discussion of xanthophyll and other carotenol esters is given in Chapter VI.

c. Formation and Preparation of Compounds Related to Fatty Acids but Having Modifications of Carboxyl Group. Some of the changes in the carboxyl group are so minor that the resulting compounds still possess many of the properties associated with the lipids. Thus, the reduction of fatty acids to aldehydes or even to alcohols yields a series of compounds which possess fat-like properties. In many cases these substances are found in nature. The corresponding hydrocarbons in which a complete removal of the oxygen of the fatty acid has occurred are sometimes present in the case of neutral fats, and are, in fact, rather important natural products. The acid anhydrides, likewise, differ little from the fatty acids, and readily revert to them. The amides lack many of the lipid properties, but are of interest because of their widespread distribution in both the plant and the animal kingdoms. The nitriles and acid chlorides are very important derivatives of fatty acids which are of particular interest because of the many reactions into which they enter. Each of these derivatives will be considered in turn and their relationship to fatty acid reviewed. Several other equally important derivatives, such as the amines, the ethers, the sulfur-containing compounds, and the ketones differ sufficiently from the fatty acids to justify their omission here. A discussion of these compounds is to be found in Ralston.⁵

⁴¹³ L. Lehrman, *J. Am. Chem. Soc.*, *64*, 2144-2146 (1942).

⁴¹⁴ R. L. Whistler and G. Hilbert, *J. Am. Chem. Soc.*, *66*, 1721-1722 (1944).

⁴¹⁵ T. J. Schoch, *J. Am. Chem. Soc.*, *60*, 2824-2825 (1938).

⁴¹⁶ T. J. Schoch and C. B. Williams, *J. Am. Chem. Soc.*, *66*, 1232-1233 (1944).

⁴¹⁷ F. F. Mikus, R. M. Hixon, and R. E. Rundle, *J. Am. Chem. Soc.*, *68*, 1115-1123 (1946).

(a) *Fatty Acid Aldehydes.* There is much evidence that the fatty acid aldehydes are normal metabolic products. Feulgen and his collaborators⁴¹⁸⁻⁴²² carried out the classical work in proving the presence and widespread distribution of the fatty acid aldehydes in tissues. Their results have been confirmed by Anchel and Waelsch,⁴²³ who isolated 0.05 to 0.2% of aldehydes (mostly C₁₆ and C₁₈) from the brain and muscle of rats and cattle. The importance of these aldehydes is further emphasized by the fact that they are likewise found in the plant kingdom, where they have been prepared from a number of the plant oils. The C₈ and C₉ aldehydes, octanal (CH₃(CH₂)₆CHO) and nonanal (CH₃(CH₂)₇CHO), are quite general components of essential oils. Decanal (CH₃(CH₂)₈CHO) has been reported in significant amounts in coriander oil,^{424,425} while dodecanal (CH₃(CH₂)₁₀CHO) has been isolated from the oil of the lily of the valley.⁴²⁶ Marcelet⁴²⁷ prepared an aldehyde-alcohol with 18 carbons from olive pulp. Many of the floral odors are to be traced to the shorter chained aldehydes, each one of which possesses a characteristic aroma. Violet leaf oil has been found to contain a doubly unsaturated aldehyde,⁴²⁸ *i.e.*, 2,6-nonadienal, which has the formula, CH₃CH₂CH:CHCH₂CH₂CH:CHCHO. Since it would appear that aldehydes play an important role as intermediates in fat oxidation and as primary tissue components in the animal, as well as being essential products in the vegetable kingdom, a fairly complete discussion of their chemistry would seem to be desirable.

a'. *Methods of Preparation of Aldehydes:* One widely used method for preparing aldehydes involves the controlled oxidation of the corresponding alcohols. When a mixture of alcohol vapor and a limited amount of air is passed over a silver catalyst⁴²⁹ at 230-300°C., the aldehydes are formed. Yields as high as 96% have been reported for octanal (from caprylic alcohol)⁴³⁰ and 82% for the higher aldehydes obtained by this procedure. Metallic nickel (5%) can be used as a catalyst in liquid-phase dehydrogenation at a carefully regulated temperature; 20% of the theoret-

⁴¹⁸ R. Feulgen and K. Voit, *Arch. ges. Physiol. Pflügers*, 206, 390-410 (1924).

⁴¹⁹ R. Feulgen, K. Inhäuser, and M. Behrens, *Z. physiol. Chem.*, 180, 161-179 (1929).

⁴²⁰ R. Feulgen and M. Behrens, *Z. physiol. Chem.*, 256, 15-20 (1938).

⁴²¹ R. Feulgen and T. Bersin, *Z. physiol. Chem.*, 260, 217-245 (1939).

⁴²² M. Behrens, *Z. physiol. Chem.*, 191, 183-186 (1930).

⁴²³ M. Anchel and H. Waelsch, *J. Biol. Chem.*, 145, 605-613 (1942).

⁴²⁴ P. P. Shorygin and V. P. Osipova, *Sintezy Dushistykh Veshchestv, Sbornik Stat'ei*, 246-247 (1939); *Chem. Abst.*, 36, 3781 (1942).

⁴²⁵ L. Y. Bryusova, R. Y. Shagalova, and N. Novikova, *Sintezy Dushistykh Veshchestv, Sbornik Stat'ei*, 247-252 (1939); *Chem. Abst.*, 36, 3781 (1942).

⁴²⁶ W. Hannemann, *Deut. Parfüm.-Ztg.*, 9, No. 8/9, 7 (Apr. 15, 1923); *Chimie & industrie*, 11, 941 (1924).

⁴²⁷ H. Marcelet, *Compt. rend.*, 206, 529-530 (1938).

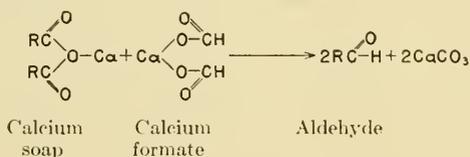
⁴²⁸ E. Späth and F. Keszler, *Ber.*, B67, 1496-1500 (1934).

⁴²⁹ C. Moureu and G. Mignonac, *Compt. rend.*, 170, 258-261 (1920).

⁴³⁰ Z. P. Aleksandrova, *J. Applied Chem. U. S. S. R.*, 10, 105-115 (1937); *Chem. Abst.*, 31, 4266 (1937).

ical amount of lauryl aldehyde has been reported to be produced by this method.⁴³¹ Other catalysts which have been used include copper, zinc, or aluminum mixed with chromium.

Another important method for the synthesis of aldehydes is by heating the calcium or barium soaps with a large excess of calcium or barium formate. The following reaction results:



Dodecanal, tetradecanal, hexadecanal, and octadecanal have been satisfactorily prepared by the calcium salt-calcium formate method.⁴³² Calcium carbonate is frequently mixed with the reactants to slow down the reaction and prevent excessive sintering. An excess of calcium formate tends to minimize ketone formation. This method is more satisfactory for the lower members of the series than for the higher aldehydes. Somewhat better yields have been claimed when the reaction is carried out in an autoclave in the presence of an inert solvent.⁴³³

A closely allied method involves the preparation of the aldehydes by heating the free fatty acids and formic acid with a decarboxylating catalyst.⁴³⁴ This method has been successfully applied to the synthesis of dodecanal,⁴³⁵ nonanal,⁴³⁶ decanal,⁴³⁷ and even of the unsaturated compound, oleic aldehyde,⁴³⁸ from the corresponding acids. Soaps at high temperatures likewise react with formaldehyde to yield aldehydes.⁴³⁹

Another important synthesis of the aldehydes is by decomposition of the ozonides of the unsaturated acids, either by direct heating in a strongly alkaline medium⁴⁴⁰ or by catalytic hydrogenation under pressure. It is also possible to obtain aldehydes having one less carbon than the original

⁴³¹ A. Halasz, *Compt. rend.*, 209, 1000-1003 (1939).

⁴³² F. Krafft, *Ber.*, 13, 1413-1418 (1880).

⁴³³ H. T. Böhme, A.-G., *Brit. Patent No.* 382,929 (Mar. 17, 1932).

⁴³⁴ I. G. Farbenind., A.-G., *Brit. Patent No.* 414,148 (Feb. 6, 1933).

⁴³⁵ S. S. Nametkin and O. M. Khol'mer, *Sintezy Dushistykh Veshchestv, Sbornik Stat'ei*, 55-57 (1939); *Chem. Abst.*, 36, 3629 (1942).

⁴³⁶ S. S. Nametkin and R. Y. Shagalova, *Sintezy Dushistykh Veshchestv, Sbornik Stat'ei*, 274-281 (1939); *Chem. Abst.*, 36, 3781 (1942).

⁴³⁷ O. Osipova, *Masloboino Zhihnoe Delo*, 11, 378-379 (1935); *Chem. Abst.*, 30, 1025-1026 (1936).

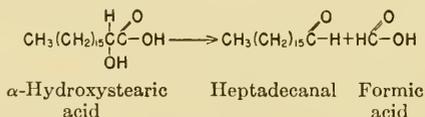
⁴³⁸ I. G. Farbenind., A.-G., *French Patent No.* 750,467 (1937); cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 815; *German Patent No.* 660,735 (Dec. 5, 1938).

⁴³⁹ A. W. Ralston and R. J. Vander Wal, *Brit. Patent No.* 509,203 (July 12, 1939); *U. S. Patent No.* 2,145,801 (Jan. 31, 1939).

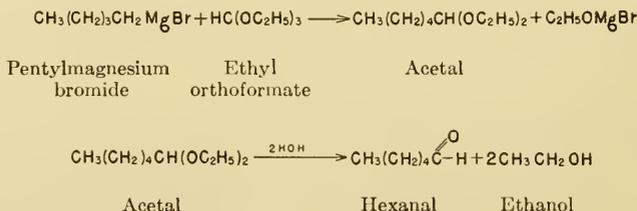
⁴⁴⁰ S. Isikawa and A. Miyata, *Science Repts. Tokyo Bunrika Daigaku*, A3, 257-263, (1939); *Chem. Abst.*, 34, 981 (1940).

aldehyde. On ozonolysis of the olefin formed by dehydration of the secondary alcohol which results from interaction of the original aldehyde and phenylmagnesium bromide, benzaldehyde and the new aldehyde are formed.⁴⁴¹ Finally, a chemical reduction (as opposed to catalytic reduction) of the ozonides will yield aldehydes. Potassium ferrocyanide⁴⁴² or zinc-acetic acid mixture⁴⁴³ may serve for such a reaction.

The hydroxy-acids are also useful as starting materials for the synthesis of aldehydes. On treatment for 24 hours with lead tetraacetate in glacial acetic acid solution, 9,10-dihydroxystearic acids gave an 85% yield of nonanal.⁴⁴⁴ Minium (Pb_3O_4) has likewise been used for the oxidative splitting of 9,10-dihydroxystearic acid to nonanal.⁴⁴⁰ Pyrolysis of ricinoleic acid (12-hydroxy-9-octadecenoic acid) results in the splitting of the acid at the hydroxyl group (between C_{12} and C_{11}) to form heptanal. When the α -hydroxy-acids are heated to their decomposition points, aldehydes having one less carbon, and formic acid, result. An example of this is the formation of heptadecanal in 50–60% yield, according to the following scheme, when 2-hydroxystearic acid is heated⁴⁴⁵ first at 200°C. and then at 270°C.



Another well-known method for the synthesis of aldehydes employs the Grignard reagent and ethyl orthoformate, $\text{CH}(\text{OC}_2\text{H}_5)_3$. Acetals are produced as intermediates, and the aldehydes result on hydrolysis of the acetals. Hexanal is formed in 45–50% yield by the application of this procedure, as follows:



Nitriles can be quantitatively converted to aldehydes, as first suggested by Stephen.⁴⁴⁶ The reaction first involves the formation of an imino

⁴⁴¹ F. G. Fischer, H. Düll, and L. Ertel, *Ber.*, *B65*, 1467–1472 (1932).

⁴⁴² C. Harries, *German Patent No.* 321,567 (June 13, 1918).

⁴⁴³ Y. Kobata, *J. Agr. Chem. Soc. Japan*, *11*, 709–714 (1935); *Chem. Abst.*, *29*, 7279 (1935).

⁴⁴⁴ C.-Y. Hsing and K.-J. Chang, *J. Am. Chem. Soc.*, *61*, 3589 (1939).

⁴⁴⁵ H. R. Le Sueur, *J. Chem. Soc.*, *85*, 827–838 (1904).

⁴⁴⁶ H. Stephen, *J. Chem. Soc.*, *127*, 1874–1877 (1925).

chloride, which is then reduced with stannous chloride. The resulting stannic aldemonium chloride is then hydrolyzed to the aldehyde.

b'. Properties of Aldehydes: The lower aldehydes are liquids which have a fruity odor, while the higher members of the series are solids which have a paraffin-like aroma when melted. In general both the melting points and the boiling points are considerably lower than for the corresponding acids. Darzens and Lévy⁴⁴⁷ found that α,α -substitution with methyl groups lowers the melting point of the resultant aldehydes as compared with the unsubstituted compounds.

There are several series of constants for each aldehyde. The first is for the free aldehyde itself, while the others are for the one or more polymers into which the aldehydes may be spontaneously converted. The properties of the aldehydes vary according to the molecular form in which they exist. In the case of the free aldehydes, the lower members are readily soluble in most organic solvents, while the higher representatives dissolve best in cold diethyl ether, chloroform, petroleum ether, benzene, ethyl acetate, and hot ethanol. The polymers are quite insoluble in most organic solvents in the cold. All the free aldehydes readily form oximes, semicarbazones, hydrazones, and such related compounds; the polymers do not react in this way. The free aldehydes readily react with potassium permanganate; the polymeric forms are not oxidized by this substance, even when boiled. On distillation under a vacuum, the polymers are quantitatively reconverted to the free aldehyde form. Although several types of polymers are possible, LeSueur⁴⁴⁸ reported a trimolecular structure. The physical constants of the saturated aldehydes are given in Table 45.

Because of the ready polymerization of the aldehydes, the early data on melting points are inaccurate. For example, heptadecanal, $\text{CH}_3(\text{CH}_2)_{15}\text{CHO}$, synthesized from α -hydroxystearic acid, was shown⁴⁴⁵ to melt at $35\text{--}36^\circ\text{C}$.; however, a solution in hot ethanol deposited crystals melting at 52°C .; after standing for 6 weeks, the compound melted at 55°C . and, on recrystallization, yielded a product melting at $77\text{--}78^\circ\text{C}$.

c'. Reactions of the Aldehydes: Aldehydes readily react with a number of reagents, giving compounds which are crystalline, and which have definite melting points as well as other characteristic physical properties. This renders it possible to identify most aldehydes from their derivatives. This circumstance is fortunate because the direct identification of aldehydes is extremely difficult. This is attributable to the impossibility of carrying out determinations of physical properties without the danger of polymerization having occurred.

The derivatives most frequently employed are the semicarbazones, the oximes, the 2,4-dinitrophenylhydrazones, the *p*-nitrophenylhydrazones,

⁴⁴⁷ G. Darzens and A. Lévy, *Compt. rend.*, 196, 184-187 (1933).

⁴⁴⁸ H. R. Le Sueur, *J. Chem. Soc.*, 87, 1888-1906 (1905).

TABLE 45
 PHYSICAL PROPERTIES OF SOME COMMON SATURATED AND UNSATURATED ALDEHYDES^a

Common name of aldehyde	Scientific name of aldehyde	Melting pt., °C.			Oxime	Boiling pt., °C.	d_4^{20} ^d	n_D^{20} ^d
		Free aldehyde	Semi-carbazone					
Valeraldehyde	Pentanal	-91.5		52	103.4	0.8095	1.3944	
Caproaldehyde	Hexanal		106	51	131; 28 ¹²	0.8335	1.4257	
Heptaldehyde	Heptanal	-43.3	109	57-58	152.8; 44.4 ⁹	0.8495	1.4217	
Caprylaldehyde	Octanal		98	60	163.4; 65 ¹¹	0.821	1.4240	
Pelargonaldehyde	Nonanal		100	64	90-91 ²⁰	0.860 ¹⁵	1.4298 ¹⁵	
Capraldehyde	Decanal		102	69	208-209; 92 ¹⁰	0.828 ¹⁵	1.4322 ²³	
—	Undecanal	-4	103	72	117 ¹⁸	0.8251 ²³	1.4350	
—	Dodecanal	11.1 ^b		73	103-104 ⁴ ; 98-99 ^{2,5}	0.8352 ¹⁵		
—	Tridecanal	14	106	80.5	156 ²³			
—	Tetradecanal	23.5	106.5	82.5	155 ¹⁰			
—	Pentadecanal	24-25	106.5	86				
—	Hexadecanal	34	107	88				
Palmitaldehyde	Heptadecanal	35-36	107-108	89.5	192-193 ²²			
Margaraldehyde	Octadecanal	38	108-109	89	203-204 ²⁶			
Stearaldehyde	9- <i>cis</i> -Octadecenal							
Oleylaldehyde	9- <i>trans</i> -Octadecenal							
Elaidylaldehyde			92		108-110 ^{0.001}	0.8509	1.4558	

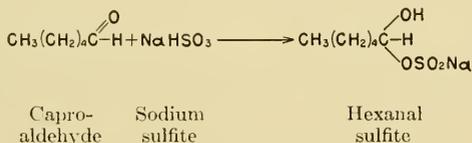
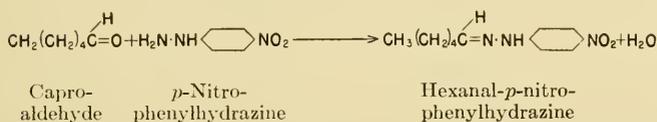
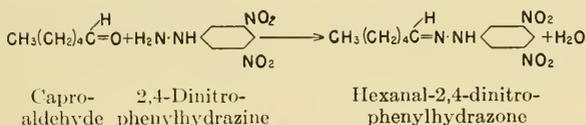
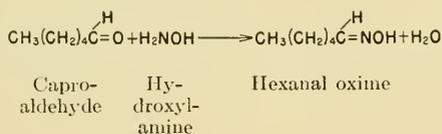
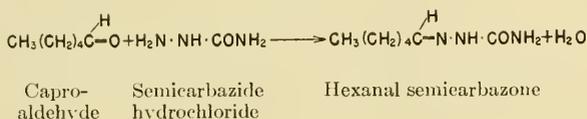
^a Adapted from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, pp. 820-821.

^b Freezing point.

^c Superior figure indicates the barometric pressure in millimeters of mercury at which the boiling point was determined.

^d Superior number indicates the temperature (°C.) recorded at *t*.

and the sulfites. The reactions of these substances with a typical aldehyde, caproaldehyde, are illustrated below:



A number of other derivatives are frequently employed for identification. These include the phenylsemicarbazones prepared by the action of phenylsemicarbazide, $\text{H}_2\text{N} \cdot \text{N}(\text{C}_6\text{H}_5) \cdot \text{CONH}_2$ and the 3,5-dinitrobenzohydrazones prepared by the use of 3,5-dinitrobenzohydrazide, $\text{H}_2\text{N} \cdot \text{NH} \cdot \text{C}_6\text{H}_3(\text{NO}_2)_2$, which is reputedly better than the 2,4-dinitrophenylhydrazine for the identification of aldehydes.⁴⁴⁹ Other closely related compounds which condense with the aldehydes in a manner similar to the above are as follows: several semicarbazides, including *p*-tolyl,⁴⁵⁰ *o*-tolyl,⁴⁵¹ and α -naphthyl;⁴⁵² benzohydrazides, which are *p*-nitro,⁴⁵³ *o*-chloro,⁴⁵⁴ *o*-nitro,⁴⁵⁵ and *m*-

⁴⁴⁹ P. P. T. Sah and T. -S. Ma, *J. Chinese Chem. Soc.*, 2, 40-46 (1934).

⁴⁵⁰ P. P. T. Sah and H. -H. Lei, *J. Chinese Chem. Soc.*, 2, 167-172 (1934).

⁴⁵¹ H. -H. Lei, P. P. T. Sah, and C. Shih, *J. Chinese Chem. Soc.*, 3, 246-250 (1935).

⁴⁵² P. P. T. Sah and S. -H. Chiang, *J. Chinese Chem. Soc.*, 4, 496-500 (1936).

⁴⁵³ P. Chen, *J. Chinese Chem. Soc.*, 3, 251-255 (1935).

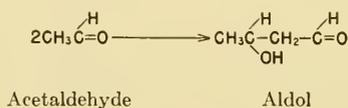
⁴⁵⁴ T. -H. Sun and P. P. T. Sah, *Science Repts. Nat. Tsing Hua Univ.*, A2, 359-363 (1934); *Chem. Abst.*, 29, 466 (1935).

⁴⁵⁵ P. P. T. Sah and C. -H. Kao, *Science Repts. Nat. Tsing Hua Univ.*, A3, 461-468 (1936); *Chem. Abst.*, 31, 3825 (1937).

bromo-⁴⁵⁶; phenylsemioxamazide⁴⁵⁷; and β -naphthylhydrazine.⁴⁵⁸

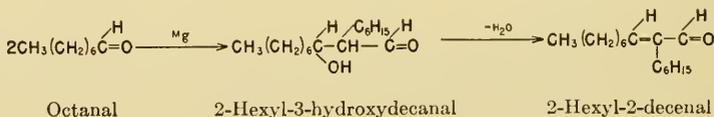
One important group of reactions of the aldehydes can be considered as polymerizations. Practically all aldehydes will undergo such a change even if kept at room temperature. Valeraldehyde spontaneously changes to a polymer melting at 83–84°C. in ether solution in the presence of potassium carbonate, while heptanal undergoes a similar reaction to produce a product melting at 51–52°C.⁴⁵⁹ As indicated earlier, the polymers which are formed are less reactive; in fact, the polymer formation frequently must be considered as a competitive reaction of the aldehydes. This removes these components from the field of activity by changing them to relatively stable polymers.

One condensation which is of especial interest biologically is the so-called *aldol condensation*. When aldehydes are heated in the presence of a catalyst, two molecules combine to a hydroxy-aldehyde. In the case of acetaldehyde, the reaction proceeds as follows:



It has been suggested that, in the animal body, the alcohol is reduced and a new condensation of acetaldehyde results. By a series of such condensations and reductions of the alcoholic groups, a long-chain aldehyde is built up which is eventually oxidized to a fatty acid. This has been suggested as a logical method for the biological synthesis of fat from a carbohydrate intermediate, acetaldehyde.⁴⁶⁰

The higher aldehydes such as caprylaldehyde likewise undergo the aldol condensation reaction to produce branched-chain alcoholic aldehydes which may be converted to unsaturated aldehydes by magnesium as follows:⁴⁶¹



⁴⁵⁶ C. -H. Kao, T. -K. Tao, C. -H. Kao, and P. P. T. Sah, *J. Chinese Chem. Soc.*, 4, 69–74 (1936).

⁴⁵⁷ P. P. T. Sah and W. -P. Han, *Science Repts. Nat. Tsing Hua Univ.*, A3, 469–476 (1936); *Chem. Abst.*, 31, 3825 (1937).

⁴⁵⁸ H. -M. Chen and P. P. T. Sah, *J. Chinese Chem. Soc.*, 4, 62–68 (1936).

⁴⁵⁹ G. Bruylants, *Ber.*, 8, 414–416 (1875).

⁴⁶⁰ A. Magnus-Lévy (1894), cited by G. Lusk, *Elements of the Science of Nutrition*, 4th ed., 1928, p. 350, 396.

⁴⁶¹ P. Shorigin, W. Issaguljanz, and A. Gussewa, *Ber.*, B66, 1431–1435 (1933).

A similar reaction which the aldehydes undergo is a condensation with ketones to form hydroxy-ketones.

The oxidation reactions are a second important group. The end product in most cases is the fatty acid, irrespective of whether a strong acid like chromic⁴⁶² or peracetic acid⁴⁶³ is employed or a slow oxidation by air takes place. In the latter case, an intermediate production of a peroxide is believed to occur. The heavy metals such as silver and iron act as excellent catalysts. The high yield of acids when the liquid aldehydes are treated with copper salts is the basis of a Canadian patent.⁴⁶⁴ Potassium permanganate is a powerful oxidant, since it can completely oxidize the aldehydes not only of the monobasic but also of the dibasic acids.⁴⁶⁵ Nitric acid is also an excellent oxidizing agent, but some nitrogen compounds are formed in addition to the fatty acids.⁴⁶⁶ The action of silver oxide, or better ammoniacal silver nitrate, as an oxidation reagent on aldehydes⁴⁶⁷ is so delicate that it may be employed to detect minute amounts of the latter compounds.

The third group of reactions of the aldehydes can be classified as reduction reactions. When aldehydes are treated with reducing agents such as zinc-hydrochloric acid or sodium-amalgam-ethanol, the corresponding alcohols are produced.⁴⁶⁸ Reichstein *et al.*⁴⁶⁹ have proposed the use of aluminum or aluminum isopropoxide for the reduction of unsaturated aldehydes to the corresponding unsaturated alcohols. In addition to the simple reduction of the aldehyde to the corresponding alcohol, other compounds may originate under special types of treatment. For example, when pressure hydrogenation is employed in the presence of a nickel catalyst on decanal, a secondary alcohol with 20 carbons can be isolated.⁴⁷⁰

Many other reactions of the aldehydes can be considered as additive reactions. In these changes, the double-bonded oxygen on the terminal carbon is usually combined with hydrogen, which permits the addition of another atom or group to the terminal carbon atom. The sulfite derivate formed when aldehydes are treated with sodium bisulfite is an example of this type of reaction. The cyanhydrin reaction is another additive reaction of wide importance in synthetic organic chemistry. Hydrogen cyanide reacts with the higher aldehydes to form aldehyde cyanhydrin, according to the following scheme:

⁴⁶² W. H. Perkin, Jr., *Ber.*, 16, 210-213 (1883).

⁴⁶³ J. D'Ans and A. Kneip, *Ber.*, 48, 1136-1146 (1915).

⁴⁶⁴ W. J. Toussaint, *Canadian Patent* No. 383,687 (Aug. 29, 1939).

⁴⁶⁵ A. Baeyer, *Ber.*, 30, 1962-1965 (1897).

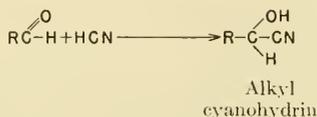
⁴⁶⁶ G. Ponzio, *J. prakt. Chem.* [2], 53, 431-432 (1896).

⁴⁶⁷ M. Delépine and P. Bonnet, *Compt. rend.*, 149, 39-41 (1909).

⁴⁶⁸ F. Krafft, *Ber.*, 23, 2360-2364 (1890).

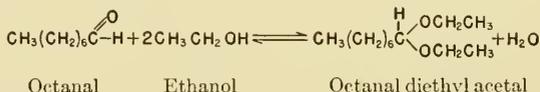
⁴⁶⁹ T. Reichstein, C. Ammann, and G. Trivelli, *Helv. Chim. Acta*, 15, 261-268 (1932).

⁴⁷⁰ J. v. Braun and G. Manz, *Ber.*, B67, 1696-1712 (1934).

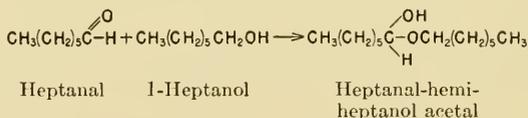


The cyanohydrins are soluble in most organic solvents such as diethyl ether, ethanol, benzene, petroleum ether, and chloroform. Their chief utility lies in the fact that they can be hydrolyzed to give a hydroxy-acid having one more carbon atom than the original aldehyde. They have been widely used in the synthesis of the carbohydrates. When the hydrolysis is only partial, the cyanohydrins yield α -hydroxy-amides. With ammonia, the aldehydes yield α -hydroxylamines.

Of the many other condensation reactions, that with the alcohols is probably best known. These condensation products are known as acetals; they are more readily formed by the longer chain aldehydes than by those having a smaller number of carbon atoms. The formation of acetals is catalyzed by acids; however, this is an equilibrium reaction, since the acetals are hydrolyzed to the aldehyde and the alcohols when boiled with mineral acids. A typical reaction of ethyl alcohol and octanal is given below:



Hemiacetals are formed when equimolecular mixtures of aldehydes and alcohols react.⁴⁷¹ A typical preparation of such a compound from 1-heptanol and heptanal is as follows:



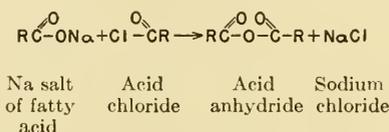
(b) *Alcohols.* The higher alcohols are found as components of the waxes, in which they are combined with the fatty acids. In addition to the large number of alcohols which can be considered to be derived from the saturated aliphatic fatty acids, there are those which correspond to the common unsaturated acids. Oleyl alcohol is one of the representatives of this group; it has a wide natural distribution. The sterols are a third group of alcohols of major importance biologically. However, since all of these alcohols are present in the so-called non-saponifiable residue, a discussion of them is included in Chapter IV.

⁴⁷¹ Anonymous, *Ann. Rept. Essential Oils, Synthetic Perfumes &c., Schimmel & Co.*, 71-74 (1933); *Chem. Abst.*, 30, 3774-3775 (1936).

the acid and acetic anhydride on an oil bath for 4 to 7 hours.⁴⁷⁷ The reflux method is also applicable to the shorter chain acids,⁴⁷⁸ and has been demonstrated to be satisfactory⁴⁷⁹ in the case of the even acids from C₈ to C₁₈. The reaction can be carried out to advantage⁴⁸⁰ under CO₂ with the highly unsaturated acids such as linoleic.

In addition to acetic anhydride, other dehydrating agents may be used. Phosphorus pentoxide gives a yield of approximately 78-79% with palmitic and stearic acids, when ketones are present as impurities.⁴⁸¹ Acetyl chloride has a satisfactory dehydrating action on caproic acid⁴⁸² or on the higher fatty acids in ether solution.⁴⁸³

A second method, which is applicable for the preparation of both simple and mixed anhydrides, involves the reaction between the sodium salt of the fatty acid and the acid chloride⁴⁸⁴ according to the following reaction:



When the sodium salt and acid chloride are derived from the same acid, a simple anhydride results.^{485,486} By varying these two components, any desired mixed anhydride can be obtained. When the sodium salts are heated with acetic anhydride under pressure, the anhydrides are also formed.⁴⁸⁷

A third procedure originally suggested by Hentschel⁴⁸⁸ involves the treatment of the sodium soaps with phosgene; in addition to the anhydride, CO₂ and 2 molecules of sodium chloride result. Phosgene has been employed on benzene suspensions of the fatty acids of tall oil for synthesis of the anhydrides.⁴⁸⁹ The pyrosulfates as such,⁴⁹⁰ or those formed from the action of SO₂ on concentrated sulfuric acid,⁴⁹¹ react with the anhydrous sodium soaps to produce the anhydrides.

⁴⁷⁷ D. Holde, J. Ripper, and F. Zadek, *Ber.*, *B57*, 103-104 (1924).

⁴⁷⁸ W. Autenrieth, *Ber.*, *34*, 168-187 (1901).

⁴⁷⁹ D. Holde and R. Gentner, *Ber.*, *B58*, 1418-1424 (1925).

⁴⁸⁰ D. Holde and R. Gentner, *Ber.*, *B58*, 1067-1071 (1925).

⁴⁸¹ G. Rankov, *Ann. univ. Sofia II, Faculté phys.-math.* [2], *33*, 221-227 (1937); *Chem. Abst.*, *32*, 3335 (1938).

⁴⁸² H. Fournier, *Bull. soc. chim.* [4], *5*, 920-926 (1909).

⁴⁸³ J. Gsell, *Chem.-Ztg.*, *31*, 99-100 (1907); *Chem. Abst.*, *1*, 1066 (1907).

⁴⁸⁴ C. Gerhardt, *Ann.*, *87*, 57-84 (1853).

⁴⁸⁵ A. Villier, *Ber.*, *9*, 1932-1933 (1876).

⁴⁸⁶ F. Krafft and W. Rosiny, *Ber.*, *33*, 3576-3579 (1900).

⁴⁸⁷ A. Michael, *Ber.*, *34*, 918-930 (1901).

⁴⁸⁸ W. Hentschel, *Ber.*, *17*, 1284-1289 (1884).

⁴⁸⁹ D. Holde and K. Schmidt, *Z. angew. Chem.*, *35*, 502-503 (1922).

⁴⁹⁰ H. Dreyfus, *French Patent No. 478,951* (Jan. 26, 1916); *Chem. Abst.*, *10*, 2278 (1916); cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 797.

⁴⁹¹ H. Dreyfus, *French Patent No. 20,261* (June 1, 1917); *Chem. Abst.*, *12*, 1195 (1918).

Another general procedure for anhydride synthesis involves the action of oxalyl chloride on the acids in benzene solution. This method is especially satisfactory with aromatic acids,⁴⁹² but it gives poor yields with the aliphatic acids.⁴⁹³ However, better results are obtained with oxalyl chlorides on the aliphatic fatty acids when their sodium soaps are used.

Ketene, $\text{CH}_2:\text{C}:\text{O}$, combines with the higher fatty acids to give a mixed acetic anhydride.⁴⁹⁴ On subsequent distillation of the mixed anhydride at high temperatures, the two symmetrical anhydrides result.

TABLE 46

MELTING POINTS AND BOILING POINTS OF SIMPLE ANHYDRIDES OF SATURATED AND UNSATURATED FATTY ACIDS

Anhydride of ^a	M.p., °C.	B.p., °C. ^b	Anhydride of	M.p., °C.
Butyric	-75	198; 85-86 ¹⁸	Arachidic ^c	77.6
Valeric	-56.1	215	Behenic ^c	81.8
Caproic	-40.6	241-243	Lignoceric ^c	86.2
Heptolic	-10.8	258; 164.5 ¹⁵	Cerotic ^c	89.4
Caprylic	0.9	280-290	Montanic ^c	92.8
Pelargonic	14.8	207 ¹⁵	Triacontanoic ^c	94.6
Capric	24.7		10-Undecenoic ^d	13.2
Undecanoic	36.7		Oleic ^e	22.1
Lauric	42.1		Elaidic ^e	46.4
Tridecanoic	49.9		Erucic ^f	46.2-46.5
Myristic	53.5		Brassicidic ^g	64
Pentadecanoic	60.6		Linoleic ^h	-3.5
Palmitic	63.9			
Margaric	67.6			
Stearic	70.7			

^a Data from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 799.

^b Superior figure indicates the barometric pressure in millimeter of mercury at which the boiling point was determined.

^c W. Bleyberg and H. Ubrich, *Ber.*, *B64*, 2504-2513 (1931).

^d F. Krafft and F. Tritschler, *Ber.*, *33*, 3580-3585 (1900).

^e D. Holde and K. Rietz, *Ber.*, *B57*, 99-102 (1924).

^f D. Holde and C. Wilke, *Z. angew. Chem.*, *35*, 105; 289-291 (1922).

^g D. Holde and K. Schmidt, *Z. angew. Chem.*, *35*, 502-503 (1922).

^h D. Holde and R. Gentner, *Ber.*, *B58*, 1067-1071 (1925).

b'. Properties of Acid Anhydrides: As noted earlier, the melting points of the anhydrides of the aliphatic acids have a wider range than do those of the fatty acids, being lower in short-chain and higher in the long-chain compounds than those of the corresponding fatty acids. All of the anhydrides below capric acid are liquids at ordinary temperatures. The more volatile short-chain compounds can be distilled at ordinary pressure

⁴⁹² R. Adams, W. V. Wirth, and H. E. French, *J. Am. Chem. Soc.*, *40*, 424-431 (1918).

⁴⁹³ R. Adams and L. H. Ulich, *J. Am. Chem. Soc.*, *42*, 599-611 (1920).

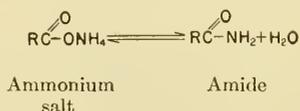
⁴⁹⁴ C. D. Hurd and M. F. Dull, *J. Am. Chem. Soc.*, *54*, 3427-3431 (1932).

without decomposition, but those composed of the long-chain fatty acids are unstable to heat and cannot be distilled even at low barometric pressures. The melting points and boiling points of some of the commoner anhydrides are included in Table 46.

The densities (d_4^t) of the symmetrical anhydrides of the saturated acids are as follows⁵: C₄, 0.9946²⁰; C₅, 0.929²⁰; C₆, 0.9279¹¹; C₇, 0.932²⁰; C₈, 0.9065^{17.5}; C₁₀, 0.8596⁷⁰; C₁₂, 0.8552⁷⁰; C₁₄, 0.8502⁷⁰; C₁₆, 0.847⁷⁰; C₁₈, 0.8443⁷⁰. The refractive indices (n_D^t) are as follows⁵: C₄, 1.4143¹⁸; C₇, 1.4312²⁰; C₈, 1.4358^{17.5}; C₁₀, 1.4234⁷⁰; C₁₂, 1.4292⁷⁰; C₁₄, 1.4335⁷⁰; C₁₆, 1.4357⁷⁰; and C₁₈, 1.4379⁷⁰. The lower dicarboxylic acids form inner anhydrides which are monomeric rings. Adipic acid apparently forms both a monomeric anhydride melting at 20°C. and a linear polymer melting at 80–85°C. The latter cannot be distilled, but it breaks down to the monomeric form. On heating to 100°C., or on standing, it can be reconverted to the linear polymer.

(e) *Fatty Acid Amides*. The amides are closely related to the fatty acids in that the hydroxyl radical of the carboxyl group has been replaced by an amino group. In some cases, one or both hydrogens of the amino group are replaced by alkyl, aryl, or other components which give rise to a large variety of substituted amides. The general formula for the fatty acid amide is R·CONH₂.

a'. Methods of Preparation of Acid Amides: The ammonium salt method is one of the oldest procedures and one which is still widely used. This method involves the removal of water by heating the ammonium salt of the fatty acid as follows:



Hofmann⁴⁹⁵ prepared stearamide by this reaction while Menshutkin⁴⁹⁶ synthesized the amides of a number of fatty acids by heating their sodium salts with ammonium chloride. A somewhat similar method involves heating the ethyl ester with an alcoholic solution of ammonia,⁴⁹⁷ but the latter reaction has the disadvantage of being reversible.⁴⁹⁸ Since the amides can lose an additional molecule of water to form the nitrile, the conditions of dehydration must be satisfactorily adjusted.

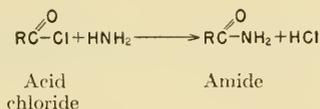
Another early method for the synthesis of the amide involves the reaction between the acid chloride and dry ammonia, or an amine, according to the following reaction:

⁴⁹⁵ A. W. Hofmann, *Ber.*, 15, 977–984 (1882).

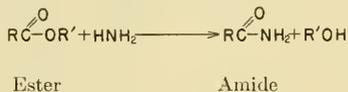
⁴⁹⁶ N. Menshutkin, *Ber.*, 17, 846–848 (1884).

⁴⁹⁷ H. Carlet, *Bull. soc. chim.* [1], 1, 73–77 (1859).

⁴⁹⁸ L. Meyer, *Ber.*, 22, 24–27 (1889).

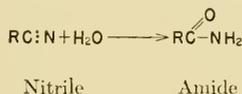


The preparation of amides directly from the neutral fats can be carried out by ammonolysis. Under such conditions, ammonia or a substituted amine acts as the hydrolysis agent.



Oda⁴⁹⁹ observed a quantitative transformation of fats into fatty acid amides when they were heated in a sealed tube with ammonia at 150°C. for one hour. Anilides are formed by heating aniline and fats in a sealed tube at 230°C. for 5 hours.⁵⁰⁰ Amides may likewise be synthesized by treatment of aliphatic esters with aqueous solutions of aliphatic amines under pressure at above 100°C., preferably⁵⁰¹ 200°C.

The nitriles have also been shown to be a possible source of the amides. This synthesis is effected by treatment with concentrated hydrochloric acid, sulfuric acid, or glacial acetic acid at low temperatures. This reaction simply involves the addition of one molecule of water as follows:



Oxidation with H₂O₂ in alkaline solution will convert the nitriles to amides, with the simultaneous liberation of oxygen.⁵⁰²

b'. Properties of Acid Amides: The amides of the fatty acids are compounds with relatively high melting points. They do not show the gradation in physical properties, with changes in chain length, that are usually the rule with homologous series. The melting points are an example of this lack of conformity. Alternation does not occur, the melting points are quite irregular, and show little variation for the higher members of the series. Thus, there is a difference of only 3.8° in the melting point of caprylamide and stearamide. On the other hand, the anilides show a somewhat greater regularity in melting point, reaching a minimum in those having 8 and 9 carbon atoms, after which there is a uniform rise with the lengthening of the chain.⁵⁰³ However, the *N*-methyl amides have been

⁴⁹⁹ R. Oda, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 24, No. 510, 171-173 (1934).

⁵⁰⁰ E. de'Conno and R. Biazzo, *Rend. accad. sci. Napoli*, [3], 21, 322-327 (1915); *J. Chem. Soc.*, 110, 788 (1916).

⁵⁰¹ C. O. Henke and W. H. Zartman, *U. S. Patent No. 2,058,013* (Oct. 20, 1936).

⁵⁰² B. Radziszewski, *Ber.*, 18, 355-356 (1885).

⁵⁰³ P. W. Robertson, *J. Chem. Soc.*, 93, 1033-1037 (1908); 115, 1210-1223 (1919).

shown to have melting points which are strikingly similar to those of the corresponding fatty acids.⁵⁰⁴ These values are included in Table 47.

TABLE 47

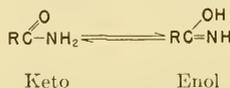
MELTING POINTS OF AMIDES, ANILIDES, AND *N*-METHYL AMIDES OF SATURATED ACIDS^a

No. of C atoms in chain	Even-chain derivatives			Odd-chain derivatives			
	Amides	Anilides	<i>N</i> -methyl amides	Amides	Anilides	<i>N</i> -methyl amides	
2	82	112	28	3	77	106	-43.0
4	115	96	- 5.2	5	106	63	-25.5
6	101	92	13.6	7	96.05 ^b	65	14.0
8	105.9 ^b	55	38.9	9	98.8 ^b	57	39.1
10	98.5 ^b	69.5 ^b	57.3	11	99	71	56.0
12	102.4 ^b	77.2 ^b	68.4	13	100	80	68.2
14	105.1 ^b	84	78.4	15			78.3
16	107.0 ^b	90.2 ^b	85.5	17			84.8
18	109.7 ^b	94	92.1				
20	108						
22	111-112	101-102					

^a Adapted from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, 1948, p. 593.

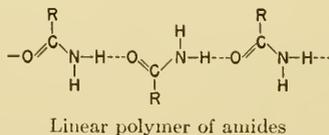
^b Freezing points.

It seems probable that the amides may exist in either of two forms, *i.e.*, the keto or the enol form, which are in equilibrium.



The irregularities in properties are believed to be ascribable to some form of molecular association. The aberrant results are chiefly observed in the lower members of the series, and the discrepancies are reduced by the introduction of a phenyl group in the molecule.⁵⁰⁵ Anilides have less tendency to form association compounds than do the corresponding amides.

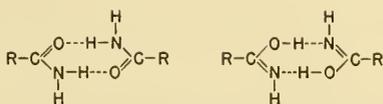
It is believed that the association of amides results in the formation of either linear or cyclic polymers. On the basis of the hydrogen-bonding characteristics of the amides, Copley *et al.*⁵⁰⁶ have suggested the following structures for these polymers:



⁵⁰⁴ G. F. D'Alelio and E. E. Reid, *J. Am. Chem. Soc.*, 59, 109-111 (1937).

⁵⁰⁵ A. N. Meldrum and W. E. S. Turner, *J. Am. Chem. Soc.*, 97, 1605-1616 (1910).

⁵⁰⁶ M. J. Copley, G. F. Zellhoefer, and C. S. Marvel, *J. Am. Chem. Soc.*, 60, 2666-2673 (1938).



Cyclic polymer structure of amides

The cyclic dimers may form larger polymers through fusion.

Of the many other series of amides and related compounds, several are of interest to the fat chemist. For example, the amides of a number of unsaturated fatty acids have been shown to be solids. Oleamide melts⁵⁰⁷ at 75–76°C., elaidamide at 93–94°C., erucamide at 82.5–83°C., and brassidamide at 94°C.⁵⁰⁸ The amides of the dibasic acids deserve attention. There are two series of such amides, depending upon whether one or two amide groups are present. The monoamides have considerably lower melting points than do the diamides. Thus, the corresponding melting points in °C. for the two types are as follows: adipic acid, monoamide,⁵⁰⁹ 125–130, diamide^{510–511}, 220; suberic acid,⁵⁰⁹ monoamide, 125–127, diamide, 216; azelaic acid,⁵⁰⁹ monoamide, 93–95, diamide, 175–176; and sebacic acid,⁵⁰⁹ monoamide, 170, diamide, 210.

The amides show considerable irregularity in solubility, which in some cases seems to be a random distribution. The discrepancies are difficult to explain, but may be related to polymer formation. The aliphatic amides do not dissolve in water but in the organic solvents. In fact, the higher amides are so hydrophobic that they are used in various waterproofing preparations. Ralston, Hoerr, and Pool⁵¹² reported variations from the normal in benzene, ethyl acetate, and 2-butanone. Lauramide was found to be more soluble than capramide, while palmitamide dissolved to a greater extent than did myristamide. The monoamides of the dicarboxylic acids are water-soluble, while the diamides are also slightly so.

The aliphatic amides are essentially neutral substances, since the basic effect of the amino group is compensated by the acidic reaction of the acyl portion of the molecule. Although the amides are relatively stable compounds, they can be hydrolyzed to ammonia and their parent fatty acid when heated with hydrochloric acid or sodium hydroxide. The reaction is especially responsive to heat, and it occurs at a rate which decreases with the lengthening of the chain. Strenuous measures must be employed to bring about hydrolysis of the high molecular weight amides. Alkaline hy-

⁵⁰⁷ O. Aschan, *Ber.*, 31, 2344–2350 (1898).

⁵⁰⁸ Y. Toyama, *J. Soc. Chem. Ind. Japan*, 25, 1053–1055 (1922); *Chem. Abst.*, 17, 3161 (1923); cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 594.

⁵⁰⁹ L. Étaix, *Ann. chim.* [7], 9, 356–409 (1896).

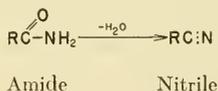
⁵¹⁰ L. Henry, *J. Chem. Soc.*, 48, 886–887 (1885).

⁵¹¹ L. Henry, *Compt. rend.*, 100, 943–946 (1885).

⁵¹² A. W. Ralston, C. W. Hoerr, and W. O. Pool, *J. Org. Chem.*, 8, 473–488 (1943).

drolisis apparently occurs by a direct substitution of a hydroxyl radical for the amino group.

c'. Reactions of Acid Amides: When the amides are heated alone or in the presence of such dehydrating agents as phosphorus pentoxide or phosphorus pentachloride, they lose one molecule of water, and are converted to the nitriles.



When the amides are subjected to hydrogenation they are converted to a mixture of primary and secondary amines which have largely lost their lipid-like properties. Thus, after reduction of such amides as lauramide in dioxane under 100–300 atmospheres of hydrogen pressure, at 175–200°C., in the presence of copper-chromium oxide as a catalyst, Adkins and Wojcik⁵¹³ obtained 40–70% of the primary amines and 25–50% of the secondary amines.

When the amides are treated with halogens in a strong alkaline solution, a substituted urea is formed which eventually gives rise to the corresponding amine. This reaction, first described by Hofmann,⁵¹⁴ presumably involves the intermediate formation of an isocyanate. The mechanism of this rearrangement has been studied extensively by Stieglitz and his associates.^{515–517} These workers believe that only nitrogen compounds which can lose constituents and form a monovalent nitrogen atom are capable of such a rearrangement.

(f) *Nitriles*. The nitriles are less closely related to the fatty acids than are the amides, since they no longer contain any oxygen atoms. They are highly reactive compounds by virtue of the unsaturated nitrogen atom. They may readily be converted to acid amides, fatty acids, amines, and a number of related compounds. They have the general formula, RC:N.

a'. Methods of Preparation of Nitriles: As has been described earlier the nitriles can be formed from the amides which, in turn, originate from the fatty acids. Wöhler and Liebig⁵¹⁸ were the first to describe this synthesis, as early as 1832. Phosphorus pentoxide is the dehydrating agent most frequently employed, but phosphorus pentasulfide has been successfully used in a number of instances.⁵¹⁹

⁵¹³ H. Adkins and B. Wojcik, *J. Am. Chem. Soc.*, **56**, 247 (1934).

⁵¹⁴ A. W. Hofmann, *Ber.*, **14**, 2725–2736 (1881).

⁵¹⁵ F. Lengfeld and J. Stieglitz, *Am. Chem. J.*, **15**, 215–222, 504–518 (1893); **16**, 370–372 (1894).

⁵¹⁶ J. Stieglitz, *Am. Chem. J.*, **18**, 751–761 (1896); **29**, 49–68 (1903); *Rev. Am. Chem. Research*, **9**, 169–170 (1903), in *J. Am. Chem. Soc.* **25** (1903).

⁵¹⁷ J. Stieglitz and R. B. Earle, *Am. Chem. J.*, **30**, 399–412, 412–421 (1903).

⁵¹⁸ F. Wöhler and J. Liebig, *Ann.*, **3**, 249–282 (1832).

⁵¹⁹ L. Henry, *Ber.*, **2**, 305–308, 494–495 (1869).

The nitrile may likewise be prepared directly from the fatty acid without the actual isolation of the amides. This reaction can be accomplished when fatty acids and ammonia interact at high temperatures in either the liquid or the vapor phase. When the fatty acids are heated in a continuous stream of ammonia at a temperature at which the amide first formed is decomposed, a quantitative transformation to the nitrile eventually obtains. The nitriles are likewise synthesized when the fatty acids or their esters are passed, in their vapor phase, with ammonia, over such contact catalysts as pumice, alumina, thoria, or silica gel at a temperature of 400°C. or higher.

The nitrile is formed when aliphatic aldehydes and ammonia are passed over thoria or aluminum.⁵²⁰ The primary and secondary amines, in the presence of a nickel catalyst, lose hydrogen, with the production of nitriles.⁵²¹ Nitriles originate when equimolecular mixtures of fatty acid esters and primary amines are heated to 500°C. in the presence of dehydrating catalysts.⁵²²

Alkyl halides have long been known to react with inorganic cyanides to give the nitriles. The compound so synthesized contains one more carbon atom than the alkyl halide. This reaction has been widely employed for the synthesis of compounds which do not occur naturally. For example, it constitutes an excellent procedure for the preparation of the fatty acids with an odd number of carbon atoms.

Hofmann⁵²³ discovered that, when the aliphatic amides or amines are treated with an excess of bromine, nitriles with one less carbon are formed. Aldoximes, such as $RCH:NOH$, lose a molecule of water in the presence of a dehydrating agent, to give rise to nitriles. Letts⁵²⁴ has demonstrated the production of nitriles along with amides when fatty acids are treated with alkali salts of thiocyanic acid. Phenylhydrazones, also, have proved to be useful starting materials since, in the presence of cuprous chloride, they yield a mixture of nitriles and amines.⁵²⁵

b'. Properties of Nitriles: The nitriles, including laurionitrile, are liquids at ordinary temperatures. They have a characteristic odor, which is most pronounced in the case of valeronitrile; the solid nitriles (myristonitrile and above) are odorless. The melting points (or freezing points) of the several nitriles in °C. are as follows: butyronitrile, -112.6; valeronitrile, -96.0; capronitrile, -79.4; caprylonitrile, -45.6; pelargononitrile, -34.2; caprinitrile, -14.5; laurionitrile, 4.0; myristonitrile, 19.2; palmitonitrile, 31.4; stearonitrile, 40.9; arachidonitrile, 48.5-49.5; doco-

⁵²⁰ A. Mailhe, *Compt. rend.*, 166, 36-38, 121-123 (1918). A. Mailhe and F. de Godon, *Compt. rend.*, 166, 215-217 (1918).

⁵²¹ A. Mailhe and F. de Godon, *Compt. rend.*, 165, 557-559 (1917).

⁵²² A. Mailhe, *Compt. rend.*, 170, 813-815 (1920).

⁵²³ A. W. Hofmann, *Ber.*, 17, 1406-1412 (1884).

⁵²⁴ E. A. Letts, *Ber.*, 5, 669-674 (1872).

⁵²⁵ A. E. Arbusow, *Ber.*, 43, 2296-2300 (1910).

sanonitrile, 53.5–54.5; pentacosanonitrile, 58–59; and hexacosanonitrile, 61–62.

The aliphatic saturated nitriles are relatively thermostable, and can be distilled without decomposition under reduced pressures. The nitriles below the C_{12} member can be distilled at ordinary pressure without destruction, but lauronitrile and the higher homologs are partially decomposed. Ralston and co-workers⁵²⁶ have recently determined the boiling points of the C_6 to C_{18} saturated nitriles at various reduced pressures. The densities are much lower than those of the fatty acids, varying at 30°C. from 0.7906 for valeronitrile to 0.8207 for myristonitrile.^{527,528}

The refractive indices are likewise lower than for the corresponding acids. They decrease as the temperature is increased, and an abrupt change in slope occurs at 40–45°C. which is also the case with the acids. The refractive indices vary at 50°C. from 1.3706 for butyronitrile to 1.4370 for stearonitrile; at 75°C., these values are 1.3590 and 1.4276, respectively.⁵²⁹

Hoerr *et al.*⁵³⁰ have recently carried out an exhaustive study on solubilities of the nitriles. The solubilities in general decrease with an increasing polarity of the solvents. They also follow the general rule of being less soluble with increasing molecular size.

Nitriles are readily hydrolyzed *via* the amides to the corresponding carboxylic acid. The lower members of the series can readily be hydrolyzed to the fatty acids by alkalis on refluxing their aqueous or alcoholic solutions.^{531,532} For splitting the higher nitriles, it may be necessary to carry out the hydrolysis under pressure, to accelerate the reaction. The breakdown may likewise be accomplished in the vapor state, with steam at 420°C., using thorium oxide or aluminum oxide as the catalyst.⁵³³

c'. Reactions of Nitriles: The hydrolysis of the nitriles can be effected by means of concentrated acids. There are two equilibria involved, *i.e.*, nitrile \rightarrow amide and amide \rightarrow acid, which respond differently to the concentration of the acid. Since the rate of the reaction will be governed by the slower of the two hydrolysis constants, the maximum conversion of the nitrile to the acid may not necessarily entail the optimum rate for both reactions. At lower acid concentrations the rate of hydrolysis of the several mineral acids is similar. At higher concentrations, hydrochloric acid has

⁵²⁶ A. W. Ralston, W. M. Selby, and W. O. Pool, *Ind. Eng. Chem.*, *33*, 682–683 (1941).

⁵²⁷ R. Merckx, J. Verhulst, and P. Bruylants, *Bull. soc. chim. Belg.*, *42*, 177–198 (1933).

⁵²⁸ B. Daragan, *Bull. soc. chim. Belg.*, *44*, 597–624 (1935).

⁵²⁹ A. Dorinson and A. W. Ralston, *J. Am. Chem. Soc.*, *66*, 361–362 (1944).

⁵³⁰ C. W. Hoerr, E. F. Binkerd, W. O. Pool, and A. W. Ralston, *J. Org. Chem.*, *9* 68–80 (1944).

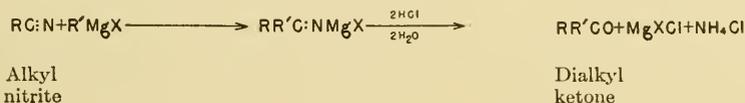
⁵³¹ E. Frankland and H. Kolbe, *Ann.*, *65*, 288–304 (1848).

⁵³² R. Adams and C. S. Marvel, *J. Am. Chem. Soc.*, *42*, 310–320 (1920).

⁵³³ A. Mailhe, *Compt. rend.*, *171*, 245–247 (1920).

the highest catalytic effect, followed in order by sulfuric, hydrobromic, and nitric acids.⁵³⁴⁻⁵³⁷ Phosphoric acid in 100% concentration⁵³³ is an effective hydrolyzing agent for the nitriles, but it has little effect in a 92.5% solution.⁵³⁹ The ethyl esters of the fatty acids are formed when the nitriles are heated under pressure at 130-140°C. with an excess of ethanol (10 parts) and one part of concentrated sulfuric acid.⁵⁴⁰

Another reaction of interest which the nitriles undergo is the formation of imino ethers (RCCl:NH) on treatment with hydrochloric acid in alcoholic solution,⁵⁴¹ which, after reduction, and hydrolysis, are converted to the corresponding aldehydes (RCHO).⁴⁴⁶ The Grignard reaction is one by which higher ketones can be readily synthesized from the nitriles according to the following reactions:



By varying the R' component of the Grignard reagent and the nitrile one can obtain any desired ketone. Amides can be formed if, instead of hydrolysis, oxidation with alkaline hydrogen peroxide is employed.⁵⁰¹ The corresponding hydroxylamines, RCH:NOH, are also readily synthesized.⁵⁴²⁻⁵⁴⁴ Substituted aminopyridines result from the action of metallic sodium on the nitriles⁵⁴⁵⁻⁵⁴⁷ while 2-alkenenitriles originate on treatment of nitriles with bromine followed by removal of HBr.⁵⁴⁸⁻⁵⁵⁰

The higher nitriles have the property of imparting "oiliness" to petrol-

⁵³⁴ B. S. Rabinovitch and C. A. Winkler, *Can. J. Research*, B20, 73-81 (1942).

⁵³⁵ B. S. Rabinovitch, C. A. Winkler, and A. R. P. Stewart, *Can. J. Research*, B20, 121-132 (1942).

⁵³⁶ J. D. McLean, B. S. Rabinovitch, and C. A. Winkler, *Can. J. Research*, B20, 168-173 (1942).

⁵³⁷ B. S. Rabinovitch and C. A. Winkler, *Can. J. Research*, B20, 221-230 (1942).

⁵³³ G. Berger and S. C. J. Olivier, *Rec. trav. chim.*, 46, 600-604 (1927).

⁵³⁹ S. C. J. Olivier, *Rec. trav. chim.*, 48, 568-570 (1929).

⁵⁴⁰ L. Spiegel, *Ber.*, 51, 296-298 (1918).

⁵⁴¹ A. Pinner, *Ber.*, 23, 2917-2919 (1890).

⁵⁴² P. Eitner and H. Wetz, *Ber.*, 26, 2340-2347 (1893).

⁵⁴³ F. Tiemann, *Ber.*, 17, 126-129 (1884).

⁵⁴⁴ E. Nordmann, *Ber.*, 17, 2746-2756 (1884).

⁵⁴⁵ E. Frankland and H. Kolbe, *Ann.*, 65, 269-287 (1848).

⁵⁴⁶ A. G. Bayer, *Ber.*, 2, 319-324 (1869).

⁵⁴⁷ E. von Meyer, *J. prakt. Chem.* [2], 22, 261-288 (1880); [2], 27, 152-156 (1883); [2], 37, 396-407 (1888); [2], 39, 188-200 (1889).

⁵⁴⁸ R. van Caillie, *Bull. soc. chim. Belg.*, 44, 438-440 (1935).

⁵⁴⁹ R. Merecx and P. Bruylants, *Bull. classe sci. Acad. roy. Belg.* [5], 19, 681-688 (1933).

⁵⁵⁰ A. Gavrilloff, *Bull. classe sci. Acad. roy. Belg.* [5], 19, 815-820 (1933).

eum lubricants,⁵⁵¹ and for this reason they have been added to mineral oils to be used as penetrating oils⁵⁵²; mixed with phosphorus pentasulfide or sulfur monochloride, they may serve as extreme pressure lubricants.⁵⁵³ The C₁₀ to C₁₈ members apparently have some use as insect repellents,⁵⁵⁴ while the lower members are useful as flotation agents with some of the metallic ores.⁵⁵⁵ Strangely enough the nitriles are largely non-toxic, in contrast to the cyanides, which have a markedly poisonous activity.

(g) *Acid Halides.* The halides of the fatty acids and the parent acids are closely related compounds; not only can they be readily formed from the fatty acids, but they quickly revert to them when exposed to water. The chlorides are best known, and some bromides have also been prepared, but the iodides and fluorides are practically unknown. The acid halides occur only as synthetic products; because of their great reactivity, they are widely used for the synthesis of products related to the fatty acids.

a'. *Methods of Preparation of Acid Halides:* The principal method of preparation of the acid chlorides is by the action of chlorinating agents on the fatty acid or the sodium soaps. The reaction may proceed in the presence or absence of solvents. The most widely employed compounds for chlorination are phosphorus pentachloride (PCl₅), phosphorus trichloride (PCl₃), thionyl chloride (SOCl₂), silicon tetrachloride (SiCl₄), and related compounds. Phosphorus pentachloride was the first reagent to be used, and an exhaustive study was made by Krafft and Bürger,⁵⁵⁶ as early as 1884, on the mechanism of its action. Many acid chlorides, including lauroyl, myristoyl, palmitoyl, and stearoyl chlorides, and later undecenoyl, oleoyl, and elaidoyl chlorides,⁵⁵⁷ have been satisfactorily prepared by the use of PCl₅. Phosphorus trichloride has the advantage that after giving up its chlorine atoms it is converted to phosphorous acid, which separates as an insoluble residue from the newly synthesized acid chlorides. Täufel and Künkele⁵⁵⁸ found that this chlorinating agent is well adapted for the preparation of oleoyl chloride from its acids.

Thionyl chloride is a useful reagent, since the end products formed from the chlorination reaction are both gaseous and thus can be readily removed. A theoretical yield of caproyl chloride⁵⁵⁹ and an 82% yield of oleoyl chlo-

⁵⁵¹ A. W. Ralston, W. O. Pool, and J. Harwood, *U. S. Patent* No. 2,053,045 (Sept. 1, 1936).

⁵⁵² A. W. Ralston, W. O. Pool, and J. Harwood, *U. S. Patent* No. 2,053,046 (Sept. 1, 1936).

⁵⁵³ A. W. Ralston, *U. S. Patents* Nos. 2,116,472 and 2,125,853 (May 3, Aug. 2, 1938).

⁵⁵⁴ A. W. Ralston and J. P. Barrett, *Oil & Soap*, 18, 89-91 (1941).

⁵⁵⁵ J. Harwood and W. O. Pool, *U. S. Patent* No. 2,166,093 (July 11, 1939).

⁵⁵⁶ F. Krafft and J. Bürger, *Ber.*, 17, 1378-1380 (1884).

⁵⁵⁷ F. Krafft and F. Tritschler, *Ber.*, 33, 3580-3585 (1900).

⁵⁵⁸ K. Täufel and F. Künkele, *Fettechem. Umschau*, 42, 27-29 (1935); *Chem. Abst.*, 29, 3307 (1935).

⁵⁵⁹ D. Bardan, *Bull. soc. chim.* [5], 1, 141-146 (1934).

ride⁵⁶⁰ were obtained when thionyl chloride was used. All of the acid chlorides from C₈ to C₁₈ have been prepared by the employment of this reagent.⁵⁶¹ Silicon tetrachloride has found an application in the synthesis of butyryl chloride,⁵⁶² as well as in that of several other lower fatty acids,⁵⁶³ but it is apparently not satisfactory for the preparation of the halides of the long-chain fatty acids.

Another procedure which can be used for the synthesis of the acid chlorides involves phosgene, COCl₂. When the acid vapors and phosgene are passed over such a contact catalyst as charcoal at a high temperature,⁵⁶⁴ or when the sodium salts are heated with phosgene in a closed vessel,⁵⁶⁵ the acid chloride results. The reaction can be effected with the higher fatty acids by passing the fatty acid vapors and phosgene over charcoal⁵⁶⁶ at 150°C. or by bubbling phosgene through the fatty acid containing a tertiary amine⁵⁶⁷ at 100°C. Lauroyl chloride and palmitoyl chloride have been prepared from the corresponding acids in 90 and 75% yields, respectively, by this latter procedure.⁵⁶⁸

Adams and Ulich⁴⁹³ have reported that the higher fatty acids react almost quantitatively with oxalyl chloride, either directly or in a solvent. Caproyl, capryl, lauroyl, myristoyl, and stearoyl chlorides have been synthesized by the use of oxalyl chloride,⁵⁶⁹ as well as a series of acid chlorides of unsaturated acids (oleoyl, elaidoyl, linoleoyl, and linolenoyl chlorides).³⁶⁰ Acid anhydrides may likewise be used as a starting material when oxalyl chloride is employed.

b'. Properties of the Acid Halides: The acid chlorides of the fatty acids with shorter chains than stearic acid are liquid at usual temperatures. They can all be distilled without decomposition but, with the higher members, this must be carried out under reduced pressure. As one might predict, the fatty acid halides are readily soluble in organic solvents; however, when the solvent contains a replaceable hydrogen, the fatty acid halide will react with it. One of the most striking properties is the pronounced reaction with water, which results in the formation of the fatty acid and hydrogen chloride. This takes place promptly with the lower members of the series, and somewhat more slowly with the longer chain compounds.

⁵⁶⁰ C. R. Noller and R. A. Bannerot, *J. Am. Chem. Soc.*, *56*, 1563-1565 (1934).

⁵⁶¹ B. F. Daubert, H. H. Fricke, and H. E. Longenecker, *J. Am. Chem. Soc.*, *65*, 2142-2144 (1943).

⁵⁶² G. Rauter, *Ann.*, *270*, 235-266 (1892).

⁵⁶³ R. E. Montonna, *J. Am. Chem. Soc.*, *49*, 2114-2116 (1927).

⁵⁶⁴ A. Hochstetter, *German Patent* No. 283,896 (Aug. 17, 1913).

⁵⁶⁵ A. Hochstetter, *German Patent* No. 284,617 (Aug. 17, 1913).

⁵⁶⁶ I. G. Farbenind., A.-G., *Brit. Patent* No. 515,963 (Dec. 19, 1939); *German Patent* No. 687,670 (Jan. 11, 1940); *Chem. Abst.*, *35*, 5911, 3268 (1941).

⁵⁶⁷ Soc. pour l'ind. chim. à Bâle, *Brit. Patent* No. 540,096 (Oct. 6, 1941); *Chem. Abst.*, *36*, 4136 (1942).

⁵⁶⁸ J. Prat and A. Étienne, *Bull. soc. chim.* [5], *11*, 30-34 (1944).

⁵⁶⁹ H. P. Averill, J. N. Roche, and C. G. King, *J. Am. Chem. Soc.*, *51*, 866-872 (1929).

Table 48 records some of the physical constants for the fatty halides which have been investigated. Densities reported⁵ are somewhat higher than for the corresponding fatty acids. The figures (d_4^4) for some of these are as follows: butyryl chloride, 1.0277²⁰; valeryl chloride, 1.0155¹⁵; heptanoyl chloride, 0.95219³⁰; caprylyl chloride, 0.94000³⁰; and nonanoyl chloride, 0.93353³⁰. The refractive index has been reported for butyryl chloride as 1.41209 (n_D^{20}) and for heptanoyl bromide⁵⁷⁰ as 1.4605 (n_D^{18}).

TABLE 48

FREEZING POINTS AND BOILING POINTS OF ACID CHLORIDES OF SOME SATURATED AND UNSATURATED ACIDS^a

Common name of chloride	Systematic name of chloride	F.p., °C.	B.p., °C. c
Butyryl	Butanoyl	— 89.0	101–102
Valeryl	Pentanoyl	—110.0	107–110
Caproyl	Hexanoyl	— 87.3	138–140
Enanthyl	Heptanoyl	— 83.8	175.2
Caprylyl	Octanoyl	— 61.0	195.6; 83 ¹⁵
Pelargonyl	Nonanoyl	— 60.5	215.4; 98 ¹⁵
Capryl	Decanoyl	— 34.5	232.3; 104–105 ⁹
Lauroyl	Dodecanoyl	— 17	150 ²²
Myristoyl	Tetradecanoyl	— 1	168 ¹⁵ ; 134 ^{2.5}
—	Pentadecanoyl	—	157 ⁵
Palmitoyl	Hexadecanoyl	12	192.5 ¹⁵
Stearoyl	Octadecanoyl	23	198–200 ¹⁵ ; 165 ^{0.4}
Oleoyl ^b	<i>cis</i> -9-Octadecenoyl	—	158–159 ^{0.5}
Elaidoyl	<i>trans</i> -9-Octadecenoyl	—	216 ¹³
Linoleoyl	9,12-Octadecadienoyl	—	167–168 ^{2.3}
—	10-Undecenoyl	—	128.5 ¹⁴

^a Largely adapted from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 809.

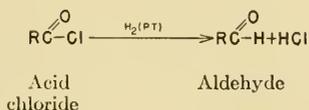
^b P. E. Verkade, *Rec. trav. chim.*, 62, 393–397 (1943).

^c The superior numbers refer to the barometric pressure in millimeters of mercury at which the boiling point was determined.

c'. Reactions of Acid Halides: These compounds are highly reactive, and hence they find wide application in the synthesis of many fatty acid derivatives. They react with alcohols to form esters (see page 114). On treatment with ammonia or amines, they form amides or substituted amides (see page 136). Nitriles can likewise result from this reaction. They are especially useful in the synthesis of ketones by the application of the Friedel-Craft reaction (see page 143).

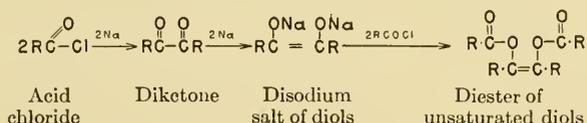
On reduction of the halides in the presence of metallic platinum, palladium, or nickel oxide, the fatty acid aldehydes are formed:

⁵⁷⁰ A. Kirrmann, *Ann. chim.* [10], 11, 223–236 (1929).

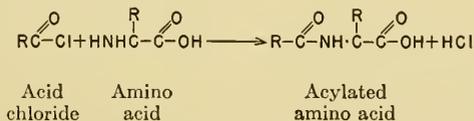


This reaction was first successfully applied by Rosenmund,⁵⁷¹ and has been used with a wide variety of acid halides^{572,573}; with the dichloride of the dibasic acid, sebacic acid, a dialdehyde results when 2% palladium-calcium carbonate is present as a catalyst.⁵⁷⁴

An entirely different reaction occurs when the acid chlorides of the higher acids are reduced in boiling ether with sodium amalgam.



Of the many other reactions in which the acid halides participate, several deserve special mention. Ketenes result from the removal of HCl from the higher members of the series.⁵⁷⁵ Fatty acid peroxides are formed when alkali or alkaline earth peroxides act on the acid chlorides.⁵⁷⁶⁻⁵⁷⁸ Probably the most interesting reaction in which the acid chlorides are involved is that with amino acids. In this reaction, the chlorine and a hydrogen of the amino group unite to form hydrogen chloride, leaving the acyl radical free to combine with the amine group:



This introduction of acyl^{577,578} (particularly acetyl) groups into amino acids and amines is a reaction which can be effected under a wide variety of biological conditions. The acetylation reaction is generally considered to be a detoxication mechanism. It is, of course, highly improbable that such a reaction in the animal body makes use of the acid chlorides.

The fatty acid halides react with the sugars to form the polyhydric esters. Thus, α -pentapalmitoylglucose has been obtained in satisfactory

⁵⁷¹ K. W. Rosenmund, *Ber.*, 51, 585-593 (1918).

⁵⁷² V. Grignard and G. Mingasson, *Compt. rend.*, 185, 1173-1176 (1927).

⁵⁷³ R. Escourrou, *Bull. soc. chim.* [5], 6, 1173-1181 (1939).

⁵⁷⁴ E. Waser, *Helv. Chim. Acta*, 8, 117-125 (1925).

⁵⁷⁵ A. Bistrzycki and A. Landtwing, *Ber.*, 42, 4720-4723 (1909).

⁵⁷⁶ W. B. Stoddard and V. R. Kokatnur, *U. S. Patent* No. 1,713,609 (June 25, 1929).

⁵⁷⁷ A. Hopwood and C. Weizmann, *Proc. Chem. Soc. London*, 26, 69-70 (1910); 27, 55-56 (1911); *J. Chem. Soc.*, 99, 571-576, 1577-1585 (1911).

⁵⁷⁸ A. Hopwood, *Proc. Chem. Soc. London*, 29, 345 (1913). C. Weizmann and A. Hopwood, *Proc. Roy. Soc. London*, A83, 455-461 (1913).

yield when glucose in pyridine and palmitoyl chloride in chloroform⁵⁷⁹ are mixed at -10°C . Many other similar derivatives have been prepared by this reaction, including the sugar, hendecastearoyl raffinose, into which 11 acyl molecules have been introduced.

(2) *Reactions of Fatty Acids Not Related to Carboxyl Group*

There are a number of reactions on the part of the fatty acids and their triglycerides which are referable to the aliphatic chain and not to the carboxyl group. Acids which possess unsaturated linkages or a reactive group such as the hydroxyl radical on the side chain readily undergo numerous changes. The principal reactions of interest to the fat chemist are hydrogenation, halogenation, and oxidation.

a. Hydrogenation. The reactions of hydrogen with organic compounds are of varied patterns. On the one hand, hydrogen may add to unsaturated linkages, thereby causing a partial or a complete saturation of the product. On the other hand, this element may bring about reduction, as for example that of a fatty acid to the corresponding alcohol. The latter process is often referred to as *hydrogenolysis* to distinguish it from the more frequent addition reaction known as *hydrogenation*. The present discussion is concerned only with the latter type of reaction.

A number of methods of hydrogenation have long been available. These include those procedures in which hydrogen is generated by water or acid with sodium, zinc, or other metals, or by the use of hydrazine or such reducing agents as hydriodic acid. All of these changes involve the production of so-called "nascent" hydrogen, which is able to react in a manner impossible for molecular hydrogen.

The advent of catalytic hydrogenation as a result of the classical experiments of Sabatier and Senderens,⁵⁸⁰⁻⁵⁸⁴ which have been summarized in the monograph of Sabatier,⁵⁸⁵ has made possible the extensive application of this technic not only to fatty acids and more especially their triglycerides, but also to a large number of related products. Although Goldschmiedt⁵⁸⁶ had demonstrated the conversion of oleic to stearic acid previous to this time, by treatment of the unsaturated acid with fuming hydriodic acid and phosphorus, no widespread commercial application of hydrogenation

⁵⁷⁹ K. Hess and E. Messmer, *Ber.*, *B54*, 499-523 (1921).

⁵⁸⁰ P. Sabatier and J. B. Senderens, *Compt. rend.*, *128*, 1173-1176 (1899).

⁵⁸¹ P. Sabatier and J. B. Senderens, *Compt. rend.*, *130*, 1761-1764 (1900).

⁵⁸² P. Sabatier and J. B. Senderens, *Compt. rend.*, *132*, 210-212, 566-568, 1254-1257 (1902).

⁵⁸³ P. Sabatier and J. B. Senderens, *Compt. rend.*, *134*, 1127-1130 (1902).

⁵⁸⁴ P. Sabatier and J. B. Senderens, *Compt. rend.*, *135*, 87-89 (1902).

⁵⁸⁵ P. Sabatier, *Catalysis in Organic Chemistry*, translated by E. E. Reid, Van Nostrand, New York, 1923.

⁵⁸⁶ G. Goldschmiedt, *Sitzber. Akad. Wiss. Wien, Math. naturw. Klasse, Abt. II*, *72*, 366-375 (1875).

was possible until the discovery of the catalytic process. The industrial development was stimulated by the research of Normann,⁵⁸⁷ who patented the catalytic transformation of liquid marine and vegetable oils into solid white fats. Ipatieff⁵⁸⁸ helped to introduce the modern methods by demonstrating that reactions were possible with high-pressure hydrogenation which could not occur under the conditions formerly employed.

In addition to the several treatises on hydrogenation already mentioned, there are a number of other reviews and monographs worthy of attention,⁵⁸⁹⁻⁵⁹⁷

(a) *Methods of Hydrogenation.* There are only four metals which have any considerable application as catalysts for hydrogenation. These are platinum, palladium, nickel, and copper. Various salts of these metals are used for the production of the catalyst, or the metals themselves are treated in various ways to improve their activity. The number of preparations of actual catalysts used is therefore quite large. Only nickel and copper compounds have extensive commercial application.

The type of catalyst employed is determined by the reaction which one desires to accelerate. The nickel catalysts are regularly used for the saturation of double bonds; copper chromite seems to serve best for the reduction of carbonyl groups, although sodium and alcohol without a catalyst are quite satisfactory also. Hydrogenation is carried out at temperatures ranging from ordinary room temperature to 400°C., while the pressures employed vary from atmospheric to 400 atmospheres. Higher pressures are to be preferred to the higher temperatures.

Nickel catalysts are poisoned by halogens, sulfur compounds, and certain nitrogenous products. Normann⁵⁹⁸ reported that sodium sulfate, sodium carbonate, sodium silicate, and even nickel sulfate partially decrease the action of reduced nickel catalysts. Other substances which lower the activity of nickel catalysts are metallic soaps, arsenic trioxide,⁵⁹⁹

⁵⁸⁷ W. Normann, *Brit. Patent No. 1515* (Jan. 21, 1903).

⁵⁸⁸ W. Ipatieff, *Ber.*, *36*, 2961-2985, 2986-3005 (1904).

⁵⁸⁹ C. Ellis, *Hydrogenation of Organic Substances*, 3rd ed., Van Nostrand, New York, 1930.

⁵⁹⁰ H. W. Lohse, *Catalytic Chemistry*, Chem. Pub. Co., Brooklyn, 1945.

⁵⁹¹ H. Adkins, *Reactions of Hydrogen with Organic Compounds over Copper-Chromium Oxide and Nickel Catalysts*, Univ. Wisconsin Press, Madison, 1937.

⁵⁹² E. F. Armstrong and K. A. Williams, *Chemistry & Industry*, *18* (59), 3-9 (1939).

⁵⁹³ E. F. Armstrong and K. A. Williams, *Chem. Age London*, *41*, 271-272, 285-288 (1939).

⁵⁹⁴ O. H. Wurster, *Ind. Eng. Chem.*, *32*, 1193-1199 (1940).

⁵⁹⁵ H. R. Mitchell, *Food Manuf.*, *18*, 369-373, 401-404 (1943); cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 360.

⁵⁹⁶ H. R. Mitchell, *Chem. Age London*, *48*, 471-475, 495-499 (1943).

⁵⁹⁷ A. E. Bailey, *Industrial Oil and Fat Products*, 2nd. Ed. Interscience, New York, 1951.

⁵⁹⁸ W. Normann, *Chem. Umschau*, *32*, 263-265 (1925); *Chem. Abst.*, *20*, 2590 (1926).

⁵⁹⁹ S. Ueno, *J. Soc. Chem. Ind. Japan*, *21*, 898-939 (1918); *Chem. Abst.*, *13*, 383-384 (1919).

organic acids such as oxalic, hydroxy-acids, glucose and protein. In the case of a mixed nickel-copper catalyst, it was found that the sodium and potassium soaps exerted a pronounced deleterious action, while magnesium, barium, calcium, lead, iron, manganese, zinc, and cadmium, when present as their soaps, had only a limited poisonous effect.⁶⁰⁰ Copper and silver soaps actually caused an augmentative action. Copper salts are added to nickel catalysts to prevent poisoning; they act as promoters, and will allow hydrogenation to proceed at a lower temperature than would otherwise be possible.⁶⁰¹ Additional compounds which are reported to have a promoting effect on nickel catalysts are the salts of beryllium, manganese, uranium, and tungsten, as well as the chromate, phosphate, molybdate and tungstate radicals.⁶⁰²

The wide variety of catalysts in use can be realized from the list of nickel compounds which have been employed. These include catalysts prepared by reduction of nickel oleate,^{603,604} nickel salts of naphthenic acids,⁶⁰⁵ nickel oxalate,⁶⁰⁶ nickel formate,^{607,608} mixed nickel and copper formates,⁶⁰⁹ and nickel carbonyl.⁶¹⁰ An active catalyst has been prepared by heating a mixture of nickel nitrate and sucrose.⁶¹¹ The most effective of all the hydrogenating catalysts is that first used by Raney,⁶¹² which is prepared by treating a nickel aluminum alloy with sodium hydroxide.^{613,614} Still other active nickel catalysts are nickel silicate and nickel borate, which are not readily poisoned.⁶¹⁵⁻⁶¹⁷ However, it is believed that the latter salt decomposes into the metal and boric acid on heating.⁶¹⁸ It has been stated

⁶⁰⁰ S. Ueno, Y. Miyake, and R. Anzai, *J. Soc. Chem. Ind. Japan*, 43, suppl., 434-435 (1940); *Chem. Abst.*, 35, 3113 (1941).

⁶⁰¹ J. Dewar and A. Liebmann, *U. S. Patent No. 1,275,405* (Aug. 13, 1918).

⁶⁰² Badische Anilin und Soda Fabrik, *Brit. Patent No. 2,306* (Jan. 28, 1914); *Chem. Abst.*, 10, 287-288 (1916).

⁶⁰³ C. H. Hausamann, *Canadian Patent No. 157,396* (Aug. 18, 1914).

⁶⁰⁴ C. Ellis, *U. S. Patent No. 1,217,118* (Feb. 20, 1917).

⁶⁰⁵ Deutsche Hydrierwerke, A.-G., *Brit. Patent No. 396,311* (Feb. 10, 1933).

⁶⁰⁶ E. C. Kayser, *U. S. Patent No. 1,236,446* (Aug. 14, 1917).

⁶⁰⁷ E. B. Higgins, *Brit. Patent Nos. 4,665* (Feb. 23, 1914) and 23,377 (Oct. 12, 1912); *Chem. Abst.*, 8, 789 (1914); 10, 974 (1916); *U. S. Patent No. 1,170,814* (Feb. 8, 1916).

⁶⁰⁸ F. Wittka, *Swiss Patent No. 151,955* (Nov. 15, 1930); *Chem. Abst.*, 26, 4828 (1932).

⁶⁰⁹ C. Ellis, *U. S. Patent No. 1,645,377* (Oct. 11, 1927).

⁶¹⁰ A. A. Shukoff, *German Patent No. 241,823* (Jan. 18, 1919).

⁶¹¹ W. P. Schuck, *Brit. Patent No. 122,192* (Jan. 6, 1919); *Canadian Patent No. 200,591* (June 1, 1920); *U. S. Patent No. 1,305,173* (May 27, 1919).

⁶¹² M. Raney, *U. S. Patents Nos. 1,628,190* (May 10, 1927) and 1,915,473 (June 27, 1933).

⁶¹³ R. Paul and G. Hilly, *Bull. soc. chim.* [5], 3, 2330-2332 (1936).

⁶¹⁴ R. Mazingo, *Org. Syntheses*, 21, 15-17 (1941).

⁶¹⁵ H. Schönfeld, *Seifensieder-Ztg.*, 41, 945-946 (1914); *Chem. Abst.*, 8, 3868 (1914).

⁶¹⁶ Müller Speisefettfabrik, A.-G., *Brit. Patent Nos. 7670* and 7671 (Mar. 26, 1914).

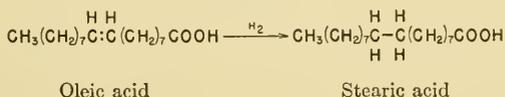
⁶¹⁷ C. Ellis, *U. S. Patent No. 1,255,590* (Feb. 5, 1918).

⁶¹⁸ W. Normann, *Seifensieder-Ztg.*, 42, 46-47 (1915); *Chem. Abst.*, 9, 1552 (1915).

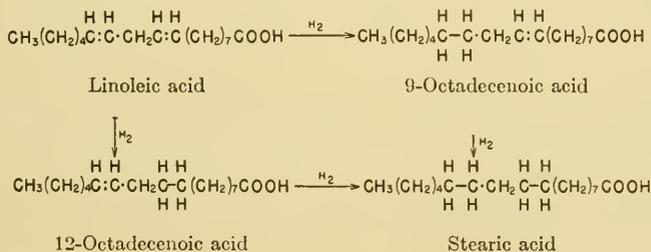
that a mixture of nickel and boron trioxide gives an active catalyst.⁶¹⁹ Nickel silicate has been reported to be the most active nickel salt when compared with the tungstate, borate, chromate, and molybdate salts.⁶²⁰

Although platinum catalysts are not generally used in commercial operations, they are useful in laboratory-scale syntheses. They are less specific than are the nickel catalysts.⁶²¹ Mixed catalysts containing platinum or palladium and nickel⁶²²⁻⁶²⁴ have been suggested. Platinum catalysts are poisoned by hydrogen sulfide, phosphine, mineral acids, carbon disulfide, chloroform, and liquid hydrocarbons.⁶²⁵

(b) *Mechanism of Catalytic Hydrogenation.* When hydrogenation is carried out with oleic acid, one mole of hydrogen is added per mole of oleic acid, with the formation of one molecule of stearic acid as follows:



When linoleic acid is used as the starting material, three products can originate: 9-octadecenoic acid, 12-octadecenoic acid, or stearic acid:



In the case of the reaction which results in the formation of stearic acid, only one end product results, since it is completely symmetric and entirely saturated. However, when linoleic acid is the starting point, the intermediate products may be either *cis* or *trans*, so that at least four different isooleic acids may result, namely *cis*- and *trans*-9-octadecenoic acids and *cis*- and *trans*-12-octadecenoic acids. It is quite obvious that the situation becomes still more complicated when a triethenoid acid such as linolenic acid is partially saturated.

Variations in the end products produced on hydrogenation of di- or poly-

⁶¹⁹ C. Bosch, A. Mittasch, and C. Schneider, *U. S. Patent* No. 1,215,334 (Feb. 13, 1917).

⁶²⁰ L. Kahlenberg and T. P. Pi, *J. Phys. Chem.*, **28**, 59-70 (1924).

⁶²¹ A. S. Richardson and A. O. Snoddy, *Ind. Eng. Chem.*, **18**, 570-571 (1926).

⁶²² E. B. Higgins, *U. S. Patent* No. 1,170,814 (Feb. 8, 1916).

⁶²³ C. Paal, *U. S. Patent* No. 1,222,660 (Apr. 17, 1917).

⁶²⁴ N. Sulzberger, *U. S. Patent* No. 1,338,709 (May 4, 1920).

⁶²⁵ F. Goldschmidt, *Seifenfabrik.*, **32**, 713-720 (1912); *Chem. Abst.*, **6**, 2550-2551 (1912).

ethenoid acids may be due to the shifting of the double bonds. Thus, the hydrogenation of linoleic acids results not only in the production of 9- or 12-octadecenoic acids but also in the formation of the 8- and 10-octadecenoic acids.⁶²⁶ The latter two isooleic acids have likewise been demonstrated by Hilditch and Vidyarthi⁶²⁷ when methyl oleate was hydrogenated in the presence of metallic nickel at 217–220°C. These results were later substantiated by Hilditch,⁶²⁸ although he used a lower temperature (180–200°C.). Van der Veen⁶²⁶ suggested that the formation of the 8- and 10-octadecenoic acids is best explained by a shift in the double bonds of oleic acid as it is formed. According to Moore⁶²⁹ such isomerization can occur only during the actual hydrogenation. On the other hand, normal oleic acid results during the hydrogenation of isooleic acids. However, although this has been considered to be an equilibrium reaction, the ratio of isooleic to oleic acid differs widely, depending upon whether oleic or isooleic acid is the initial material hydrogenated. This fact would indicate that at least one component of the isooleic acid mixture can revert to the original oleic acid. The extent of the reaction in either direction varies with the experimental conditions. A high temperature and, to a lesser extent, a large amount of catalyst, are conducive to the formation of a higher proportion of the iso-acids than of ordinary oleic acid. Ethyl esters are reported to form more iso-acids than is the case with the triglycerides.⁶³⁰

In addition to the shifting of the double bond during hydrogenation, geometrical isomerism is also a feature. Thus, when methyl oleate is partially hydrogenated to the extent of 30%, elaidic acid (*trans*-9-octadecenoic acid) is produced, as well as the aforementioned positional isomers.⁶²⁷ Conversely, when elaidic acid esters are hydrogenated, the corresponding oleic acid esters result.^{4,630} This transformation proceeds more smoothly with the ethyl esters than with the triglycerides.⁶³⁰

The so-called "selective" hydrogenation was first described by Moore and associates⁶³¹ in 1917, and has been amply confirmed^{632–634} since the pioneer investigations. According to this concept, *cis-cis*-9,12-octadecadienoic or its esters are practically completely transformed to the monoethenoid acid before a saturation of the second double bond occurs. According to

⁶²⁶ H. Van der Veen, *Chem. Umschau*, 38, 89–96 (1931); *Chem. Abst.*, 25, 2972 (1931).

⁶²⁷ T. P. Hilditch and N. L. Vidyarthi, *Proc. Roy. Soc. London*, A122, 552–563 (1929).

⁶²⁸ T. P. Hilditch, *Chem. Umschau*, 37, 354–356 (1930); *Chem. Abst.*, 25, 1800 (1931).

⁶²⁹ C. W. Moore, *J. Chem. Soc. Ind.*, 38, 320–325T (1919).

⁶³⁰ A. Steger and H. W. Scheffers, *Chem. Umschau*, 38, 45–53 (1931); *Chem. Abst.*, 25, 2315 (1931).

⁶³¹ H. K. Moore, G. A. Richter, and W. B. Van Arsdell, *J. Ind. Eng. Chem.*, 9, 451–462, (1917).

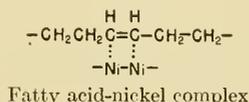
⁶³² A. S. Richardson, C. A. Knuth, and C. H. Milligan, *Ind. Eng. Chem.*, 16, 519–522, (1924); 17, 80–83 (1925).

⁶³³ T. P. Hilditch and C. W. Moore, *J. Soc. Chem. Ind.*, 42, 15–17 T (1923).

⁶³⁴ D. R. Dhingra, T. P. Hilditch, and A. J. Rhead, *J. Soc. Chem. Ind.*, 51, 195–198T, (1932).

Hilditch and Vidyarthi,⁶³⁵ over 90% of methyl linoleate or methyl linoleate is transformed into the monoethenoid compound before any saturated esters are formed. In the case of the various monoethenoid acids, it has been shown that hydrogenation occurs most readily in the 9,10-position and with most difficulty when it is adjacent to the carboxyl group, *i.e.*, in 2,3-position.⁶³⁶ This is similar to the ease with which halogens are added when the unsaturated linkage occupies the different positions.⁶³⁷

The mechanism whereby catalysts effect the addition of hydrogen is not known. However, it is supposed that the catalyst first reacts with the double bond to form an adsorption complex at the surface of the molecule:



This compound then reacts with hydrogen to regenerate the catalyst with the evolution of heat and with the simultaneous production of the compound containing the two added hydrogen atoms.

b. Halogenation. Chlorine, bromine, iodine, and fluorine combine readily with unsaturated acids and their esters, including the triglycerides, to yield saturated compounds. The reaction between fluorine and the saturated hydrocarbons may proceed with explosive violence, with the production of hydrogen fluoride or carbon fluorides.⁶³⁸ The reaction will occur even when inert carbonaceous material such as lampblack is exposed to fluorine.⁶³⁹ However, when oleic acid is fluorinated, two difluorostearic acids, which melt at 81° and 95°C., respectively,⁵ are produced by a normal reaction. Elaidic acid under similar conditions gives rise to a difluorostearic acid⁵ having a melting point of 84–85°C. Lead tetrafluoride serves as an excellent fluorinating agent.⁶⁴⁰

An example of simple halogenation of unsaturated acids is afforded by the determination of the iodine number. Since free iodine reacts with fat very slowly, it is usually combined with other substances to facilitate the reaction. One of the earliest reagents for this use was the so-called Hübl solution, which contains an alcoholic solution of iodine in the presence of mercuric chloride.⁶⁴¹ Later modifications were the Wijs reagent,^{642,643} in

⁶³⁵ T. P. Hilditch and N. R. Vidyarthi, *Proc. Roy. Soc. London*, A122, 563–570 (1929).

⁶³⁶ G. V. Pigulevskii and P. A. Artamonov, *J. Gen. Chem. U. S. S. R.*, 12, 510–517, (1942); *Chem. Abst.*, 37, 2716 (1943).

⁶³⁷ G. Ponzio and C. Gastaldi, *Gazz. chim. ital.*, 42, II, 92–95 (1912).

⁶³⁸ B. Humiston, *J. Phys. Chem.*, 23, 572–577 (1919).

⁶³⁹ H. Moissan, *Compt. rend.*, 110, 276–279 (1890).

⁶⁴⁰ O. Dimroth and W. Bockemüller, *Ber.*, 64, 516–522 (1931).

⁶⁴¹ Baron Hübl, *Dinglers Polytech. J.*, 253, 281–295 (1884).

⁶⁴² J. J. A. Wijs, *Ber.*, 31, 750–752 (1898).

⁶⁴³ J. J. A. Wijs, *Z. Untersuch. Nahr. Genussm.*, 1, 561 (1898).

which iodine monochloride is employed in glacial acetic acid, and the Hanus method,⁶⁴⁴ which involves the use of iodine monobromide in place of the iodine monochloride. The Hanus solution is more easily prepared than the Wijs reagent, and is more stable, according to Bull.⁶⁴⁵ The Wijs reagent lasts about a month while the Hanus solution remains satisfactory for at least a year. Both the Hanus and Wijs procedures are in current use in the United States, but the latter method is employed more in England and Europe. According to Bull.⁶⁴⁵ the results are 2 to 4% lower with the Hanus than with the Wijs technic, according to Lewkowitsch, 5 to 6% lower.⁶⁴⁶ The Association of Official Agricultural Chemists has adopted the Hanus method as the official one, while the Committee on Analysis of Commercial Fats and Oils of the Division of Industrial Chemists and Chemical Engineers of the American Chemical Society has accepted the Wijs procedure as standard.⁶⁴⁷ A comparison of the three methods mentioned above has been reported by Hunt⁶⁴⁸ and by Tolman and Munson.⁶⁴⁹ Excellent chlorinating reagents are sulfur monochloride, (SOCl), thionyl chloride (SOCl₂), phosphorus trichloride (PCl₃), phosphorus pentachloride (PCl₅), hypochlorous acid (HOCl), oxalyl chloride (COCl-COCl), phosgene (COCl₂), and halogen hydrides.

(a) *Factors Altering the Addition of Halogens.* One of the most important factors which control the rate of reaction of the monoethenoid acids is the position of the double bond. Ponzio and Gastaldi⁶³⁷ were able to introduce only 50% of the theoretical amount of iodine into 2-octadecenoic acid, even after 70 hours' reaction, while Eckert and Halla⁶⁵⁰ have found iodine values of 9.0, 16.3, and 27.0 for 2-, 3-, and 4-octadecenoic acids, respectively, compared with the theoretical value of 89.9.

In the case of the di- or polyethenoid acids, the rate and completeness of the addition of the halogens depend not only on the position of the unsaturated linkages in the aliphatic chain, but also on their position relative to each other. Most nonconjugated polyethenoid acids add halogens normally. In the case of linoleic acid, bromine adds first to the 12,13-linkage and then to the 9,10-positions,⁶⁵¹ but the total amount added is 100% of that possible. A stepwise addition of bromine has been noted for linolenic acid, as well, the order being the 15,16-, then the 12,13-, and

⁶⁴⁴ J. Hanus, *Z. Untersuch Nahr. Genussm.*, **4**, 913-920 (1901).

⁶⁴⁵ H. B. Bull, *The Biochemistry of the Lipids*, Wiley, New York, 1937.

⁶⁴⁶ J. I. Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes*, 6th ed., Vol. I, Macmillan, London, 1921, p. 409; 1913 ed. p. 408.

⁶⁴⁷ Anonymous, "Standard Methods for the Sampling and Analysis of Commercial Fats and Oils," *Ind. Eng. Chem.*, **11**, 1161-1168 (1919).

⁶⁴⁸ F. W. Hunt, *J. Soc. Chem. Ind.*, **21**, 454-456 (1902).

⁶⁴⁹ L. M. Tolman and L. S. Munson, *J. Am. Chem. Soc.*, **25**, 244-251 (1903).

⁶⁵⁰ A. Eckert and O. Halla, *Monatsh.*, **34**, 1815-1824 (1913).

⁶⁵¹ Y. Toyama and T. Tsuchiya, *J. Soc. Chem. Ind. Japan*, **38**, suppl., 35-36, 36-38B (1935); *Chem. Abst.*, **29**, 2509 (1935).

finally the 9,10-linkages.⁴ When the double bonds are conjugated, the addition of the halogens is not complete. For example, 9,10,12,13-octadecadienoic acid adds only one mole of halogen immediately, while the second mole combines slowly and the reaction is still incomplete after many hours. In the case of a doubly conjugated acid such as elaeostearic acid, halogens add normally to only two of the three unsaturated linkages.

c. Oxidation of Aliphatic Chains. The unsaturated fatty acids are quite resistant to oxidation, while the unsaturated derivatives are quite susceptible to a variety of oxidizing agents. When they are oxidized in the animal body under optimum conditions, or outside the body under a high oxygen pressure in a bomb, the end products are exclusively carbon dioxide and water. However, in the case of less stringent measures, many intermediate products have been reported for the saturated acids. The unsaturated acids are oxidized with considerable ease, forming hydroxy-acids, ketones, and finally breaking down by rupture of the double bonds to shorter chain mono- or di-carboxylic acids.

(a) *Oxidation of Saturated Acids.* The saturated acids are not oxidized by air, but they are attacked when treated with oxygen at elevated temperatures in the presence of catalysts. When stearic acid is treated in this manner, some of the products identified include shorter chain mono-carboxylic acids, dicarboxylic acids, lactones, lactonic acids, and carbon dioxide.⁶⁵² Hydroxy-acids have been reported in substantial amounts on oxidation of stearic acid⁶⁵³ or when palmitic acid was treated with hydrogen peroxide in the presence of cupric salts.⁶⁵⁴ When capric, caprylic, caproic, valeric, and butyric acids were treated with H_2O_2 in the presence of catalysts, carbon dioxide was found to be an end product.⁶⁵⁵ Acetic acid, a resistant intermediate product, was also found.

Oxidation of the saturated acids with concentrated nitric acid leads to the formation of dicarboxylic acids. Myristic acid gives rise to oxalic, succinic, glutaric, pimelic, adipic, and suberic acids.⁶⁵⁶ Dieterle⁶⁵⁷ reported suberic and sebacic acids from stearic acid, while Carrette⁶⁵⁸ found only succinic and glutaric acids. It would thus seem that the acids which result depend upon the intensity of the oxidation. Potassium permanganate in acetone has been shown to change caproic and heptanoic acids to shorter

⁶⁵² A. H. Salway and P. N. Williams, *J. Chem. Soc.*, 121, 1343-1348 (1922).

⁶⁵³ E. Zerner, *Naturprodukte*, 1923, 83-94; *Chem. Abst.*, 18, 112 (1924).

⁶⁵⁴ I. Smedley-MacLean and M. S. B. Pearce, *Biochem. J.*, 28, 486-494 (1934).

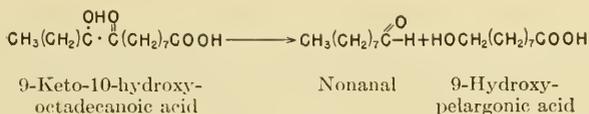
⁶⁵⁵ R. H. Allen and E. J. Witzemann, *J. Am. Chem. Soc.*, 63, 1922-1927 (1941).

⁶⁵⁶ H. Noerdlinger, *Ber.*, 19, 1893-1899 (1836).

⁶⁵⁷ W. I. Dieterle, *Über die Oxydationsprodukte reiner Stearinsäure durch Salpetersäure.*

II. Beitrag zur Kenntnis der Adipinsäure. Bern, G. F. Rapp, Cannstatt, 1883, 66 pp.; cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 388.

⁶⁵⁸ H. Carrette, *Compt. rend.*, 102, 692-693 (1886).



The reactions between air and the unsaturated acids are of considerable importance because they form the basis for several interesting properties of the fats. In the first place, rancidity results from the development of this reaction. Before rancidity can be detected by odor or taste, the formation of peroxides occurs; this corresponds to the so-called induction period. The presence of these peroxides can be quantitatively determined by organoleptic tests which depend upon the liberation of iodine from hydrogen iodide.^{670,671} An extensive discussion of rancidity, as well as of conditions which promote or retard it, is included in Chapter III.

The production of a hard film of paint likewise depends on the oxidation of the double bonds. Thus, trilinolenin is most suitable as a component of drying oils, while the triglyceride of the monoethenoid acid, oleic, is useless for this purpose. Elaeostearic acid is even more efficient as a constituent in a drying oil than is the corresponding non-conjugated triethenoid acid, linolenic, because of the conjugated position of the double bonds.

(c) *Oxidation with Various Oxidizing Agents.* There are a number of different oxidizing agents which can be used for oxidation of the unsaturated acids. The end products vary with the reagent employed. Although concentrated nitric acid was formerly the most common oxidizing agent, it has been used but little in recent years.

The results of chromic acid, when used with the unsaturated acids, differ according to the conditions of the oxidation. When the reaction is carefully controlled, hydroxy- and keto-acids result. With more strenuous oxidation procedures, the end products of oleic acid may be the monobasic saturated acids from pelargonic to acetic, and the dicarboxylic acids⁶⁷² from C₉ to C₂. However, when oleic acid was refluxed with 2% potassium dichromate acidified with sulfuric acid, no reaction occurred for 4 days, but after 10 days, pelargonic (nonanoic) and azelaic acids were produced.⁶⁷² Silver chromate is an excellent oxidizing agent which decomposes fatty acids completely to carbon dioxide.⁶⁷³ Chromic anhydride, on the other hand, does not effect as complete a destruction as the silver salt, since the extent of breakdown depends upon the molecular configuration. Simon⁶⁷³ calculated the *oxidation deficiency* by a formula employing the data obtained by oxidation with both silver chromate and chromic anhydride

⁶⁷⁰ A. Taffel and C. Revis, *J. Soc. Chem. Ind.*, 50, 87-91T (1931).

⁶⁷¹ C. H. Lea, *Proc. Roy. Soc. London*, B108, 175-189 (1931).

⁶⁷² F. G. Ehmed, *J. Chem. Soc.*, 73, 627-634 (1898).

⁶⁷³ L. G. Simon, *Compt. rend.*, 174, 1706-1708 (1922); 175, 1070-1072 (1922); 179, 975-977 (1924); 180, 673-675, 833-836, 1405-1407 (1925).

This constant is of some value in indicating the complexity of molecular structure.

Potassium permanganate is probably the most widely used oxidizing agent for unsaturated acids. When the reaction is carried out in an aqueous alkaline medium at 0° to 30°C., the products formed are predominantly polyhydroxy-acids, which are usually geometric isomers of the original acids.⁶⁷⁴ If an excess of alkali is avoided, the end products of the oxidation of oleic acid with permanganate are 9-hydroxy-10-ketostearic and 9-keto-10-hydroxystearic acids.^{675,676} On the other hand, when a non-aqueous medium such as acetone is used,⁶⁷⁷ or when an acidic solution is employed at an elevated temperature, fission results through the rupture of the fatty acids at the double bonds.⁶⁷⁷

The polyhydroxy-acids which were shown by Hazura⁶⁷⁸ to be produced by alkaline permanganate oxidation of a number of unsaturated acids are listed in Table 49.

TABLE 49
PRODUCTS OF ALKALINE PERMANGANATE OXIDATION OF UNSATURATED ACIDS OBTAINED BY HAZURA^a

Acid oxidized		Principal oxidation product	
Name	Formula	Hydroxy acid	Formula
Oleic	C ₁₈ H ₃₄ O ₂	Dihydroxystearic	C ₁₈ H ₃₄ O ₂ (OH) ₂
Elaidic	C ₁₈ H ₃₄ O ₂	Isodihydroxystearic	C ₁₈ H ₃₄ O ₂ (OH) ₂
Ricinoleic	C ₁₈ H ₃₃ O ₂ (OH)	Trihydroxystearic	C ₁₈ H ₃₃ O ₂ (OH) ₃
Linoleic	C ₁₈ H ₃₂ O ₂	Sativic	C ₁₈ H ₃₂ O ₂ (OH) ₄
Linolenic	C ₁₈ H ₃₀ O ₂	Linusic	C ₁₈ H ₃₀ O ₂ (OH) ₆
Erucic	C ₂₂ H ₄₂ O ₂	Dihydroxybehenic	C ₂₂ H ₄₂ O ₂ (OH) ₂
Brassicidic	C ₂₂ H ₄₂ O ₂	Isodihydroxybehenic	C ₂₂ H ₄₂ O ₂ (OH) ₂
Undecenoic	C ₁₁ H ₂₀ O ₂	Dihydroxyundecanoic	C ₁₁ H ₂₀ O ₂ (OH) ₂

^a K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 394 (from K. Hazura, *Monatsh.*, 8, 260-270 (1887)).

Hydrogen peroxide and related peroxides react with the unsaturated acids. Although no reaction would be detected with oleic acid at ordinary temperatures,⁶⁷⁹ Hilditch⁶⁸⁰ demonstrated the formation of dihydroxystearic acid when oleic acid was refluxed for 8 hours with hydrogen peroxide. Benzoyl peroxide, (C₆H₅CO)₂O₂, oxidized oleic acid to dihydroxystearic acid after treatment in dry ether at 2-3°C. for 2 days.⁶⁸¹ Such per acids

⁶⁷⁴ A. Saytzeff, *J. prakt. Chem.* [N.S.], 33, 300-318 (1886).

⁶⁷⁵ D. Holde and J. Marcusson, *Ber.*, 36, 2657-2662 (1903).

⁶⁷⁶ G. King, *J. Chem. Soc.*, 1936, 1788-1792.

⁶⁷⁷ E. F. Armstrong and T. P. Hilditch, *J. Soc. Chem. Ind.*, 44, 43-47T (1925).

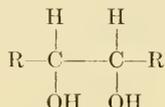
⁶⁷⁸ K. Hazura, *Monatsh.*, 8, 260-270 (1887).

⁶⁷⁹ K. Hazura, *Monatsh.*, 9, 469-474 (1888).

⁶⁸⁰ T. P. Hilditch, *J. Chem. Soc.*, 129, 1828-1836 (1926).

⁶⁸¹ G. V. Pigulevskii and M. A. Petrov, *J. Russ. Phys. Chem. Soc.*, 58, 1062-1066 (1926); *Chem. Abst.*, 22, 943 (1928).

tion. For example, periodic acid, H_5IO_6 , prepared by the interaction of sulfuric acid and barium or potassium periodate, brings about fission of 9-keto-10-hydroxystearic and 9-hydroxy-10-ketostearic acids between carbons 9 and 10⁶⁷⁶; this also occurs with dihydroxystearic acid.⁶⁹² Lead tetraacetate, a reagent developed by Criegee⁶⁹³⁻⁶⁹⁵ for the cleavage of glycols of the form:



acts in a highly specific manner to cause fission between the carbons containing the two hydroxyl groups. 9,10-Dihydroxystearic acid is decomposed by lead tetraacetate to *n*-nonanal ($\text{CH}_3(\text{CH}_2)_7\text{CHO}$) and azelaic semialdehyde ($\text{CHO}(\text{CH}_2)_7\text{COOH}$).⁴⁴⁴ The rate at which polyhydroxyacids are oxidized by Criegee's reagent is quite variable. The two low-melting forms of 9,10,12,13-tetrahydroxystearic acids are more rapidly oxidized than the high-melting forms, while the same variation is noted for the low-melting (m.p., 95°C.) and high-melting (m.p., 132°C.) forms of 9,10-dihydroxystearic acid.⁶⁹⁶

d. Other Reactions of Aliphatic Chain of Fatty Acids. Hydrohalogenation, *i. e.*, the addition of a halogen halide, HX , to an unsaturated carbon-to-carbon linkage, is a common reaction. This occurs with the acids of the four halogens. The reaction proceeds most readily with hydrogen iodide, is somewhat slower with hydrogen bromide, and may require several days for completion with hydrogen chloride.^{697,698} The relationship of hydrogen fluoride is less clear. The hydrohalogenation can occur in the presence or absence of solvents. Two isomers are possible with a monothenoid acid in which the positions of the hydrogen and halide atoms are reversed.

The position taken by the halide is influenced by several factors. It usually adds to the carbon having the fewer hydrogens.⁶⁹⁹ The position of the double bond in relation to the carboxyl has some effect; in α - or β -unsaturated acids, the halide adds to the carbon more remote from the carboxyl, while with the γ -unsaturated acid the relationship is re-

⁶⁹² G. King, *J. Chem. Soc.*, 1942, 387-391.

⁶⁹³ R. Criegee, *Ber.*, 64, 260-266 (1931).

⁶⁹⁴ R. Criegee, *Ann.*, 481, 263-302 (1930).

⁶⁹⁵ R. Criegee, L. Kraft, and B. Rank, *Ann.*, 507, 159-197 (1933).

⁶⁹⁶ T. P. Hilditch and H. Jaspersen, *Nature*, 147, 327 (1941).

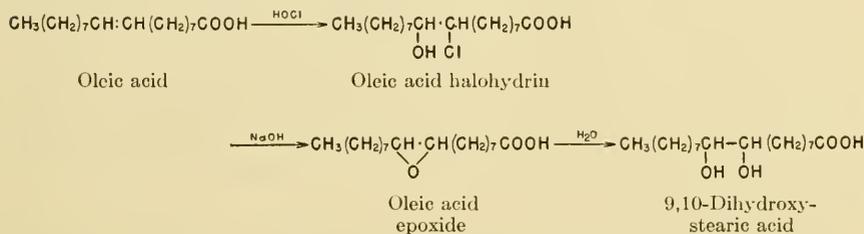
⁶⁹⁷ S. Piotrowski, *Ber.*, 23, 2531-2533 (1890).

⁶⁹⁸ A. Albitskiĭ, *J. Russ. Phys. Chem. Soc.*, 31, 100-103 (1899); cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 444.

⁶⁹⁹ W. Markownikoff, *Ann.*, 153, 228-259 (1870).

versed.^{5,700-702} The solvent may be a deciding factor as regards the position which the halogen occupies.⁷⁰³

Hypochlorous acid, HOCl, as well as the bromine and iodine counterparts, combine with the unsaturated linkage to give the halohydrins; in the presence of aqueous or alcoholic alkalis, the halohydrins are converted to the epoxide which, on hydrolysis, yields the dihydroxy-acid.⁷⁰⁴⁻⁷⁰⁶ The reaction between oleic and hypochlorous acid is pictured below:



Many other reactions are possible with saturated and unsaturated fatty acids which are of more interest to the general organic chemist than to the fat chemist. These reactions include substitution of the halides in the aliphatic chain, dehalogenation, and dehydrohalogenation, the reaction with sulfuric acid to form sulfonic acids, and many others. For a discussion of the reactions of greatest interest, the reader is referred to Markley⁴ and to Ralston.⁵

5. Chemistry of Glycerol

Approximately 10% of the fat molecule consists of glycerol, which occurs in combination with the fatty acids. Glycerol (or glycerine in the earlier literature) is a trihydric alcohol having the formula CH₂OH·CHOH·CH₂OH. In contradistinction to the fats and to the higher fatty acids, glycerol is largely insoluble in most of the fat solvents such as chloroform, benzene, petroleum ether, or anhydrous diethyl ether; on the other hand, it is readily soluble in water, and is miscible with it in all proportions. The same is true for ethyl alcohol. In some cases, glycerol also acts more satisfactorily as a solvent for a number of salts than does alcohol or water.

⁷⁰⁰ E. Erlenmeyer, *Ber.*, 13, 305-310 (1880).

⁷⁰¹ R. Fittig, *Ber.*, 13, 955-956 (1880); *Ann.*, 200, 21-96 (1880); 208, 111-121 (1881); 255, 1-144 (1889); 283, 47-148, 269-341 (1894).

⁷⁰² F. Fichter and W. Langguth, *Ann.*, 313, 371-381 (1900).

⁷⁰³ J. Walker and J. S. Lumsden, *J. Chem. Soc.*, 79, 1191-1197 (1901).

⁷⁰⁴ A. Albitskiĭ, *J. Russ. Phys. Chem. Soc.*, 31, 76-100 (1899); cited by K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, pp. 343-344.

⁷⁰⁵ A. Albitzky, *J. prakt. Chem.* [2], 61, 65-94 (1900).

⁷⁰⁶ B. H. Nicolet and T. C. Poulter, *J. Am. Chem. Soc.*, 52, 1186-1191 (1930).

Glycerol is practically odorless, but has a sweet taste. The usual article of commerce is a liquid at ordinary temperature; it is extremely viscous. Pure anhydrous glycerol forms a crystalline solid which melts at 20°C. It cannot be separated from water by distillation, since the polyhydric alcohol is steam-distillable.

Glycerol decomposes slowly at the boiling temperature. However, the rate of decomposition may be greatly accelerated when a dehydrating agent such as acid potassium sulfate (KHSO_4) is present on heating. Under such conditions, two molecules of water are lost from each glycerol molecule, and the characteristic product, acrolein ($\text{CH}_2:\text{CHCHO}$), is formed. The latter compound can readily be recognized in extremely small amounts by its penetrating and irritating odor. The formation of acrolein has been widely accepted as a satisfactory qualitative test for free glycerol and for fats. For more detailed information on the chemistry of glycerol, the reader is referred to Lawrie.⁶

Because glycerol is a triatomic alcohol, each molecule is able to combine with three fatty acid molecules. Such compounds are referred to as triglycerides. They comprise practically the entire bulk of the natural fats and oils. However, mono- and diglycerides are also well known. These are compounds in which only one or two fatty acids are combined with a glycerol molecule. Although such compounds are present in an extremely small proportion, if at all, in natural products, they can be readily synthesized. They presumably confer special properties on fats, and are sometimes added to them to render the fats adaptable for special uses. They are present in the so-called "high ratio" shortenings.

The wide variety of fats found in nature is possible because of the polyhydric nature of glycerol. In addition to the possibility of variation due to the formation of mono-, di-, or triglycerides, a further isomerism of the mono- and diglycerides may occur, depending upon the hydroxyl on which the combination occurs. There are two different monoglycerides (α or β) and two distinct diglycerides ($\alpha\alpha'$ and $\alpha\beta$); α and α' represent the terminal carbons and β represents the middle one.

The "mixed" triglycerides offer the greatest opportunity for variations. These are compounds in which more than one type of fatty acid is present in the molecule. The properties of such mixed triglycerides may not always be predictable from those of their component constituents. The types and properties of some of these mixed triglycerides are discussed later (Chapter III).

Although the bulk of the fatty acids in nature are found in combination with glycerol, many other compounds enter into stable union with them. In these cases, also, the combination occurs through the carboxyl group whereby the acidic properties of the fatty acid are dissipated. Examples of such compounds are the higher alcohols which form waxes with fatty acids.

Such cyclic alcohols as the sterols also form esters with the fatty acids. Less important quantitatively are vitamins A and D, which are ordinarily present in ester form with fatty acids. A different combination obtains with sphingomyelin and the cerebrosides, where the fatty acids are combined through the carboxyl group with an amino group of a basic compound such as sphingosine. A stable combination is formed whereby the acidic properties of the fatty acid and the basicity of the sphingosine are both effectively neutralized.

CHAPTER III

THE CHEMISTRY OF NEUTRAL FATS

I. Introduction

Although the chemistry and the metabolism of fats are usually considered to be identical with those of their component fatty acids, there are many reasons why they should be discussed separately. Actually, fatty acids are of minor importance *per se* in the naturally occurring fats and oils. In fats prepared from animal or vegetable sources, free fatty acids comprise only a fractional part of the total present as the triglyceride. The free acids may be found in appreciable amounts only in rancid fats or in freshly prepared oils from sources like the olive, where a high concentration of lipase is present in the pulp from which the oil is processed. On the other hand, the fat which is found in the storage organs of animals is largely neutral fat or is in the form of the corresponding phospholipids. The question has been raised by Fraser¹ whether or not the hydrolysis of the fat to fatty acid (or soap) and glycerol is a necessary prerequisite to absorption of fat from the gastrointestinal tract, as has been generally accepted for many years.

It has long been recognized that certain properties of neutral fats are quite distinct from those of the component fatty acids. In the case of the simple triglycerides, the melting points and solubilities are quite comparable to those of the component fatty acids. On the other hand, where mixed triglycerides occur, *i.e.*, those in which several varieties of fatty acids are combined with a single glycerol molecule, the melting point of the resulting compound may vary greatly from that of a mixture of the component fatty acids. In addition, the melting point may show marked variation according to the relative positions of the component fatty acid residues.

Isomerism is considerably more complex in the neutral fat than in the single fatty acid. In the case of the triglyceride one is concerned not only with such positional isomerism of the fatty acids as the location of the unsaturated linkage and such types of stereoisomerism as *cis-trans* arrangement, but also with optical isomerism resulting from unsymmetrical arrangements of the fatty acids, as well as with other variations which occur when dissimilar fatty acid residues occupy the different positions in glycerol.

The most marked difference in properties between the triglycerides and

¹ A. C. Fraser, *Physiol. Revs.*, 26, 103-119 (1946).

the fatty acids is to be found in those reactions which are referable to the free carboxyl group. The triglycerides are unable to react with the alkalis or with the various metallic salts to form soaps without a preliminary saponification. The formation of fatty acid aldehydes and alcohols, acid amides, cyanides, and related compounds is precluded unless a preliminary saponification and separation of the resulting fatty acids has been effected.

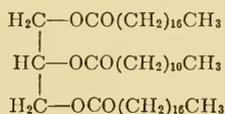
Physical properties, such as solubility, show marked variations between the triglycerides and the fatty acids. For example, the lower fatty acids such as acetic, propionic, and butyric are soluble in water, while the corresponding triglycerides are largely insoluble. In the case of the long-chain fatty acids such as palmitic and stearic, solubility can readily be effected in dilute aqueous alkali in the cold state, with the formation of water-soluble soaps. Neutral fats, on the other hand, cannot be dissolved in a similar solvent in the cold state except over long periods, and then only after hydrolysis of the molecule has been produced. Even at boiling temperature a considerable period may elapse before solution is effected, and in this case, also, it will not occur until after saponification has taken place.

The preparation of pure triglycerides from natural mixtures and the separation and identification of the isomeric triglycerides are much more difficult than in the case of the fatty acids. Fractionation of the natural triglycerides by distillation under reduced pressure can usually not be carried out, and this procedure offers no such satisfactory means of separation as it does with the methyl esters of the fatty acids. It is only by such means as differences in solubility and melting point that partial separation of specific triglycerides may be accomplished.

2. Nomenclature

The synthetic mono-, di-, and triglycerides and the natural fats consist of combinations of glycerol and the component fatty acids in ester linkages. Two isomers are possible with the monoglycerides, depending upon whether the ester combination is on one of the terminal carbon atoms of glycerol (in which case they are called unsymmetrical or α -monoglycerides) or on the central carbon of the glycerol, when the compound is known as a symmetrical or a β -monoglyceride. In the case of the diglycerides containing a single kind of fatty acid, two isomers may occur also. In one case, the acid constituents are combined with the two outside carbons of glycerol; these isomers are called symmetrical or α, α' -diglycerides. In the second type, the fatty acids are esterified with the middle carbon and one of the outside carbons of glycerol. These esters are spoken of as unsymmetrical or α, β -diglycerides. When the two substituent acids are different, four isomers

are possible. The simple triglycerides usually carry the name of the component acid with a prefix of *tri* and a suffix of *ein* replacing the terminal *ic*. In the case of the mono- or diglycerides, the same suffix is used with the appropriate *mono* or *di* preceding the name of the acid. With mixed triglycerides containing two molecules of one acid, the prefix *di* and the suffix *ein* are given to this acid, while the name of the single acid precedes, with a suffix *yl* replacing the terminal *ic* of the acid. Another method of terminology employs the suffix *yl* for both constituents and ends the name with the term *glycerol*. Still another modification involves numbering the glycerol carbons 1,2,3 instead of α, β, α' . Thus, the following names may correctly be applied to the following mixed triglyceride:



β -lauryl- α, α' -distearin, 2-lauryl-1,3-distearin, 2-laurodistearin, or β -lauryl- α, α' -distearyl-glycerol. The glycerides are frequently referred to without letter or number designation as "symmetrical" or "unsymmetrical." The above compound could be called symmetrical lauryl-distearin. In the case of the diglyceride, α, α' -dipalmitin, the term symmetrical dipalmitin may be used, while the α - β -isomer would obviously be known as unsymmetrical dipalmitin.

3. Synthesis of the Glycerides

(1) Monoglycerides

The monoglycerides have assumed considerable importance because of their use in the preparation of the so-called "high ratio" shortenings. The two series of compounds, α and β , require different methods for synthesis.

a. α -Monoglycerides. The classical method for the synthesis of α -monoglycerides was developed by Emil Fischer,^{2,3} although Berthelot⁴ had, many years earlier, prepared a monostearin by heating an excess of glycerol with stearic acid in a sealed tube at 200°C. However, since this method also yielded di- and triglycerides with longer periods of heating, it is evident that the product obtained was probably impure.

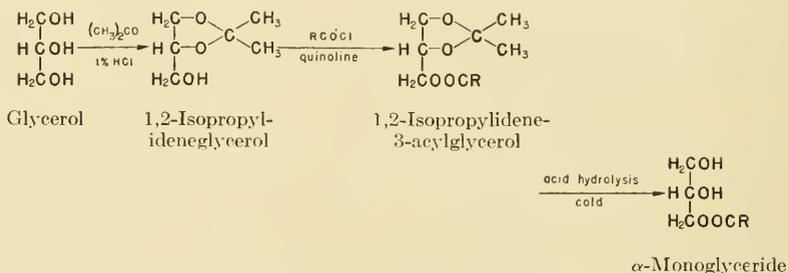
Fischer's method involves the condensation of glycerol with acetone to give 1,2-isopropylidenglycerol (acetoneglycerol), which is subsequently esterified with an acyl chloride in the presence of cold quinoline. The

² E. Fischer, *Ber.*, 53, 1621-1633 (1920).

³ E. Fischer, M. Bergmann, and E. Bärwind, *Ber.*, 53, 1589-1605 (1920).

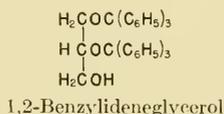
⁴ M. Berthelot, *Chimie organique fondée sur la synthese*, Vol. 2, 1860. Cited by B. F. Daubert and C. G. King, *Chem. Revs.*, 29, 271 (1941).

acetone is then removed by cold acid hydrolysis to give the α -monoglyceride, as illustrated below. Daubert and King⁵ state in their recent re-



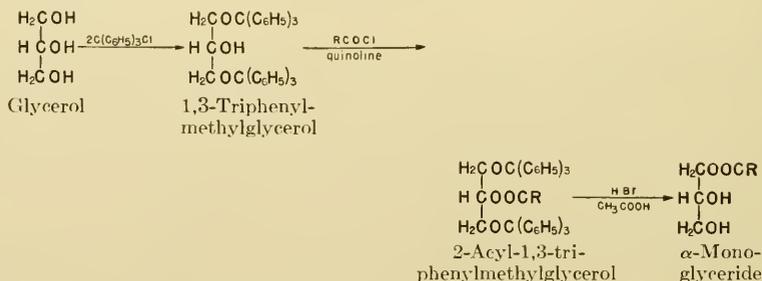
view that a large number of investigators have used the Fischer synthesis successfully.

Daubert and King⁶ employed 1,2-benzylidene-glycerol in place of 1,2-isopropylidene-glycerol for preparing pure α -monoglycerides. The reac-



tions are analogous to those with 1,2-isopropylidene-glycerol. The starting product, 1,2-benzylidene-glycerol, is the predominant product when trityl acts upon glycerol.

α -Monoglycerides are likewise formed when 1,3-triphenylmethyl-(trityl)-glycerol is acylated with an aliphatic group and the product is subjected to hydrolysis.⁷ Because of the greater stability of the α -carbon over the β -carbon, an aliphatic acyl group migrates from the β - to the α -position during hydrolysis. Verkade and co-workers⁸ have confirmed the earlier investigation of Jackson and King.⁷



⁵ B. F. Daubert and C. G. King, *Chem. Revs.*, 29, 269-285 (1941).

⁶ B. F. Daubert and C. G. King, *J. Am. Chem. Soc.*, 60, 3003-3005 (1938).

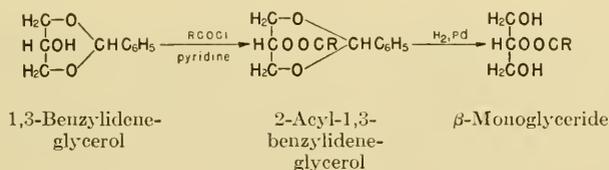
⁷ D. T. Jackson and C. G. King, *J. Am. Chem. Soc.*, 55, 678-680 (1933).

⁸ P. E. Verkade, J. van der Lee, and W. Meerburg, *Rec. trav. chim.*, 54, 716-724 (1935).

Still another procedure by which α -monoglycerides have been synthesized is by the direct esterification of glycerol in the presence of syrupy phosphoric acid. This method has been reported by Schuette *et al.*^{9,10} for the preparation of α -monoacetin and α -monobutylin, but it is unsatisfactory for acids containing more than four carbon atoms. A somewhat different method has been used by Young and Black,¹¹ who started with the triglyceride. Thus, when trilaurin is heated with glycerol and sodium phosphate under anhydrous conditions, α -monolaurin is formed.

Baer and H. O. L. Fischer¹² prepared optically active 1,2-acetoneglycerols from D- and L-mannitol by splitting with lead tetraacetate followed by reduction in ethyl acetate solution with hydrogen under pressure, using the Raney nickel catalyst. From the optically active acetoneglycerol isomers, the corresponding α -monoglycerides were prepared by the method of E. Fischer, Bergmann, and Bärwind.³ Several other technics which involve direct esterification have also been proposed.¹³⁻¹⁶

b. β -Monoglycerides. The first satisfactory procedure for the preparation of β -monoglycerides was introduced by Bergmann and Carter.¹⁷ This method involves the catalytic reduction of esterified 1,3-benzylidene-glycerol with hydrogen under pressure, as shown below. Bergmann and



Carter¹⁷ prepared β -monoacetin and β -monopalmitin successfully by the above procedures. Stimmel and King¹⁸ have also found it satisfactory in the preparation of the β -monoglycerides of capric, lauric, myristic, and stearic acids.

An equally effective technic for preparing the symmetrical monoglycerides involves the catalytic detritylation of the esterified 1,3-ditrityl ethers of glycerol¹⁹ according to the following scheme:

⁹ H. A. Schuette and J. T. Hale, *J. Am. Chem. Soc.*, **52**, 1978-1981 (1930).

¹⁰ P. G. Gilchrist and H. A. Schuette, *J. Am. Chem. Soc.*, **53**, 3480-3484 (1931).

¹¹ H. H. Young and H. C. Black, *J. Am. Chem. Soc.*, **60**, 2603-2605 (1938).

¹² E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **123**, 463-473 (1939).

¹³ I. Bellucci, *Gazz. chim. ital*, **42**, II, 283-305 (1913).

¹⁴ I. Bellucci and R. Manzetti, *Atti accad. Lincei*, **20**, I, 125-128 (1911).

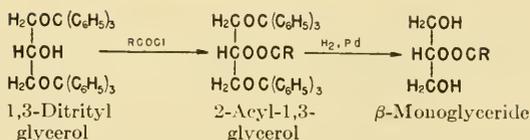
¹⁵ G. Gianoli, *Seifensieder-Ztg.*, **39**, 578 (1911); *Chem. Abst.*, **5**, 2573 (1911).

¹⁶ T. P. Hilditch and J. G. Rigg, *J. Chem. Soc.*, **1935**, 1774-1778.

¹⁷ M. Bergmann and N. M. Carter, *Z. physiol. Chem.*, **191**, 211-221 (1930).

¹⁸ B. F. Stimmel and C. G. King, *J. Am. Chem. Soc.*, **56**, 1724-1725 (1934).

¹⁹ B. F. Daubert, *J. Am. Chem. Soc.*, **62**, 1713-1714 (1940).



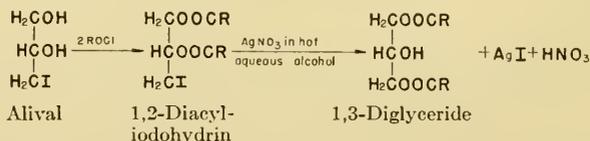
The earlier method of Grün²⁰ for preparing the β -monoglyceride, which involves treating 1,3-dichlorohydrin with an acyl chloride and subsequently removing the chlorine, has been shown by Fairbourne^{21,22} to be unreliable. Similarly, the acid hydrolysis of β -acylditrylglycerols results in the α - rather than in the expected β -monoglyceride.⁷

Eckey and Formo²³ have recently described a method involving *directed interesterification* for the production of monoglycerides. When cottonseed oil was mixed with an excess of glycerol, and sodium methoxide in xylene was used as the catalyst, a considerable amount of monoglyceride resulted. When the reaction was allowed to continue for 3 days at 26.7°C., 27% of the fat was separated as the crystalline monoglyceride. When the latter product was purified by recrystallization from petroleum ether at 4.4°C., it was shown to be relatively pure.

(2) Diglycerides

Two types of diglycerides are known, namely the symmetrical or α, α' -compound, and the unsymmetrical or α, β -isomer. A further differentiation involves the classification of those diglycerides into the simple diglycerides (where the two fatty acid residues are the same) and the mixed diglycerides (in which two different fatty acid molecules are involved).

a. Symmetrical Diglycerides. The method of Fischer, Bergmann, and Bärwind³ has been quite widely used for the synthesis of the diglycerides of the α, α' -type. This involves a reaction between alival (α -iodohydrin) and the acyl chloride.



A similar reaction for the preparation of symmetrical diglycerides employs trityl.^{7,23,24} After hydrolysis of 1-trityl-2,3-diacylglycerol with hydrobromic acid in chloroform, the symmetrical rather than the unsymmetrical diglyceride is the final product.

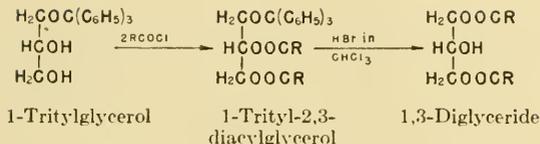
²⁰ A. Grün and E. Theimer, *Ber.*, 40, 1792-1801 (1907).

²¹ A. Fairbourne and G. E. Foster, *J. Chem. Soc.*, 127, 2759-2764 (1925).

²² A. Fairbourne and G. E. Foster, *J. Chem. Soc.*, 129, 3146-3151 (1926).

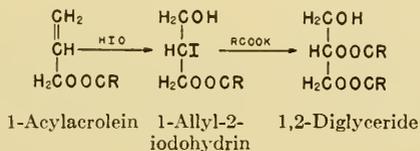
²³ E. W. Eckey and M. W. Formo, *J. Am. Oil Chemists' Soc.*, 26, 207-211 (1949).

²⁴ P. E. Verkade and J. van der Lee, *Rec. trav. chim.*, 55, 267-277 (1936).



The original Grün method which involves the treatment of 1,3-glycerol disulfate with acyl chloride²⁵ is apparently somewhat unsatisfactory^{26,27} because of: (a) the high temperatures employed, (b) the uncertainties of the structure of the intermediate halohydrins and disulfuric acid esters, and finally because of (c) possibilities of rearrangement during the course of the reactions.

b. Unsymmetrical Diglycerides. Because of the unstable character of the acyl group on the β -position, a number of methods designed to prepare α,β -diglycerides have actually yielded the symmetrical derivatives. This criticism apparently applies to the Grün procedure,²⁷ as well as to those of a number of other workers.²⁸⁻³³ Golendeev³⁴ has reported a method for the synthesis of unsymmetrical diglycerides from allyl esters where a shift from the β - to the α' -carbon probably does not occur. This is outlined below:



This would apparently be satisfactory for the preparation of mixed, unsymmetrical diglycerides.

The first entirely satisfactory procedure for making the unsymmetrical diglycerides is that suggested by Daubert and King.³⁵ This involves the catalytic detritylation of 1,2-diacyl-3-tritylglycerol by hydrogen in neutral solution as shown below. No shift of the acyl group from the β - to the α' -

²⁵ A. Grün, *Ber.*, **33**, 2284-2287 (1905).

²⁶ H. P. Averill, J. N. Roche, and C. G. King, *J. Am. Chem. Soc.*, **51**, 866-872 (1929).

²⁷ A. Fairbourn, *J. Chem. Soc.*, **137**, 369-382 (1930).

²⁸ A. Grün and P. Schacht, *Ber.*, **40**, 1778-1791 (1907).

²⁹ F. Guth, *Z. Biol.*, **44**, 78-110 (1903).

³⁰ R. R. Renshaw, *J. Am. Chem. Soc.*, **36**, 537-545 (1914).

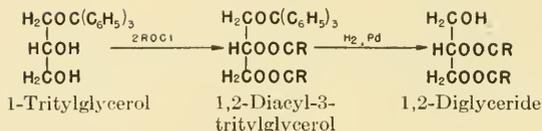
³¹ R. Delaby and P. Dubois, *Compt. rend.*, **187**, 767-769 (1928).

³² M. V. Humnicki and M. J. Lunkiewicz, *Bull. soc. chim.*, **45**, 422-428 (1929).

³³ C. Weizmann and L. Haskelberg, *Compt. rend.*, **189**, 104-106 (1929).

³⁴ P. Golendeev, *J. Gen. Chem. U. S. S. R.*, **6**, 1841-1846 (1936); *Chem. Abst.*, **31**, 4274 (1937). Cited by A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 560.

³⁵ B. F. Daubert and C. G. King, *J. Am. Chem. Soc.*, **61**, 3328-3330 (1939).



position occurs under these circumstances. Another workable method used by the same workers is the catalytic reduction of esterified 1-glyceryl-benzyl carbonate.

(3) Triglycerides

Chevreul,³⁶ in 1823, concluded that natural fats are primarily glyceryl esters of palmitic, stearic, and oleic acids, which he believed were present as the simple triglycerides, tripalmitin, tristearin, and triolein. However, Berthelot³⁷ first suggested that fats are probably composed to a considerable extent of mixed triglycerides. This was finally proved to be the case when Fritzweiler³⁸ and later Klimont³⁹⁻⁴⁴ isolated several types of mixed triglycerides from various natural fats. However, simple triglycerides, also, can be separated from fats. The synthetic compounds of high purity are readily available.

a. Simple Triglycerides. Berthelot⁴ demonstrated the production of tristearin by the heating of stearic acid with glycerol over a prolonged period in a sealed tube at 200°C. Garner⁴⁵ developed a method for obtaining an almost quantitative yield of pure triglycerides by heating equivalent amounts of glycerol and fatty acids at a temperature of 200°C. in an atmosphere of carbon dioxide. Several other procedures have been proposed.⁴⁶⁻⁴⁸ These methods are useless for the synthesis of mixed triglycerides.

b. Mixed Triglycerides. As has been noted earlier, it has long been recognized that natural fats contain a large proportion of mixed triglycerides. Although a number of these have been separated in pure form

³⁶ M. E. Chevreul, *Recherches chimiques sur les corps gras* (1823). Cited by B. F. Daubert and C. G. King, *Chem. Revs.*, **29**, 270 (1941).

³⁷ M. Berthelot, *Ann. chim. phys.*, **41**, 216-319 (1854).

³⁸ R. Fritzweiler, *Arb. kaiserl. Gesundh.*, **18**, 371-377 (1902); *Chem. Zentr.*, **1902**, **I**, 1113.

³⁹ J. Klimont, *Ber.*, **34**, 2636 (1901).

⁴⁰ J. Klimont, *Monatsh.*, **23**, 51-59 (1902).

⁴¹ J. Klimont, *Monatsh.*, **26**, 563-569 (1905).

⁴² J. Klimont, *Z. Untersuch. Nahr. u. Genussm.*, **12**, 359-360 (1906).

⁴³ J. Klimont, *Monatsh.*, **25**, 929-932 (1904).

⁴⁴ J. Klimont and E. Meisels, *Monatsh.*, **30**, 341-346 (1909).

⁴⁵ T. L. Garner, *J. Soc. Chem. Ind.*, **47**, 278-280 (1928).

⁴⁶ E. B. Herschberg, *J. Am. Chem. Soc.*, **61**, 3587-3588 (1939).

⁴⁷ R. K. Newman, J. M. Trikojus, and G. Harker, *Proc. Roy. Soc. N. S. Wales*, **59**, 293-300 (1926).

⁴⁸ P. E. Verkade, J. van der Lee, and W. Meerburg, *Rec. trav. chim.*, **51**, 850-852 (1932).

by fractional crystallization, until recently there was little evidence of the positional configuration of the molecules. However, since 1927, the structure of a number of natural mixed triglycerides has been elucidated by the Hilditch school, while the synthesis of a large group of such mixed triglycerides by King and his associates has helped to identify the natural compounds of unknown structure. Practically all the mixed triglycerides which have been separated from natural sources are composed of only two fatty acids, one of which is present to the extent of two molecules. Likewise, the products which have been synthesized have consisted almost entirely of this type of compound. Little is known of triglycerides which contain three different fatty acid components, although Wynter Blyth and Robertson⁴⁹ prepared a solid glyceride from butter fat, which they identified as an oleyl-butyryl-palmitin.

Two types of mixed glycerides are known, namely, the symmetrical, in which the single acid molecule is in the β -position, and the unsymmetrical form, where the single fatty acid occupies the α -position.

It has been pointed out by Longenecker⁵⁰ that a very large number of different mixed triglycerides is possible in any natural fat. If one represents the number of different kinds of acids as n , then the theoretical number of combinations resulting from an even distribution of acids in the

TABLE I

NUMERICAL RELATIONSHIP BETWEEN COMPONENT FATTY ACIDS AND GLYCERIDES^a

Number of fatty acids, n	Theoretical combinations, n^3	Number of glycerides, $\frac{1}{2}(n^3 + n^2)$
1	1	1
2	8	6
3	27	18
4	64	40
5	125	75
6	216	126
7	343	196
8	512	288
9	729	405
10	1000	550

^a H. E. Longenecker, *Chem. Revs.*, 29, 201-224 (1941).

molecules would be n^3 . However, since the α - and α' -positions on glycerol are equivalent, the total number of molecules chemically distinguishable would be $\frac{1}{2}(n^3 + n^2)$. The numbers of such possible compounds in relation to the component acids are given in Table I.

⁴⁹ A. Wynter Blyth and G. H. Robertson, *Proc. Chem. Soc. London*, 5, 5 (1889).

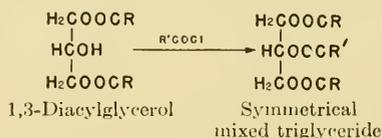
⁵⁰ H. E. Longenecker, *Chem. Revs.*, 29, 201-224 (1941).

The following simple and mixed triglycerides would be possible where only two fatty acids (such as palmitic and stearic) are available:

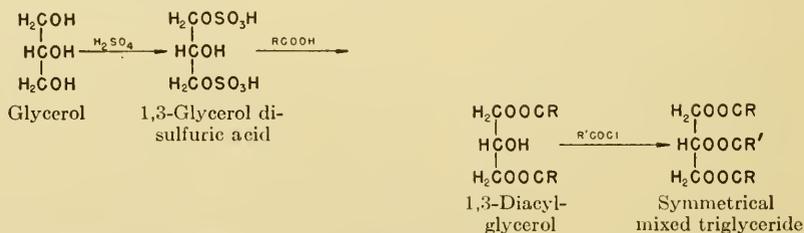
1. tripalmitin
2. α -stearyl- α' , β -dipalmitin
3. β -stearyl- α , α' -dipalmitin
4. α' -stearyl- α , β -dipalmitin
5. α -palmityl- α' , β -distearin
6. β -palmityl- α , α' -distearin
7. α' -palmityl- α , β -distearin
8. tristearin

However, since the positions α and α' are equivalent, 2 and 4 as well as 5 and 7 are identical; this leaves the remaining 6 (1, 2, 3, 5, 6, and 8) as the different possible compounds.

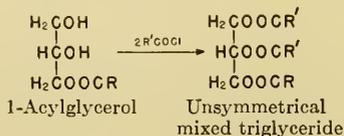
(a) *Symmetrical Mixed Triglycerides*. The Fischer method,³ employing alival, which is useful for the production of symmetrical diglycerides, can be used to produce symmetrical mixed triglycerides. It is only necessary to treat the diglyceride with the acyl chloride as indicated in the accompanying equation. The method of Grün and associates,^{20,25,23} who used



symmetrical diglycerides prepared from glycerol disulfuric acid for acylation, is described below.



(b) *Unsymmetrical Mixed Triglycerides*. The most satisfactory method for the preparation of the unsymmetrical triglycerides is by the acylation



of the α -monoglyceride.⁵¹ The Fischer method given above has been sat-

⁵¹ J. C. Irvine, J. McDonald, and C. W. Soutar, *J. Chem. Soc.*, 107, 337-351 (1915).

isfactorily used by a large number of workers for the preparation of mixed triglycerides.^{6,52-57}

(4) *Synthesis of Fats from Petroleum*

The shortage of natural fats in Germany prior to and during World War II afforded a stimulus to the development of the synthetic fat industry. The background information necessary for establishing such commercial production of food fats had been available for many years. Gessner,⁵⁸ as early as 1865, had demonstrated the oxidation of hydrocarbons by air, and Schaal⁵⁹ had patented a similar process in 1885. Much additional information was obtained shortly after World War I in the laboratories of Lever Brothers and Unilever in England, by Francis and co-workers.⁶⁰⁻⁶² These investigators studied the relative activity of a variety of oxidizing agents and catalysts. They came to the conclusion that the most economical and efficient synthesis of fatty acids was brought about by the oxidation of the paraffins at a high temperature with air. However, little was done with this information in England, because the low cost of natural fats precluded the commercial production of the more expensive synthetic products.

Interest in this field was revived in 1935 in Germany by the demonstration that the by-product, "gatsch," could serve as a source of fatty acids. This Fischer "gatsch" originated, to the extent of 5 to 12%, in the synthesis of petroleum products by the treatment of carbon monoxide with hydrogen at a high temperature under a pressure of 10 atmospheres, by the so-called Fischer-Tropsch process. It could readily be separated from the hydrocarbons, gasoline, Diesel oil, and "macroparaffins" by distillation. It consisted of soft waxy hydrocarbons melting in the 340-450°C. range.

The process, started in 1937 by Deutsche Fettsäure Werke in Witten, Germany, involves the following main steps: After oxidation in an alumi-

⁵² H. C. Black and C. A. Overley, *J. Am. Chem. Soc.*, *61*, 3051-3052 (1939).

⁵³ G. F. Converse and E. H. Shaw, *Proc. S. Dakota Acad. Sci.*, *17*, 31-33 (1937).

⁵⁴ C. Amberger and K. Bromig, *Biochem. Z.*, *130*, 252-266 (1922).

⁵⁵ O. E. McElroy and C. G. King, *J. Am. Chem. Soc.*, *56*, 1191-1192 (1934).

⁵⁶ H. E. Robinson, J. N. Roche, and C. G. King, *J. Am. Chem. Soc.*, *54*, 705-710 (1932).

⁵⁷ P. E. Verkade and J. van der Lee, *Proc. Acad. Sci. Amsterdam*, *37*, 812-818 (1934).

⁵⁸ O. Gessner, *Practical Treatise on Coal, Petroleum, and Other Distilled Oils* (1865). Cited by F. A. O. Report, *Synthetic Fats, Their Potential Contribution to World Food Requirements*, Washington, D. C., Nov. 3, 1948, p. 1.

⁵⁹ E. Schaal, "Verfahren zur Oxidation von Petroleum und ähnlichen Kohlenwasserstoffen zu Säuren und zur Herstellung von Seifen und Estern dieser Säuren," *Patentschrift*, *32*, 705 (1885). Cited by F. A. O. Report, *Synthetic Fats*, Washington, D. C., Nov. 3, 1948, p. 1.

⁶⁰ F. Francis, *J. Chem. Soc.*, *121*, 496-505 (1922).

⁶¹ F. Francis and J. C. Pope, *J. Chem. Soc.*, *121*, 506-511 (1922).

⁶² F. Francis and R. H. Coysh, *J. Chem. Soc.*, *121*, 511-513 (1922).

num vessel at 110°C. under atmospheric pressure with air and a 0.2% solution of potassium permanganate as a catalyst, the material is saponified by soda ash or caustic soda, and the resulting soap is separated by settling, autoclaving, and flash distillation. The soap is transformed to fatty acids with sulfuric acid, and the fatty acids are fractionated by steam distillation. The second and third fractions, which include the C₁₀ to C₁₈ fatty acids, are used for the production of synthetic fats. After esterification with glycerol by the use of the Bellucci method, which employs a zinc catalyst and a temperature of 120–180°C., a synthetic triglyceride is obtained which can be used for the preparation of margarine.⁶³

In addition to the fatty acids produced by the Witten works (FSW), other sources of starting material were the "Riebeckparaffin" obtained from the tar of brown coal and the "Tiefemperatur-Hydrierungsparaffin" (TTH). Production of synthetic fats was likewise carried out by the Oppau plant of I. G. Farbenindustrie-A.-G., as well as at Ludwigshafen by the Badische Anilin und Soda Fabrik (BASF).

The physical properties of the synthetic fats vary with the nature of the starting material. Samples are reported as brown or colorless, odorless, and having melting points^{64–66} varying from 31° to 47°C. Table 2 summarizes the available data on several types of synthetic fats.

TABLE 2
SOME CONSTANTS FOR VARIOUS FATS SYNTHESIZED FROM PETROLEUM PRODUCTS^a

Synthetic fat	Iodine No.	Melting point, °C.	Unsaponifiable fraction, %	Free fatty acid	Saponification No.
Witten fat	11	31	0.3	0.1	230
Riebeck-paraffin fat	2	47	} <1.0	0.4	220
TTH-paraffin fat	2	39		0.2	225
Fischer-paraffin fat (BASF)	5	34		0.3	230
Fischer-paraffin fat (BASF) (without iso-acids)	2	39.5		0.3	225

^a Data adapted from *Synthetic Fats. Their Potential Contribution to World Food Requirements*, Food and Agriculture Organization of the United Nations, Nov. 3, 1948.

The synthetic fats are triglycerides chiefly of the C₁₀ to C₁₈ fatty acids. They contain small amounts of di- and monoglycerides, just as is the case

⁶³ F. A. O. Report, *Synthetic Fats, Their Potential Contribution to World Food Requirements*, Washington, D. C., Nov. 3, 1948.

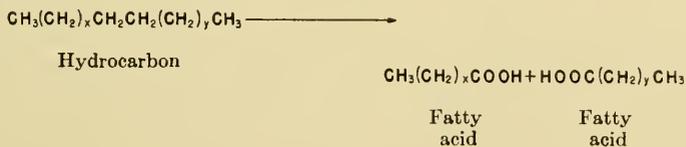
⁶⁴ J. W. Vincent, "Aspects of the Synthetic Fatty Acids and the Synthetic Fat Industries in Germany," U. S. Dept. of Commerce, *Publication Board Report No. 49,196*, Washington, D. C. (July, 1946). *Bios Final Report*, 805, Item 22. *Bibliog. Scientific and Industrial Repts.*, 4, (Jan.–May, 1947), p. 1043.

⁶⁵ P. N. Williams, *Chemistry & Industry*, 19, 251–255 (1947).

⁶⁶ G. Schiller, *Z. Lebensm. Untersuch. Forsch.*, 88, 174–190 (1948). Cited by F. A. O. Report, *Synthetic Fats*, Washington, D. C., Nov. 3, 1948, p. 3.

with many hydrogenated fats produced in the United States. The main chemical difference between the synthetic fats from petroleum and the natural fats and those hydrogenated from vegetable oils is that the former contain approximately 50% odd-chain fatty acids while, of course, all natural fats consist solely of even-chain fatty acids. Moreover, considerable amounts of branched-chain acids have also been reported in the synthetic fats; 2-, 3-, and 4-methylated acids have been detected, and it is probable that the ethyl-branched chain acids occur in small amounts. Dicarboxylic acids from C_{10} to C_{14} are likewise present^{67,68} to the extent of 3 to 4% of the crude fatty acid mixture obtained by oxidation of petroleum products, but they are almost completely eliminated from the refined fat. Other compounds present in small amounts include hydroxy- and keto-acids, lactones, as well as aliphatic alcohols and ketones.⁶³ Finally, the synthetic fat contains practically no unsaturated acids.

Thomas and Weitzel⁶⁹ have suggested that the reactions involved in the formation of fatty acids from paraffins require several steps. The primary reaction results in an unsaturation at the middle of the molecule of the carbon-to-carbon linkage. This is followed either by an oxidative splitting of the olefin so produced to the acids, or by the intermediate formation of a peroxide.⁷⁰ A schematic example of this process as suggested by Weitzel⁷¹ follows:



Since the maximum desirable length of the fatty acids for soaps or for food fats is 18 carbons, the hydrocarbons should not exceed 35 carbons in length. The assumption that oxidation occurs on the tertiary carbon atoms of the paraffin at the site of the branched chain^{68,72} is apparently incorrect, since considerable amounts of iso-acids have been demonstrated.⁶⁸

The question of toxicity of the synthetic fats is not as yet completely

⁶⁷ W. Keil, *Z. physiol. Chem.*, **274**, 175-185 (1942).

⁶⁸ H. Appel, H. Böhm, W. Keil, and G. Schiller, *Z. physiol. Chem.*, **282**, 220-244 (1947).

⁶⁹ K. Thomas and G. Weitzel, in R. Kuhn, *Fiat Rev. German Sci., Biochemistry*, **1**, 1-58 (1947).

⁷⁰ A. Imhausen, *Kolloid-Z.*, **103**, 105-108 (1943).

⁷¹ G. Weitzel, *Fette u. Seifen*, **46**, 21 (1939). Cited by K. Thomas and G. Weitzel in R. Kuhn, *Fiat Rev. German Sci., Biochemistry*, **1**, 30 (1947).

⁷² G. Weitzel, *Angew. Chem.*, **51**, 531-546 (1938). Cited by K. Thomas and G. Weitzel, in *Fiat Rev. German Sci., Biochemistry*, **1**, 30 (1947).

settled. Although the earlier results of Thomas and Weitzel^{73,74} indicated the toxicity of the iso-acids as demonstrated by rat growth tests, later results⁷⁵ indicate that the fats from the Fischer-Tropsch process are almost completely utilized, in spite of the fact that they must have contained an iso-acid content⁷⁶ of 35%. In tests on man, Thomas⁷⁵ found an average loss of 3-4 grams of iso-acid in the urine after consumption of synthetic fats in amounts of 100-220 g., although in one case the value rose to 9 g. Thomas believes that only those fatty acids containing more than one branched chain fail to be metabolized. On the other hand, it is claimed⁶³ that the Witten fat can be purified by a process involving extraction with methyl formate and related solvents so that a product is produced which has no inhibitory effect on growth.⁷⁷ This is believed to be safe for consumption by human subjects. More information is needed before the problem of toxicity can be definitely settled. This is especially the case in view of the possible unreliability of some of the data obtained during the war. Since the Nazis considered the production of synthetic fats as a triumph for German science, unfavorable reports were withheld or suppressed. Moreover, the authors who reported favorable results tended in some instances to be overenthusiastic in their commendation of the synthetic fats.

In the Witten plant, 40,000 tons of "gatsch" were processed per year into 31,000 tons of fatty acids. Only 5% of the latter, or 1550 tons, were available for the production of edible fats. The claim that the cost of synthetic fat was decreased to that of natural fat during 1943-1944 was based upon the assumption that the by-product, "gatsch," costs nothing. On the basis of assigning a fair charge for this source material, Vincent⁶⁴ has estimated the cost as \$708 per ton in Germany (35.4 cents per pound), and \$821 per ton in England (41.1 cents per pound). In addition to the review of Thomas and Weitzel,⁶⁹ the reader is referred to the monograph of Wittka.⁷⁸

⁷³ K. Thomas and G. Weitzel, *Deut. med. Wochschr.*, 71, 18-21 (1946); *Chem. Abst.*, 41, 1342 (1947).

⁷⁴ K. Thomas and G. Weitzel, *Z. physiol. Chem.*, 282, 180-185 (1947); *Süddeut. Apoth.-Ztg.*, 87, 255-256 (1947); *Chem. Abst.*, 43, 5097 (1949).

⁷⁵ K. Thomas, Unpublished results cited in F. A. O. Report, *Synthetic Fats*, Washington, D. C., Nov. 3, 1948, p. 4.

⁷⁶ H. Kraut, Ä. Weischer, and R. Hügel, *Biochem. Z.*, 316, 96-107 (1943); 317, 187-192 (1944).

⁷⁷ H. Appel, H. Böhm, W. Keil, and G. Schiller, *Z. physiol. Chem.*, 274, 186-205 (1942).

⁷⁸ F. Wittka, *Moderne Fettchemische Technologie. II. Gewinnung der höheren Fettsäuren durch Oxydation der KWSe*, Barth, Leipzig, 1940; *Chem. Abst.*, 39, 2000 (1945); *Seifensieder-Ztg.*, 66, 666-668, 669-700 (1939); *Chem. Abst.*, 33, 9603 (1939); *Allgem. Oel- u. Fett-Ztg.*, 88, 358-360, 397-399 (1941); *Chem. Abst.*, 37, 2201, 3959 (1943). Cited by K. Thomas and G. Weitzel, in R. Kuhn, *Fiat Rev. German Sci., Biochemistry*, 1, 35 (1947).

4. Composition of Natural Fats and Oils

There are an almost infinite number of different fats and oils, each of which possesses a specific composition. One of the conditions which render this phenomenon possible is the wide variation found in the total number of varieties of fatty acids represented in any one fat. Another contributing factor is that the proportions between any given groups of fatty acids may show an almost limitless number of variations. But even with two fats containing the same series of fatty acids in exactly the same proportions, noticeable alterations in the physical properties and the chemical characteristics of such fats may still be predicted. This may be attributed to the wide variety of mixed triglycerides which are possible; since these components make up a large proportion of the natural fat, variations in them would necessarily change the character of the component fat.

Hilditch has determined the composition of some 600 different kinds of fats. The results of these investigations are included in his comprehensive monograph.⁷⁹ Included in his study are 100 fats of aquatic origin, 80 fats from land animals, and 420 fats from various plant species. Some of the important conclusions have been summarized in the recent review of Longenecker.⁵⁰

The results of Hilditch have demonstrated the close connection in the type and the proportion of the fatty acids in plants and animals having a close taxonomic relationship. The most intricate structures and the most complex forms of fatty acids have invariably been shown to occur in the simplest forms of plant and animal life. In fact, Hilditch and Lovern⁸⁰ have called attention to the fact that a simplification in structure and number of the component fatty acids seems to be associated with an ascending evolutionary scale.

(1) Component Fatty Acids in Natural Fats and Oils

a. Analysis of Fats. The estimation of the proportion of the several fatty acids composing a fat is a relatively simple procedure as compared with the determination of the arrangement of the fatty acids in the triglyceride molecules. A number of different procedures are now available for the quantitative analysis of the fatty acids.

(a) *Fractionation into Saturated and Unsaturated Acids.* A preliminary separation of the fatty acids into two portions, depending upon the solubility of their lead soaps in alcohol,⁸¹⁻⁸³ is usually the first step. After saponifica-

⁷⁹ T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947.

⁸⁰ T. P. Hilditch and J. A. Lovern, *Nature*, 137, 478-481 (1936).

⁸¹ T. P. Hilditch, *Chem. Products*, 3, 78-81 (1940); *Chem. Abst.*, 35, 1653 (1941).

⁸² T. P. Hilditch and J. Priestman, *Analyst*, 56, 354-367 (1931).

⁸³ E. Twitchell, *Ind. Eng. Chem.*, 13, 806-807 (1921).

tion of the fat or oil, the mixture of fatty acids is taken up in alcohol and treated with lead hydroxide. The lead salts of the saturated acids are insoluble, while those of the unsaturated acids are, in general, soluble in alcohol. However, this difference does not hold for the short-chain saturated acids (C_6 - C_{12}), which dissolve quite well in alcohol. Hence, this procedure for the separation of saturated and unsaturated acids is not satisfactory for fatty acid mixtures from butter fat, coconut oil, or palm oil in which large proportions of saturated short-chain acids are present. On the other hand, not all unsaturated acids have lead salts which are readily soluble in alcohol. Thus, petroselinic and other isooleic acids, elaeostearic acid, erucic acid, and hydroxy-acids may also be present to some extent in the alcohol-insoluble fraction. Even lead oleate may occur to the extent of 1-2% in the precipitate. Data obtained by the use of this so-called Twitchell method⁸³ must therefore be accepted with reservations.

(b) *Fractional Distillation of Fatty Acid Mixtures.* The separation of fatty acids from each other by fractional distillation, particularly at reduced pressures, has been widely employed in the analyses of fats.⁸⁴⁻⁹² This procedure is of little use in the separation of most triglyceride mixtures, because of the complexity of their composition. However, in the case of the free fatty acids or of their methyl or ethyl esters, a relatively sharp separation into the individual components may be effected.

Although, as recently as 1924, Channon and his associates⁹³ stated that the fractionation method is of little quantitative use, many recent improvements have made it considerably more precise. Of particular importance has been the development of the electrically heated packed column with an evacuated jacket to protect it against temperature fluctuations.⁹⁴ By means of this innovation, Jantzen and Tiedcke were able to separate the methyl esters of palmitic and stearic acids, as well as the high-melting acids of peanut oil. Further modifications were incorporated by Bush and Schwartz,⁹⁵ as well as by Lepkovsky and his collaborators.^{96,97}

⁸⁴ E. F. Armstrong, J. Allan, and C. W. Moore, *J. Soc. Chem. Ind.*, 44, 63-68T (1925)

⁸⁵ E. F. Armstrong, J. Allan, and C. W. Moore, *J. Soc. Chem. Ind.*, 44, 143-144T (1925).

⁸⁶ W. F. Baughman and G. S. Jamieson, *J. Am. Chem. Soc.*, 42, 152-157 (1920).

⁸⁷ H. E. Longenecker, *J. Soc. Chem. Ind.*, 56, 199-202 T (1937).

⁸⁸ A. W. Weitkamp and L. C. Brunstrum, *Oil & Soap*, 18, 47-50 (1941).

⁸⁹ A. E. Bailey, *Industrial Oil and Fat Products*, 2nd Ed., Interscience, New York, 1951.

⁹⁰ F. A. Norris and D. E. Terry, *Oil & Soap*, 22, 41-46 (1945).

⁹¹ F. C. Williams and J. O. Osburn, *J. Am. Oil Chemists' Soc.*, 26, 663-668 (1949).

⁹² A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948.

⁹³ H. J. Channon, J. C. Drummond, and J. Golding, *Analyst*, 49, 311-327 (1924).

⁹⁴ E. Jantzen and C. Tiedecke, *J. prakt. Chem.*, 127, 277-291 (1930).

⁹⁵ M. T. Bush and A. M. Schwartz, *Ind. Eng. Chem., Anal. Ed.*, 4, 142-143 (1932).

⁹⁶ H. M. Evans, R. E. Cornish, S. Lepkovsky, R. C. Archibald, and G. Feskov, *Ind. Eng. Chem., Anal. Ed.*, 2, 339-343 (1930).

⁹⁷ S. Lepkovsky, G. V. Feskov, and H. M. Evans, *J. Am. Chem. Soc.*, 58, 978-981 (1936).

The latter investigators were able to separate caprylic, capric, lauric, myristic, palmitic, and erucic acids from each other, although more satisfactory results obtained when the methyl esters were fractionated. The methyl esters of saturated and unsaturated acids, as well as the methyl esters of certain dicarboxylic acids,⁹⁸ can be separated by distillation.⁹⁹

Many types of packing are used in the fractionating columns. These include Lessing rings made of 50-mesh copper screen,¹⁰⁰ glass modifications of the single-turn and double-turn wire helices previously used,¹⁰¹ and spiral continuous wire coil packings in fractionating columns having separate sleeve-like metal vacuum jackets.¹⁰² Klem¹⁰³ successfully employed the last type for the difficult separation of methyl palmitate, methyl stearate, methyl oleate, and methyl elaidate from each other. Fenske and co-workers,¹⁰⁴ after investigating many types of packing, have reported best results with wire or glass helices, carding teeth, and jack-chains. A number of other workers have found wire helices especially efficient.¹⁰⁵⁻¹⁰⁷ Methyl palmitate and methyl stearate were fractionated very effectively by Schoenheimer and Rittenberg¹⁰⁸ by means of tightly fitting metal helices. Price and McDermott¹⁰⁹ have described a simple method for preparing glass helices, while Longenecker⁸⁷ used single-turn glass helices in the investigation of the acids of beef tallow, peanut oil, the liquid esters of butter fat and for separating the methyl esters of the butter fatty acids.¹¹⁰ By far the most effective packing is the conical type developed by Stedman,¹¹¹ which has been further described by Bragg.¹¹² In industrial columns, a very efficient bubble-cap type is employed, but this cannot be satisfactorily used on the laboratory scale. For a further discussion of fractionating columns, the reader is referred to Longenecker¹¹³ and Ralston.⁹²

⁹⁸ F. Rennkamp, *Z. physiol. Chem.*, **260**, 276-278 (1939).

⁹⁹ L. Keffler and J. H. McLean, *J. Soc. Chem. Ind.*, **54**, 362-367T (1935).

¹⁰⁰ F. C. Whitmore and A. B. Lux, *J. Am. Chem. Soc.*, **54**, 3448-3454 (1932).

¹⁰¹ C. D. Wilson, S. T. Parker, and K. C. Laughlin, *J. Am. Chem. Soc.*, **55**, 2795-2796 (1933).

¹⁰² W. J. Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **5**, 119-135 (1933).

¹⁰³ A. Klem, *Nature*, **142**, 616 (1938).

¹⁰⁴ M. R. Fenske, C. O. Tongberg, and D. Quiggle, *Ind. Eng. Chem.*, **26**, 1169-1177 (1934).

¹⁰⁵ M. R. Fenske (to Pennsylvania State College), *U. S. Patent* No. 2,037,317 (Apr. 14, 1936).

¹⁰⁶ C. O. Tongberg, S. Lawroski, and M. R. Fenske, *Ind. Eng. Chem.*, **29**, 957-958 (1937).

¹⁰⁷ M. R. Fenske, S. Lawroski, and C. O. Tongberg, *Ind. Eng. Chem.*, **30**, 297-300 (1938).

¹⁰⁸ R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, **120**, 155-165 (1937).

¹⁰⁹ R. W. Price and W. C. McDermott, *Ind. Eng. Chem., Anal. Ed.*, **11**, 289-290 (1939).

¹¹⁰ T. P. Hilditch and H. E. Longenecker, *J. Biol. Chem.*, **122**, 497-506 (1937).

¹¹¹ D. F. Stedman, *Can. J. Research*, **B15**, 383-400 (1937).

¹¹² L. B. Bragg, *Ind. Eng. Chem., Anal. Ed.*, **11**, 283-287 (1939).

¹¹³ H. E. Longenecker, *Oil & Soap*, **17**, 53-57 (1940).

High-vacuum procedures have practically always been used for the fractionations of fatty acids. Caldwell and Hurtley¹¹⁴ used a procedure of this nature for the distillation of fatty acids of butter and coconut oil. Palmitic and stearic acids were first separated by vacuum distillation.^{115,116} In recent work vacuum and fractional distillation have been frequently employed by using the methyl and ethyl esters,¹¹⁷ as in the case of palm kernel oil,⁹³ coconut butter (copra),¹¹⁸ cod-liver oil,¹¹⁹ butter,^{93,120-125} cottonseed oil,¹²⁶ human milk fat,¹²⁷ hydrogenated herring oil,¹²⁸ chaulmoogra oil,¹²⁹ peanut oil,¹³⁰ and brain lipids.¹³¹ Molecular distillation which involves a much higher degree of vacuum is seldom employed for the isolation of fatty acids, but it usually finds an application in the distillation of triglycerides or of other high-boiling compounds.

For the identification of the resulting fractions, saponification equivalents, iodine and thiocyanogen numbers, refractive indices, ultraviolet, infrared, or Raman spectral patterns, and, in special cases, other physical and chemical constants, are employed. Such procedures are discussed in detail by Harper *et al.*,¹³² Hilditch,¹³³ and Longenecker.¹¹³

(c) *Non-solvent Crystallization.* In some cases higher melting fatty acids can be separated from those of lower melting point by chilling. This procedure has an exceedingly limited application and is generally quite unsatisfactory.

(d) *Crystallization from Solvents.* Several types of procedure can be listed under this category. The most widely employed method is that

¹¹⁴ K. S. Caldwell and W. H. Hurtley, *J. Chem. Soc.*, 95, 853-861 (1909).

¹¹⁵ F. Krafft, *Ber.*, 36, 4339-4344 (1903).

¹¹⁶ H. Kreis and A. Hafner, *Ber.*, 36, 2766-2773 (1903).

¹¹⁷ E. B. Holland, *J. Ind. Eng. Chem.*, 3, 171-173 (1911).

¹¹⁸ A. Haller and Youssoufian, *Compt. rend.*, 143, 803-806 (1906).

¹¹⁹ H. Bull, *Ber.*, 39, 3570-3576 (1906).

¹²⁰ I. Smedley, *Biochem. J.*, 6, 451-461 (1912).

¹²¹ E. B. Holland, J. C. Reed, and J. P. Buckley, *J. Agr. Research*, 6, 101-113 (1916).

¹²² E. B. Holland and J. P. Buckley, *J. Agr. Research*, 12, 719-732 (1918).

¹²³ E. B. Holland, M. E. Garvey, H. B. Pierce, A. C. Messer, J. G. Archibald, and C. O. Dunbar, *J. Agr. Research*, 24, 365-398 (1923).

¹²⁴ C. Crowther and A. Hynd, *Biochem. J.*, 11, 139-163 (1917).

¹²⁵ A. W. Bosworth and J. B. Brown, *J. Biol. Chem.*, 103, 115-134 (1933).

¹²⁶ V. J. Meyer, *Chem.-Ztg.*, 31, 793-794 (1907); *Chem. Abst.*, 1, 2945-2946 (1907).

¹²⁷ A. W. Bosworth, *J. Biol. Chem.*, 106, 235-244 (1934).

¹²⁸ A. Grün, *Chem. Umschau*, 26, 101-103 (1919); *Chem. Abstr.*, 14, 643 (1920).

¹²⁹ T. Hashimoto, *J. Am. Chem. Soc.*, 47, 2325-2333 (1925).

¹³⁰ W. D. Cohen, *Verslag Akad. Wetenschappen Amsterdam*, 34, 462-467 (1925).

¹³¹ J. B. Brown, *J. Biol. Chem.*, 83, 783-791 (1929).

¹³² D. A. Harper, T. P. Hilditch, and J. T. Tereleski, *J. Soc. Chem. Ind.*, 56, 310-315T (1937).

¹³³ T. P. Hilditch, *J. Soc. Chem. Ind.*, 52, 169-171 (1933); *Proc. Pacific Sci. Congr., 5th Congr., Can.*, 5, 3647-3661 (1933). G. Hefter and H. Schönfeld, *Chemie und Technologie der Fette und Fettprodukte*, 2nd ed., Vol. I, Springer, Berlin, 1937, pp. 60, 149. T. P. Hilditch and H. E. Longenecker, *J. Biol. Chem.*, 122, 497-506 (1938); *Fette u. Seifen*, 43, 97-100 (1936); *Chem. Abst.*, 30, 7494-7495 (1936).

which involves fractional crystallization of the fatty acids generally from acetone solutions at low temperatures, as developed in the laboratory of J. B. Brown.¹³⁴⁻¹⁴¹ By its use, many naturally occurring unsaturated acids such as oleic, linoleic, linolenic, erucic, and ricinoleic acids have been separated from each other, as well as from the saturated acids.

Another application of fractional crystallization from solvents involves the separation of the hexabromides from the tetrabromides. These addition products of the unsaturated acids are both insoluble in petroleum ether, but to different extents. However, the usefulness of the method is diminished by the fact that variations in solubility obtain in the presence of mixtures.

Finally, the separation of the barium, magnesium, and lead soaps of the solid acids from those of the liquid acids, as described in an earlier section, is another example of crystallization from solvents.

(e) *Chromatographic Separation.* One of the most recently developed fields of fatty acid analysis is partition chromatography. Various types of procedures have been suggested, and the particular method to be used depends somewhat upon the fatty acids to be separated. By the use of a silica gel column, Smith¹⁴² has been able to resolve chloroform+1% butanol solutions of formic, acetic, propionic, *n*-butyric, and *n*-valerianic acids into their component compounds. Although the butanol-chloroform-water system has been shown by others^{143,144} to give a satisfactory separation of C₁ to C₄ acids, it is not effective for longer chain acids. When the silica gel tube is buffered, such a system may be used for the C₂ to C₈ acids including *n*- and *iso*-valeric acids.¹⁴⁵ Peterson and Johnson¹⁴⁶ were able to isolate the C₂ to C₁₀ acids by employing benzene-aqueous sulfuric acid in Celite-packed tubes. The adsorption technic with an aluminum oxide column has been used to separate oleic from linoleic acids.¹⁴⁷ A methanol-isooctane mixture has found application in partition chromatography of the saturated acids of intermediate length.¹⁴⁸ The analysis of mixtures of C₁₁ to C₁₉ acids can be accomplished by the use of furfuryl

¹³⁴ J. B. Brown, *J. Biol. Chem.*, *90*, 133-139 (1931).

¹³⁵ D. L. Cramer and J. B. Brown, *J. Biol. Chem.*, *151*, 427-438 (1943).

¹³⁶ J. S. Frankel and J. B. Brown, *J. Am. Chem. Soc.*, *63*, 1483 (1941).

¹³⁷ R. C. Millican and J. B. Brown, *J. Biol. Chem.*, *154*, 437-449 (1944).

¹³⁸ J. B. Brown and G. G. Stoner, *J. Am. Chem. Soc.*, *59*, 3-6 (1937).

¹³⁹ J. S. Frankel, W. Stoneburner, and J. B. Brown, *J. Am. Chem. Soc.*, *65*, 259-262 (1943).

¹⁴⁰ F. A. Smith and J. B. Brown, *Oil & Soap*, *22*, 277-283 (1945).

¹⁴¹ J. B. Brown, *Chem. Revs.*, *29*, 333-354 (1941).

¹⁴² E. L. Smith, *Biochem. J.*, *36*, xxii-xxiii (1942).

¹⁴³ S. R. Elsdon, *Biochem. J.*, *40*, 252-256 (1946).

¹⁴⁴ L. L. Ramsey and W. I. Patterson, *J. Assoc. Official Agr. Chem.*, *28*, 644-656 (1945).

¹⁴⁵ V. Moyle, E. Baldwin, and R. Scarisbrick, *Biochem. J.*, *43*, 308-317 (1948).

¹⁴⁶ M. H. Peterson and M. J. Johnson, *J. Biol. Chem.*, *174*, 775-789 (1948).

¹⁴⁷ C. L. Reinbold and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, *25*, 117-120 (1948).

¹⁴⁸ L. L. Ramsey and W. I. Patterson, *J. Assoc. Official Agr. Chem.*, *31*, 139-150 (1948).

alcohol and 2-aminopyridine as the immobile solvent, with *n*-hexane as the mobile phase on a silica gel column.¹⁴⁹

Although the elution technic, when applied to chromatography, does not afford complete separations,¹⁵⁰⁻¹⁵² a modified procedure, called "displacement analysis," appears to be effective. By the use of silica gel columns with heptane as the solvent, Claesson¹⁵³ has been able to separate saturated, unsaturated, and branched-chain acids. By the use of cetylpyridinium chloride or picric acid as the displacing agent on a column of charcoal or Hyflo Super Cel, Holman and Hagdahl¹⁵⁴ demonstrated a fair separation of acids from C₁₂ to C₂₂. In a later study, the same workers¹⁵⁵ effected a separation of C₁ to C₂₂ acids by using the fatty acids themselves as the displacing agents.

(f) *Countercurrent Distribution*. This technic, which recently came into prominence as a new analytical procedure,^{156-157a} has been used for the quantitative estimation of the C₂ to C₅ acids.¹⁵⁸ When an isopropyl ether-2.2 *M* phosphate buffer system at a pH of 5.17 was employed, the accuracy was within 2 to 3%.

b. Animal Fats. The animal fats differ in composition from the vegetable fats in containing a larger variety of fatty acids. Both saturated and unsaturated fatty acids of the C₂₀, C₂₂, and even the C₂₄ series are found in many animal fats, in addition to the usual components of all fats, namely, the fatty acids of the C₁₆ and C₁₈ series, palmitic, stearic, and oleic acids. In addition to the latter three acids, vegetable fats frequently contain large proportions of linoleic acid, while this diethenoid acid is seldom found in appreciable quantity in animal fats unless it has been fed to the animal previously.

Gross differences in composition are to be noted between the fat from aquatic animals and from those species which live on land. The fats of marine origin tend to be more complex, and contain only 15 to 20% of the saturated acids. Palmitic acid is the predominant member of this group, but small amounts of stearic, myristic, lauric, capric, and even caprylic

¹⁴⁹ L. L. Ramsey and W. I. Patterson, *J. Assoc. Official Agr. Chem.*, **31**, 441-452 (1948).

¹⁵⁰ H. G. Cassidy, *J. Am. Chem. Soc.*, **62**, 3073-3076, 3077-3079 (1940).

¹⁵¹ H. G. Cassidy, *J. Am. Chem. Soc.*, **63**, 2735-2739 (1941).

¹⁵² H. P. Kaufmann and W. Wolf, *Fette u. Seifen*, **50**, 519-521 (1943); *Chem. Abst.*, **39**, 205 (1945).

¹⁵³ S. Claesson, *Rec. trav. chim.*, **65**, 571-575 (1946).

¹⁵⁴ R. T. Holman and L. Hagdahl, *Arch. Biochem.*, **17**, 301-310 (1948).

¹⁵⁵ R. T. Holman and L. Hagdahl, *J. Dairy Sci.*, **32**, 700 (1949).

¹⁵⁶ L. C. Craig, *J. Biol. Chem.*, **155**, 519-534 (1944).

¹⁵⁷ L. C. Craig, C. Golumbic, H. Mighton, and E. Titus, *J. Biol. Chem.*, **161**, 321-332 (1945).

^{157a} L. C. Craig and D. Craig, in *Technique of Organic Chemistry*, Vol. III, A. Weissberger, ed., Chap. IV, "Extraction and Distribution," pages 171-311, Interscience, New York, 1950.

¹⁵⁸ Y. Sato, G. T. Barry, and L. C. Craig, *J. Biol. Chem.*, **170**, 501-507 (1947).

acids, have been reported.¹⁵⁹ The fats from the higher land animals contain more saturated acids, and stearic acid plays a more prominent role. In the case of the unsaturated acids, both the marine and the terrestrial animals possess many representatives between C₁₆ and C₂₄. However, the aquatic forms have a much larger proportion of the longer chain compounds, while most of the unsaturated fatty acids in the higher land forms are concentrated in the C₁₆ and C₁₈ series.

(a) *Composition of Fats from Aquatic Forms.* Strangely enough, there seems to be a very considerable alteration in the type of fat which is found in the salt-water and the fresh-water species. In general, the sea-water forms are more complex and have high proportions of C₁₈, C₂₀, and C₂₂ unsaturated acids. On the other hand, the fresh-water animals contain a larger content of C₁₆ and C₁₈ unsaturated acids, with little C₂₀ and practically no C₂₂ acids. However, in both cases, the C₂₀ and C₂₂ acids which are found are very highly unsaturated, containing from three to six unsaturated linkages. Such differences cannot be shown to exist in the case of the algae, in which the properties are in accordance with the color grouping rather than with the origin.¹⁶⁰ On the other hand, Lovern¹⁶¹ found that a marked difference obtains between the fat of the marine form of copepod, *Calanus finmarchicus*, and that of a fresh-water copepod. This variation in composition is shown by the lower content of saturated acids, and of the C₁₆ and C₁₈ unsaturated acids. A relatively large amount of C₂₂ unsaturated acids occurs in the salt-water copepod but is practically absent from the fresh-water forms.

Marked differences in the composition of the fat of the marine and of the fresh-water fishes have been demonstrated by the extensive studies of Lovern¹⁶²⁻¹⁶⁹ and others.¹⁷⁰⁻¹⁷² The fresh-water fishes have a practically constant content of palmitic acid (20%) and an extremely high content of C₁₈ unsaturated acids (40% and more), while the figures for C₂₀ and C₂₂ unsaturated acids are lower than in the marine forms. Lovern^{164,169} has suggested that the variation in composition of fresh-water and marine

¹⁵⁹ N. Nobori, *J. Soc. Chem. Ind. Japan*, 43, 59-60, 110, 340B (1940).

¹⁶⁰ J. A. Lovern, *Biochem. J.*, 30, 387-390 (1936).

¹⁶¹ J. A. Lovern, *Biochem. J.*, 29, 847-849 (1935).

¹⁶² J. A. Lovern, *Biochem. J.*, 31, 755-763 (1937).

¹⁶³ J. A. Lovern, *Biochem. J.*, 32, 676-680 (1938).

¹⁶⁴ J. A. Lovern, *Biochem. J.*, 26, 1978-1984 (1932).

¹⁶⁵ J. A. Lovern, *Biochem. J.*, 30, 2023-2026 (1936).

¹⁶⁶ J. A. Lovern, *Biochem. J.*, 28, 1955-1960 (1934).

¹⁶⁷ J. A. Lovern, *Biochem. J.*, 29, 1894-1897 (1935).

¹⁶⁸ J. A. Lovern, *Biochem. J.*, 28, 1961-1963 (1934).

¹⁶⁹ J. A. Lovern, *Biochem. J.*, 30, 20-24 (1936).

¹⁷⁰ H. N. Brocklesby, *Biol. Board Canada, Progress Repts.*, No. 30, 19-20 (1936).

¹⁷¹ H. N. Brocklesby and K. F. Harding, *J. Fish Research Board Canada*, 4, 59-62 (1938).

¹⁷² E. F. Armstrong and J. Allan, *J. Soc. Chem. Ind.*, 43, 207-218T (1924).

TABLE 3
COMPOSITION OF FAT OF ZOOPLANKTON AND A MARINE AND A FRESH-WATER COPEPOD^a

Genera and species	Saturated acids with C atoms ^b				Unsaturated acids with C atoms ^b				
	<14	14	16	18	14	16	18	20	22
Fresh-Water Forms									
Zooplankton									
<i>Daphnia galeata</i>	1	3.5	13	1.7	3	21.1	45	12	—
					(>2.0)	(>2.0)	(5.0)	(8.0)	
<i>Diaptomus gracilis</i>	—	2.6	20	1.6	—	15.7	34.6	25.5	—
						(>2.0)	(4.5)	(7.1)	
Copepod									
<i>Cyclops strenuus</i>	—	6	15.6	1	3	30	25	15.6	3.5
					(>2.7)	(3.0)	(5.2)	(8.6)	(7.1)
Salt-Water Form									
Copepod.									
<i>Calanus finmarchicus</i>	—	8	10.6	1.3	1.6	12	17	24.5	25.1
					(2.0)	(2.4)	(5.1)	(7.8)	(8.1)

^a Adapted from J. A. Lovern, *Biochem. J.*, 29, 847-849 (1933).

^b Figures in parentheses represent unsaturation in hydrogen atoms.

forms may be only a reflection of a difference in food habits rather than of alterations in fat metabolism. Other possible causes might be seasonal or environmental variations. A comparison of the analytical data on the zooplanktons and copepods is given in Table 3, and that for the fishes is included in Tables 4 and 5.

The salmon plays a unique role among the fishes, for which reason it is included both in the fresh water and in the marine group. For their first 2 or 3 years of life, the salmon live in fresh water, where they are known as "salmon parr." The body fat of these animals approaches the composition of fresh-water forms more nearly at this time than at any other stage. Thus, C_{14} unsaturated acids are present and the C_{16} unsaturated acids are at the highest level, while the C_{22} unsaturated compounds are at the lowest point in the life cycle. Just before the end of their fresh-water sojourn, they become silvery and are referred to as "smolts." They swim downstream and out to sea, and then the body fat begins to change to that typical of marine fishes. After maturing, the adult fish feed heavily in the sea and return to the rivers for spawning. By this time their body fat has become typically marine, with a larger proportion of the longer chain unsaturated acids. During the journey upstream, the fishes fast. A considerable amount of fat is transferred from the body depots to the gonads. After spawning, most male fishes perish in the river, but some females are able to return to the sea. These so-called "kelts" have been shown to be extremely emaciated. A body fat content as low as 0.3% has been reported, in contrast to a level of 13 to 14% in salmon fresh from the sea. For further details, the reader is referred to the extensive studies of Lovern.^{166,168,169}

A number of other unique features characterize the fat deposits of fishes. For example, the liver serves as the main depot for fat storage in many species, contrasted with the minor part it plays in most terrestrial forms. In the case of some of the *Elasmobranch* families and the *Physeteridae* (sperm whales), glycerol is found not only in ester linkage with the fatty acids but also at the same time in ether linkage with some of the higher alcohols such as batyl, selachyl, and chimyl alcohols. (For a discussion of the chemistry of these alcohols, see Chapter IV.) Such a possible mixed ether-ester triglyceride might have the composition⁸¹ shown in the accompanying

Octadecenyl alcohol.....	$H_2C-OCH_2(CH_2)_7CH:CH(CH_2)_7CH_3$
Oleic acid.....	$H\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-OCO(CH_2)_7CH:CH(CH_2)_7CH_3$
Gadoleic acid.....	$H_2C-OCO(CH_2)_7CH:CH(CH_2)_9CH_3$

formula. The presence of these glyceryl ethers has been reported by the

Genera and species	Common name	Flesh fat, %	Unsatoni- fiable material in oil, %	Saturated acids with C atoms ^b			Unsaturated acids with C atoms ^b												
				14	16	18	14	16	18	20	22	24							
<i>Scombridae</i>																			
<i>Thunnus thynnus</i> ¹	Tunny	23	0.7	4.2	18.6	3.5	—	6.2	26.0	23.5	18.0	—	—	—	—	—	—	—	—
<i>Pluronectidae</i>																			
<i>Hippoglossus hippoglossus</i> ¹ ..	Halibut	4-7	1.3	4.0	14.8	0.7	—	6.5	23.8	26.9	23.3	—	—	—	—	—	—	—	—
<i>Rhombus maximus</i> ¹	Turbot	4	2.1	3.4	15.1	2.1	0.3	8.9	21.7	26.6	21.9	—	—	—	—	—	—	—	—
<i>Petromyzonidae</i>																			
<i>Petromyzon fluviatilis</i> ¹	River lamprey or lampern	8.5	2.3	9.5	17.6	0.7	—	10.9	35.3	15.3	10.7	—	—	—	—	—	—	—	—

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 40.

^b Figures in parentheses represent unsaturation in hydrogen atoms.

^c J. A. Lovern, *Biochem. J.*, **32**, 676-680 (1938).

^d T. P. Hilditch and S. P. Pathak, *Biochem. J.*, **42**, 316-320 (1948).

^e J. A. Lovern, *Biochem. J.*, **26**, 1978-1984 (1932).

^f H. N. Brocklesby, *Biol. Board Can., Prog. Repts.*, No. 30, 19-20 (1936).

^g H. N. Brocklesby and K. F. Harding, *J. Fisheries Research Board Can.*, **4**, 59-62 (1938).

^h E. F. Armstrong and J. Allan, *J. Soc. Chem. Ind.*, **43**, 207-218T (1924).

ⁱ J. A. Lovern, *Biochem. J.*, **28**, 1955-1960 (1934).

^j J. A. Lovern, *Biochem. J.*, **31**, 755-763 (1937).

^k Also contains 0.4% of arachidic acid.

^l J. A. Lovern, *Biochem. J.*, **30**, 2023-2026 (1936).

TABLE 5
 COMPONENT ACIDS (WEIGHT PER CENT) OF FATS OF FRESH-WATER FISHES^a

Genera and species	Common name	Flesh fat, %	Unsaponifiable material, %	Saturated acids with C atoms ^b			Unsaturated acids with C atoms ^b				
				14	16	18	14	16	18	20	22
<i>Salmonidae</i>											
<i>Salmo salar</i> , young	Salmon	3.9	5.0	2.7 ^d	17.7	3.3	3.1	21.7	30.0	12.9	9.9
<i>Salmo trutta</i> ^e	Brown trout	—	—	3.1	19.0	4.5	(>2.0)	(2.3)	(3.8)	(8.3)	(10.2)
<i>Coregonus pollan</i> ^f	Pollan	—	1.5	2.9	14.3	1.9	1.5	11.5	38.3	15.0	8.2
<i>Esocidae</i>											
<i>Esox lucius</i> ^f	Pike	—	4.0	4.7	13.2	0.5	0.8	(2.6)	(3.9)	(7.8)	(10.1)
<i>Percidae</i>											
<i>Percia fluviatilis</i> ^f	Perch	—	6.0	3.5	12.5	2.0	1.1	19.8	40.0	13.5	6.1
<i>Cyprinidae</i>											
<i>Cyprinus carpio</i> ^f	Carp	—	3.5	3.7	14.6	1.9	1.0	(2.0)	(3.2)	(7.4)	(9.1)
<i>Ctenopharyngodon idaltus</i> ^g	Grass-feeding carp	3.7	2.0	1.5	13.6	2.5	1.5	20.8	38.4	15.3	6.3
<i>Hypophthalmichthys nobilis</i> ^g	Mud-feeding carp	—	4.4	2.6	18.0	1.9	0.7	(2.0)	(2.8)	(7.5)	(7.5)
<i>H. molitrix</i> ^g	Mud-feeding carp	2	9.5	—	17	6	—	19.4	40.5	13.8	7.1
		2	2.4	0.8	21.3	1.1	0.6	(2.0)	(3.2)	(6.8)	(9.2)

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 42.

^b Figures in parentheses represent unsaturation in hydrogen atoms.

^c J. A. Lovren, *Biochem. J.*, 28, 1961-1963 (1934).

^d 0.7% lauric acid also present.

^e J. A. Lovren, *Biochem. J.*, 31, 755-763 (1937).

^f J. A. Lovren, *Biochem. J.*, 26, 1978-1984 (1932).

^g J. A. Lovren, *Biochem. J.*, 29, 1894-1897 (1935).

Hilditch group,¹⁷³⁻¹⁷⁶ including Lovern,¹⁶² by Japanese workers,^{177,178} and by others.^{179,180}

The fat from dolphins and porpoises, as well as from other members of the *Delphinidae*, is unique in containing a considerable proportion of the branched-chain compound, isovaleric acid. The amount varies in different portions of the body, being highest in the head (particularly the jaw) and blubber, and being absent from the liver, lungs, and heart. Lovern¹⁶¹ has reported that a sample of the jaw fat of the porpoise (*Phocaena communis*) contained 25.3% of isovaleric acid, which corresponds to a molar percentage of 44.7%. Isovaleric acid is likewise present in the head fat of another marine mammal, the white whale (*Delphinapterus leucas*),¹⁸² to the extent of 25.1%.

Whale oil contains sizable amounts of C₂₀ and C₂₂ unsaturated acids and is a typical marine fat. The samples obtained from Arctic regions which have been investigated by C. W. Moore and C. H. Clarke, according to Armstrong and Allan,¹⁷² have a higher unsaturation (and a lower proportion of saturated acids) than the oil obtained from the Antarctic.^{176,177,183,184}

(b) *Composition of Fats from Land Animals.* With the increasing complexity of the animals, the component fats become simpler. The transformation is a gradual one. In the case of the amphibia and reptiles, there is little change in the total unsaturated acids as compared with those of the aquatic forms, but a marked reduction in acids of the C₂₂, C₂₀, and C₁₆ series, with a proportional increase in the C₁₈ unsaturated acids.¹⁸⁵⁻¹⁸⁹

A considerable amount of experimental work has been carried out on the composition of the fat of birds. Only small amounts of C₂₀-C₂₂ unsaturated acids were found to be present in the fats from light Sussex hens, while palmitic acid comprised about 25% and stearic acid 7% of the total

¹⁷³ T. P. Hilditch and A. Houlbrooke, *Analyst*, *53*, 246-257 (1928).

¹⁷⁴ T. P. Hilditch and J. A. Lovern, *J. Soc. Chem. Ind.*, *47*, 105-111T (1928).

¹⁷⁵ T. P. Hilditch and J. A. Lovern, *J. Soc. Chem. Ind.*, *48*, 359-365T (1929).

¹⁷⁶ T. P. Hilditch and J. A. Lovern, *J. Soc. Chem. Ind.*, *48*, 365-368T (1929).

¹⁷⁷ M. Tsujimoto, *Chem. Umschau Fette Öle Wachse Harze*, *39*, 50-52 (1932); *Chem. Abst.*, *26*, 3126 (1932).

¹⁷⁸ M. Tsujimoto, *J. Soc. Chem. Ind.*, *51*, 317-323T (1932).

¹⁷⁹ E. André and A. Bloch, *Compt. rend.*, *195*, 627-629 (1932).

¹⁸⁰ T. H. Wang and C. H. Kan, *J. Chinese Chem. Soc.*, *4*, 393-401 (1936).

¹⁸¹ J. A. Lovern, *Biochem. J.*, *28*, 394-402 (1934).

¹⁸² N. V. Williams and N. Y. Maslov, *Schriften Zentr. Forsch.-Inst. Lebensmittelchem. U. S. S. R.*, *4*, 150-156 (1935); *Chem. Abst.*, *30*, 4707 (1936).

¹⁸³ I. Tveraæn and A. Klem, *Hvalrædets Skrifter*, No. 11, Kr. 8, 5-48 (1935).

¹⁸⁴ T. P. Hilditch and J. T. Tereleski, *J. Soc. Chem. Ind.*, *56*, 315-322T (1937).

¹⁸⁵ T. G. Green and T. P. Hilditch, *Biochem. J.*, *32*, 681-686 (1938).

¹⁸⁶ T. P. Hilditch and K. S. Murti, *J. Soc. Chem. Ind.*, *58*, 351-353T (1939).

¹⁸⁷ T. P. Hilditch and H. S. Paul, *Biochem. J.*, *31*, 227-228 (1937).

¹⁸⁸ E. Klenk, *Z. physiol. Chem.*, *221*, 259-263, 264-270 (1933).

¹⁸⁹ E. Klenk, F. Ditt, and W. Diebold, *Z. physiol. Chem.*, *232*, 54-63 (1935).

fat. Hexadecenoic acid made up 7%, oleic acid 38%, and linoleic acid¹⁹⁰ 22%. A surprising uniformity in the composition of abdominal, gizzard, and neck fats was noted both in young and in old birds. In the case of sea birds, a considerable increase in the proportion of C₂₀-C₂₂ unsaturated acids obtains, with a corresponding decrease of the C₁₈ unsaturated acids and palmitic acid. Thus, the fat of these birds resembles that of the aquatic animals more than that of the land species. Lovern¹⁹¹ has suggested that this atypical composition may result from the diet of sea food; furthermore, sea birds may have no specific requirements, and any type of depot fats may serve equally well.

The fat of rats and pigs has been the subject of many investigations. Where the proportion of fat in the diets is sufficiently low, so as not to influence the type of fat laid down, the fat from the rat and pig is composed of 25 to 30% of palmitic acid and only 5 to 10% of stearic acid. The unsaturated acid is chiefly oleic, which may comprise 45 to 50% of the total; considerable amounts of palmitoleic acid also occur, with only traces of the higher acids. When the composition of the fat is altered by diet, no change takes place in the proportion of stearic acid, but a marked decrease in both palmitic and palmitoleic acids obtains. The new food fat may replace the fatty acids which have disappeared. Such fatty acids as lauric, myristic, linoleic, linolenic, and arachidonic can all be more or less effectively added to the body fat. Beadle *et al.*,¹⁹² in fact, have recently proved that as much as 27.6% of the fatty acids present in rats, and 11.4% of that composing pig fat, may consist of trienoic acids (chiefly linolenic) if a large proportion of this type of unsaturated acid is fed.

Less information is available as to the fat composition in the higher species. In the case of beef and mutton tallow, only three fatty acids are present in any appreciable amounts, namely, palmitic, stearic, and oleic acid. These are present to the approximate extent of 25-30%, 20-25%, and 40-45%, respectively.¹⁹³ The composition of the fat of steers is much less influenced by the feeding of unsaturated acids than is that of the rat and pig.¹⁹⁴ Although arachidic acid is normally not present in the fat of the pig, definite amounts are found after the animals have received large amounts of peanuts over a period of time.¹⁹⁵

It was recognized at an early date that human fat contains palmitic,

¹⁹⁰ T. P. Hilditch, E. C. Jones, and A. J. Rhead, *Biochem. J.*, **28**, 786-795 (1934).

¹⁹¹ J. A. Lovern, *Biochem. J.*, **32**, 2142-2144 (1938).

¹⁹² B. W. Beadle, O. H. M. Wilder, and H. R. Kraybill, *J. Biol. Chem.*, **175**, 221-229 (1948).

¹⁹³ A. Banks and T. P. Hilditch, *J. Soc. Chem. Ind.*, **51**, 111-114T (1932).

¹⁹⁴ B. H. Thomas, C. C. Culbertson, and F. Beard, *Proc. Am. Soc. Animal Production*, **27**, 193-199 (1934).

¹⁹⁵ N. R. Ellis and H. S. Isbell, *J. Biol. Chem.*, **69**, 219-238 (1926).

stearic, and oleic acids,¹⁹⁶ while Eckstein,¹⁹⁷ in 1925, proved the presence of traces of lauric and myristic acids also, and of less than 1% of linoleic,

TABLE 6
TYPICAL DEPOT FATS (WEIGHT PER CENT) OF DEPOT FATS OF HIGHER LAND ANIMALS

Animal	Saturated acids			Unsaturated acids ^a				
	C ₁₄	C ₁₆	C ₁₈	C ₁₄ -2H	C ₁₆ -2H	Oleic	Linoleic	C ₂₀
Frog ^b	4	11	3	—	15	52 (2.5)	15 (6)	
Lizard ^c	4	29	10	—	12	40 (2.7)	5 (5.5)	
Chicken ^d	0.1	25.6	7.0	—	7.0	38.4	21.3	0.6 (6.8)
Rat ^e								
Low-fat diet	3.1	26.7	0.4	0.9	15.6	47.2	2.2	0.3 (8)
5% fat diet	1.8	24.4	4.1	0.3	4.8	44.3	18.6	1.4 (8)
High corn oil diet	0.7	12.2	2.9	—	6.9	44.8	32.3	—
High coconut oil diet	44.5 ^f	19.9	4.0	2.0 ^g	4.4	23.8	1.4	—
Pig (back fat)								
Low-fat diet ^h	0.8	25.9	12.2	0.2	2.0	48.1	7.8	3.0 (8)
Low-fat diet ⁱ alone	1.7	25.5	13.7	—	—	50.2	8.9	—
+4% cottonseed oil	1.1	25.0	21.1	—	—	39.5	13.3	—
+8% cottonseed oil	1.1	13.8	26.5	—	—	31.8	26.8	—
Mutton tallow ^j	4.6	24.6	30.5	—	—	36.0	4.3	—
Goat ^k	3.5	25.5	28.1	—	—	38.4	—	2.4 (8)
Beef tallow ^l	3.3	24.9	24.1	0.4	2.4	41.8	1.8	0.5 (8)
Horse								
Abdominal ^m	—	28	5	—	—	50	13 ^o	—
Body ⁿ	—	29	7	—	—	55	7 ^p	—
Reindeer loin ^q	7	35	20 ^r	—	—	37	—	—
Camel hump ^s	—	37	16	—	—	47	—	—

^a Figures in parentheses represent unsaturation in hydrogen atoms.

^b E. Klenk, *Z. physiol. Chem.*, 221, 259-263, 264-270 (1933).

^c T. P. Hilditch and H. Paul, *Biochem. J.*, 31, 227-228 (1935).

^d T. P. Hilditch, E. C. Jones, and A. J. Rhead, *Biochem. J.*, 28, 786-795 (1934).

^e H. E. Longenecker, *J. Biol. Chem.*, 128, 645-658 (1939); 129, 13-22 (1939); 130, 167-177 (1939).

^f Including 27.2% C₁₂ acid.

^g Including 0.6% C₁₂ unsaturated acid.

^h T. P. Hilditch, C. H. Lea, and W. H. Pedelty, *Biochem. J.*, 33, 493-504 (1939).

ⁱ N. R. Ellis, C. S. Rothwell, and W. O. Pool, *J. Biol. Chem.*, 92, 385-398 (1931).

^j G. Collin, T. P. Hilditch, and C. H. Lea, *J. Soc. Chem. Ind.*, 48, 46-50T (1929).

^k D. R. Dhingra and D. N. Sharma, *J. Soc. Chem. Ind.*, 57, 369-370T (1938).

^l T. P. Hilditch and H. E. Longenecker, *Biochem. J.*, 31, 1805-1819 (1937).

^m H. A. Schuette, T. M. Garvin, and E. J. Schwoegler, *J. Biol. Chem.*, 107, 635-639 (1934).

ⁿ A. Heiduschka and A. Steinruck, *J. prakt. Chem.*, 102, 241-266 (1921).

^o 4% of linolenic acid in addition.

^p 2% of linolenic acid in addition.

^q W. F. Baughman, G. S. Jamieson, and R. S. McKinney, *Oil & Fat Industries*, 6, 11-12 (1929).

^r 1% C₂₀ saturated acid in addition.

^s E. F. Armstrong and J. Allan, *J. Soc. Chem. Ind.*, 43, 207-218T (1924).

¹⁹⁶ J. Jaekle, *Z. physiol. Chem.*, 36, 53-84 (1902).

¹⁹⁷ H. C. Eckstein, *J. Biol. Chem.*, 64, 797-806 (1925).

linolenic, and arachidonic acids, respectively. Wagner¹⁹⁸ could not confirm the presence of linolenic acid, although he did prove that linoleic and arachidonic acids are present. Cramer and Brown¹³⁵ reported important amounts of tetradecenoic as well as of hexadecenoic acid in all five samples of human fat which they examined. The composition of typical animal fats is included in Table 6, while the results of Cramer and Brown¹³⁵ on human fat are included in Table 7.

TABLE 7
FATTY ACID CONTENT OF HUMAN FAT (WEIGHT PER CENT)^a

Fatty acid	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Lauric	0.1	0.6	—	—	0.9
Myristic	2.7	5.9	2.6	2.6	3.9
Palmitic	24.0	25.0	24.7	25.4	25.7
Stearic	8.4	5.8	7.7	7.7	5.2
Tetradecenoic	0.2	0.6	0.4	0.4	0.5
Hexadecenoic	5.0	6.7	7.3	5.6	7.6
Octadecenoic	46.9	45.4	45.8	44.8	46.6
Octadecadienoic	10.2	8.2	10.0	11.0	8.7
Arachidonic	1.0	1.0	0.4	0.3	0.6
Other C ₂₀	1.5	0.8	1.1	2.2	0.3

^a D. L. Cramer and J. B. Brown, *J. Biol. Chem.*, 151, 427-438 (1943), p. 428.

(c) *Composition of Milk Fats.* The composition of the milk fats shows a certain uniformity in various species. Thus, the myristic and palmitic acids are in fair agreement in samples from mature animals of a number of species, being 7-12% and 23-31%, respectively. Oleic acid, also, shows considerable uniformity, varying only from 28 to 41% in all types reported.

However, there are a number of important differences between human milk fat and that from the lower animals. The chief one is the extremely low content of butyric, caproic, and caprylic acids in human milk in contrast to their relatively high proportion in goat and cow milk. Human milk also contains a larger proportion of octadecadienoic acid than is found in any other milk fats examined, but this might be attributable to diet. The presence of C₁₀₋₁₆ unsaturated fatty acids in milk fat from the cow, goat, and human subject seems to be slight but definite. The function of these monoethenoid acids is unknown. Achaya and Banerjee¹⁹⁹ recently reported a maximum of 15.4% of butyric acid in the milk fat of the Indian buffalo. This represents by far the highest concentration of this acid found in natural fats. The composition of milk fats from several varieties of animals is included in Table 8.

¹⁹⁸ O. Wagner, *Biochem. Z.*, 174, 412-419 (1926).

¹⁹⁹ K. T. Achaya and B. N. Banerjee, *Biochem. J.*, 40, 664-669 (1946).

TABLE 8
COMPOSITION OF MILK FAT FROM VARIOUS SPECIES

Species of milk fat	Saturated acids with C atoms, weight per cent										Monounsaturated acids with C atoms, weight per cent									
	C ₄	C ₆	C ₈	C ₁₀	C ₁₂	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀	C ₃₂	C ₃₄	C ₃₆	C ₃₈	C ₄₀	
Buffalo ^c	4.1	1.4	0.9	1.7	2.8	10.1	31.1	11.2	0.9	—	—	—	—	—	—	—	—	—	—	—
Indian buffalo ^d	15.4	1.1	1.4	1.5	1.9	9.2	31.9	12.5	0.1	0.1	0.1	0.6	3.0	16.8 ^e	—	—	—	—	—	—
Pasture, oil cake.....	11.5	—	0.1	0.5	0.8	4.8	25.1	19.0	1.1	0.03	—	—	—	—	—	—	—	—	—	—
High cottonseed oil.....	2.1	0.9	0.6	1.4	4.6	7.3	29.3	11.1	—	—	—	—	—	—	—	—	—	—	—	—
Camel ^g	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cow	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Spring pasture ^h	3.5	1.7	1.3	3.1	4.1	11.1	27.3	11.5	0.6	—	—	—	—	—	—	—	—	—	—	—
Autumn fed ^h	3.1	1.7	1.6	2.1	3.4	6.9	29.0	7.6	0.9	—	—	—	—	—	—	—	—	—	—	—
Winter, stall fed ⁱ	3.9	1.5	0.7	1.9	3.7	8.4	22.0	15.0	0.7	—	—	—	—	—	—	—	—	—	—	—
Early summer pasture ^h	3.3	1.3	1.2	2.2	4.0	10.4	26.1	6.5	—	—	—	—	—	—	—	—	—	—	—	—
Winter, stall fed ^j	3.7	2.0	1.0	2.6	1.7	9.3	25.4	10.7	0.4	0.2	—	—	—	—	—	—	—	—	—	—
Colostrum ^k	2.6	1.6	0.5	1.6	3.2	9.5	31.7	11.8	0.6	0.1	0.2	0.7	2.7	28.5	2.5	1.8	—	—	—	—
Goat ^l	2.1	1.9	2.7	7.9	3.5	10.2	28.7	8.1	0.4	0.2	—	—	—	—	—	—	—	—	—	—
Goat ^m	3.0	2.3	3.9	8.6	4.6	11.4	24.6	9.3	0.1	—	—	—	—	—	—	—	—	—	—	—
Human	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Colostrum, 1-2 days ⁿ	0.2	0.1	0.8	3.5	0.9	2.8	24.6	9.9	4.9	0.2	0.1	0.1	1.8	36.0	7.5 ^o	6.4	—	—	—	—
Mature ⁿ	0.4	0.1	0.3	2.2	5.5	8.5	23.2	6.9	1.1	0.1	0.1	0.6	3.0	36.5	7.8 ^p	3.3	—	—	—	—
Sheep ^m	3.3	2.8	3.8	7.8	5.4	12.2	23.5	6.9	1.9	—	—	—	—	—	—	—	—	—	—	—
Mare ^q	1.1	1.9	4.4	7.9	6.8	7.4	15.4	2.4	0.2	1.3	1.2	1.9	7.2	16.3 ^r	14.0	4.0	—	—	—	—
Cow ghee ^o	3.3	2.1	1.0	2.3	3.7	5.8	30.0	11.2	—	—	—	—	—	—	—	—	—	—	—	—

^a Linoleic acid.
^b Total unsaturated C₂₀₋₂₂ acids.
^c R. Bhattacharya and T. P. Hilditch, *Analyst*, 56, 161-170 (1931).
^d K. T. Achaya and B. N. Banerjee, *Biochem. J.*, 40, 664-669 (1946).
^e 1.2% linoleic and 3.3% gadoleic acids.
^f 1.0% linoleic and 0.6% gadoleic acids.
^g D. R. Dhingra, *Biochem. J.*, 28, 73-78 (1934).
^h T. P. Hilditch and J. J. Sleightholme, *Biochem. J.*, 24, 1098-1113 (1930).
ⁱ H. K. Dean and T. P. Hilditch, *Biochem. J.*, 27, 889-897 (1933).
^j T. P. Hilditch and H. Paul, *Biochem. J.*, 30, 1905-1914 (1936).
^k A. R. Baldwin and H. E. Longenecker, *J. Biol. Chem.*, 155, 407-412 (1944).
^l R. W. Riemenschneider and N. R. Ellis, *J. Biol. Chem.*, 113, 219-233 (1936).
^m D. R. Dhingra, *Biochem. J.*, 27, 851-859 (1933).
ⁿ A. R. Baldwin and H. E. Longenecker, *J. Biol. Chem.*, 154, 255-265 (1944).
^o 0.3% of octadecatrienoic acid in addition.
^p 0.4% of octadecatrienoic acid in addition.
^q T. P. Hilditch and H. Jasperson, *Biochem. J.*, 33, 443-447 (1944).
^r 6.6% octadecadienoic acid also present.

c. Vegetable Fats. In most cases, vegetable fats have fewer varieties of component fatty acids than do those from animal sources. Palmitic and oleic acids are the predominant acids in the vegetable lipids, just as they are in the animal fats; however, the other chief acid in the plant group, *i.e.*, linoleic acid, is present in extremely limited amounts in the animal. On the other hand, stearic acid, which plays such a prominent role in the animal kingdom, is absent or present in very small amounts in most of the vegetable fats; only one series of plant lipids, the members of which are mostly of tropical origin, contains appreciable quantities of stearic acid.

The fatty acid content of the plant products is related to the family and species. In listing the composition of the various fats, it has seemed best to arrange them according to their botanical classification, by the system employed by Hilditch.⁸⁰ In this procedure the fats especially rich in certain fatty acids are grouped together. In the present tables, only those fats of fairly wide commercial application are included in the compilations.

(a) *Fats with a High Content of Linoleic Acid.* Seed oils have the highest proportion of linoleic acid of any of the common vegetable fats. In some cases, the linoleic acid may exceed 75% of the total fatty acid content. This acid is especially rich in the seeds of the large trees such as the pine and walnut. Certain herbaceous plants, including flax, perilla, and hemp, also contain a high proportion of linoleic acid coupled with considerable amounts of linolenic acid. The composition of some of these oils is summarized in Table 9. Another group of fats also high in linoleic acid but which contains no linolenic acid, or only traces of it, is listed in Table 10. In Table 11 the fats which are high not only in oleic and linoleic acids but also in palmitic acid are assembled.

(b) *Fats Containing Elaeostearic, Petroselinic, or Erucic Acid.* Several important unsaturated acids are frequently found in certain fats, in addition to the more common oleic, linoleic, and linolenic acids. Elaeostearic acid (9,10,11,12,13,14-octadecatrienoic acid) is the best known of these, on account of its application in the paint and varnish industry. Because of the conjugate position of the double bonds, this acid is especially effective as a "drying oil." Tung oil may contain as much as 80–95% of the fatty acids in the form of elaeostearic acid.^{200–205}

²⁰⁰ H. P. Kaufmann and J. Baltés, *Ber.*, 69, 2676–2679 (1936).

²⁰¹ J. Van Loon, *Farben-Ztg.*, 35, 1767–1769 (1930); *Chem. Abst.*, 24, 4178 (1930). Cited by T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 167.

²⁰² R. S. McKinney and G. S. Jamieson, *Oil & Soap*, 12, 92–93 (1935).

²⁰³ R. S. McKinney and G. S. Jamieson, *Oil & Soap*, 14, 2–3 (1937).

²⁰⁴ R. S. McKinney and G. S. Jamieson, *Oil & Soap*, 15, 30–32 (1938).

²⁰⁵ A. Steger and J. Van Loon, *J. Soc. Chem. Ind.*, 47, 361–363T (1928).

TABLE 9
COMPOSITION OF SOME SEED FATS CONTAINING PRINCIPALLY OLEIC, LINOLEIC, AND LINOLENIC ACIDS^a

Family and species	Common name	Saturated acid, weight per cent			Unsaturated acid, weight per cent		
		C ₁₆	C ₁₈	C ₂₀	Oleic	Linoleic	Linolenic
<i>Coniferae (Pinaceae)</i>							
<i>Pinus monophylla</i> ^b	Mexican piñon nut oil	8.8	8.8	—	64.0	27.0	—
<i>Pinus peuce</i> ^c	Fir seed oil	0.7	—	—	42.7	49.5	7.7
<i>Pinus sabiniana</i> ^d	Digger pine seed oil	5.5	5.5	—	51	43.5	—
<i>Juglandaceae</i>							
<i>Hicoria pecan</i> ^e	Pecan nut oil	3.3	1.9	—	78.7	16.1	—
<i>Juglans regia</i> ^f	Walnut seed oil	4.6	0.9	—	17.6	73.0	3.2
	Walnut seed oil	3.5 ^g	1.9	—	35.0	57.0	3.0
<i>Moraceae</i>							
<i>Cannabis sativa</i> ^h	Hempseed oil	10.1	10.1	—	16.0	46.0	28.0
<i>Cannabis sativa</i> ⁱ	Hempseed oil	5.8	1.7	1.1	6.0	70.0	15.0
<i>Labiatae</i>							
<i>Perilla octinoides</i> ^j	Perilla seed oil	6.7	6.7	—	6.6	37.0	44.0
<i>Passifloraceae</i>							
<i>Passiflora edulis</i> ^k	Passion fruit oil (purple granada)	7.1	1.8	0.4	19.9	62.3	5.8
<i>Linaceae</i>							
<i>Linum usitatissimum</i> ^l	Linseed oil (flax)	8.3	8.3	—	17.3	21.8	46.5
<i>Linum usitatissimum</i> ^m	Linseed oil (flax)	10.3	10.3	—	21.0	15.0	53.0
<i>Linum usitatissimum</i> ⁿ	Linseed oil (flax)	5.4	3.5 ^o	0.6	8.0	43.0	40.0

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, pp. 156-157.^b M. Adams and A. Holmes, *Ind. Eng. Chem.*, **5**, 285-287 (1913).^c O. von Friedrichs, *J. Soc. Chem. Ind.*, **39**, No. 8, 304A (1920).^d J. Semb, *J. Am. Pharm. Assoc.*, **24**, 609-613 (1935).^e G. S. Jamieson and S. I. Gertler, *Oil & Fat Industries*, **6**, 23-24 (1929).^f G. S. Jamieson and R. S. McKinney, *Oil & Fat Industries*, **6**, 21-23 (1929).^g Also contains 0.5% C₁₄.^h H. P. Kaufmann and S. Juschkevitch, *Z. angew. Chem.*, **43**, 90-92 (1930).ⁱ H. N. Griffiths and T. P. Hilditch, *J. Soc. Chem. Ind.*, **53**, 75-81T (1934).^j H. P. Kaufmann, *Allgem. Oel-u. Fett-Ztg.*, **27**, 39-40 (1930); *Chem. Abst.*, **24**, 2000 (1930).^k G. S. Jamieson and R. S. McKinney, *Oil & Soap*, **11**, 193 (1934).^l A. Eibner and F. Brose, *Chem. Umschau*, **35**, 157-166 (1928); *Chem. Abst.*, **22**, 4852 (1928).^m P. J. Gay, *J. Soc. Chem. Ind.*, **51**, 126-129T (1932).ⁿ H. N. Griffiths and T. P. Hilditch, *J. Soc. Chem. Ind.*, **53**, 75-81T (1934). T. P. Hilditch and E. C. Jones, *ibid.*, **53**, 13-21T (1934).^o Also contains 0.2% C₁₄.

TABLE 10
COMPOSITION (WEIGHT PER CENT) OF SOME SEED OILS CONTAINING PRINCIPALLY OLEIC OR LINOLEIC ACIDS OR BOTH^a

Family and species	Common name of oil	Saturated acid				Unsaturated acid		
		C ₁₄	C ₁₆	C ₁₈	C ₂₀	Oleic	Linoleic	Linolenic
<i>Betulaceae</i>								
<i>Corylus avellana</i> ^b	Filbert	0.2	3.2	1.7	—	91.0	4.0	—
<i>Fagaceae</i>								
<i>Fagus sylvatica</i> ^c	Beechnut	—	5.2	3.7	—	81.0	10	0.4
<i>Fagus sylvatica</i> ^d	Beechnut	—	12.0	12.0	—	48	38.0	2.9
<i>Urticaceae</i>								
<i>Celtis occidentalis</i> ^e	Hackberry seed	—	—	5.3	—	16.0	78.0	—
<i>Papaveraceae</i>								
<i>Papaver somniferum</i> ^f	Poppyseed (opium)	—	4.8	2.9	—	30.1	62.2	—
<i>Vitaceae</i>								
<i>Vitis riparia</i> ^g	Wild grapesced	—	3.4	1.9	—	44.0	50.0	—
<i>Vitis vinifera</i> ^h	Grapesced	—	6.5	2.3	0.1	33.0	46.0	2.4
<i>Vitis vinifera</i> ⁱ	Grapesced	—	12.0	12.0	—	16.7	71.4	—
<i>Theaceae</i>								
<i>Thea sinensis</i> ^j	Teaseed	0.3	7.6	0.8	0.6	83.3	7.4	—
<i>Thea sinensis</i> ^k	Chinese teaseed	—	6	6	—	90	10	—
<i>Camellia japonica</i> ^l	Japanese rose	—	10.6	10.6	—	82.6	2.1	—
<i>Pedaliaceae</i>								
<i>Sesamum indicum</i> ^m	Sesame	—	7.8	4.7	0.4	49.4	37.7	—
<i>Sesamum indicum</i> ⁿ	Sesame	—	9.1	4.3	0.8	45.4	40.4	—
<i>Compositae</i>								
<i>Carthamus tinctorius</i> ^o	Safflowerseed	—	6	4	—	38	51	1
<i>Carthamus tinctorius</i> ^p	Safflowerseed	—	4.2	1.6	0.4	26.3	67.4	0.1
<i>Carthamus tinctorius</i> ^q	Safflowerseed	—	7.9	7.9	—	13.0	78.0	1.0
<i>Helianthus annuus</i> ^r	Sunflowerseed	—	3.5	2.9	0.6*	34.1	58.5	—
<i>Helianthus annuus</i> ^s	Sunflowerseed	—	3.7	1.6	0.7	42.0	52.0	—
<i>Helianthus annuus</i> ^t	Sunflowerseed	—	10	10	—	36.0	54.0	—
<i>Rosaceae</i>								
<i>Prunus amygdalus</i> ^v	Almond kernel	1.2	4.5	—	—	77.0	17.3	—
<i>Prunus armeniaca</i> ^w	Apricot kernel	—	2.6	1.2	—	64.4	31.8	—
<i>Prunus cerasus</i> ^x	Cherry, sour, kernel	0.2	4.3	2.9	0.7	49.5	42.3	—
<i>Prunus domestica</i> ^y	Plum kernel	—	5.9	5.9	—	69	21.0	—
<i>Prunus laurocerasus</i> ^z	Laurel cherry kernel	1.8	9.9	1.7	—	73.4	13.2	—
<i>Prunus lusitana</i> ^z	Portuguese laurel cherry kernel	1.3	6.6	2.2	—	57.9	32.0	—
<i>Rubus caesius</i> ^{aa}	Blackberry seed	—	5.0	—	—	17	80.0	3.0

Family and species	Common name of oil	Saturated acid				Unsaturated acid		
		C ₁₄	C ₁₆	C ₁₈	C ₂₀	Oleic	Linoleic	Linolenic
<i>Cucurbitaceae</i>								
<i>Cucumis melo</i> ^b	Muskmelon seed	0.3	10.3	4.6	—	27.5	57.3	—
<i>Cucurbita pepo</i> ^c	Pumpkin seed	—	5.9	7.1	—	40.9	46.1	—
<i>Citrullus vulgaris</i> ^d	Watermelon seed	—	12.6 ^e	15.0	—	43.0	26.0	—
<i>Echinocystis oreagnaf</i> ^f	Mock cucumber seed	—	6.79	3.72	—	58.5	22.9	—

^a Adapted from T. P. Hilditch, *The Chemical Constitution of Natural Fats*, 2nd ed., Wiley, New York, 1947, pp. 158-160, 166-168.

^b H. A. Schuette and C. Y. Chang, *J. Am. Chem. Soc.*, **55**, 3333-3335 (1933).

^c A. Heiduschka and P. Roser, *J. prakt. Chem.*, **104**, 137-160 (1922).

^d E. Delvaux, *Fette u. Seifen*, **43**, 183-184 (1936); *Chem. Abst.*, **31**, 3720 (1937).

^e H. A. Schuette and R. G. Zehmpfenning, *Oil & Soap*, **14**, 269-270 (1937).

^f A. Eihner and B. Wibeltz, *Chem. Umschau*, **31**, 109-120, 121-127 (1924); *Chem. Abst.*, **18**, 3728 (1924).

^g G. D. Beal and C. K. Beebe, *Ind. Eng. Chem.*, **7**, 1054 (1915).

^h G. S. Jamieson and R. S. McKinney, *Oil & Soap*, **12**, 241 (1935).

ⁱ H. P. Kaufmann and H. Fiedler, *Fette u. Seifen*, **44**, 286-289 (1937); *Chem. Abst.*, **31**, 7677 (1937).

^j T. P. Hilditch and E. C. Jones, *J. Soc. Chem. Ind.*, **53**, 13-21T (1934). H. N. Griffiths and T. P. Hilditch, *ibid.*, **53**, 75-81T (1934).

^k S. Ueno and T. Ueda, *J. Soc. Chem. Ind. Japan*, **41**, suppl., 326-328B (1938).

^l H. P. Kaufmann and J. Balties, *Fette u. Seifen*, **45**, 152 (1938); *Chem. Abst.*, **32**, 4810 (1938).

^m G. S. Jamieson and W. F. Baughman, *J. Am. Chem. Soc.*, **46**, 775-778 (1924).

ⁿ T. P. Hilditch, M. B. Ichaporia, and H. Jasperson, *J. Soc. Chem. Ind.*, **57**, 363-368 (1938).

^o J. Zukevanik, *Acta Univ. Asiae Mediae Tashkent*, **6**, 3-19 (1928). Cited from T. P. Hilditch, *The Chemical Constitution of Natural Fats*, p. 160.

^p G. S. Jamieson and S. I. Gertler, *Oil & Fat Industries*, **6**, 11-13 (1929).

^q H. P. Kaufmann and H. Fiedler, *Fette u. Seifen*, **44**, 420-423 (1937); *Chem. Abst.*, **32**, 816 (1938).

^r G. S. Jamieson and W. F. Baughman, *J. Am. Chem. Soc.*, **44**, 2952-2957 (1922).

^s Also contains 0.4% C₂₄.

^t J. Pieraerts, *Mat. grasses*, **17**, 7280-7284, 7340-7343 (1925); *Chem. Abst.*, **20**, 1096 (1926).

^u A. Eihner, *Farbe u. Lack*, **31**, 463-464, 472-473 (1926); *Chem. Abst.*, **21**, 184 (1927).

^v H. M. Thompson, *Unpublished data*, 1933. Cited by T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, p. 166.

^w G. S. Jamieson and R. S. McKinney, *Oil & Soap*, **10**, 147-149 (1933).

^x G. S. Jamieson and S. I. Gertler, *Oil & Fat Industries*, **7**, 371-372 (1930).

^y E. Delvaux, *Fette u. Seifen*, **43**, 183-184 (1936); *Chem. Abst.*, **31**, 3720 (1937).

^z E. E. Jones, *Unpublished data*, 1931. Cited from T. P. Hilditch, *The Chemical Constitution of Natural Fats*, p. 166.

^{aa} R. Krizian, *Ren. Fett-u. Harz-Ind.*, **15**, 7-9, 29-30 (1908); *Chem. Abst.*, **2**, 1352 (1908). Cited by T. P. Hilditch, *The Chemical Constitution of Natural Fats*, p. 166.

^{ab} W. F. Baughman, D. Brauns, and G. S. Jamieson, *J. Am. Chem. Soc.*, **42**, 2398-2402 (1920).

^{ac} J. L. Riebsomer and G. A. Nesty, *J. Am. Chem. Soc.*, **56**, 1784-1785 (1934).

^{ad} J. Pieraerts, *Bull. Sci. Pharmacol.*, **24**, 204-210 (1917).

^{ae} Also contains 2.5% C₂₂.

^{af} G. Einhorn, A. Miiski, and E. Kalashnikov, *Maslobojno Zhirovoe Delo*, **45**, 44-48 (1929); *Chem. Abst.*, **24**, 2625 (1930).

TABLE II
COMPOSITION (WEIGHT PER CENT) OF SEED OILS CONTAINING PRINCIPALLY PALMITIC, OLEIC, AND LINOLEIC ACIDS^a

Family name and species	Common name of oil	Saturated acids				Unsaturated acids	
		C ₁₄	C ₁₆	C ₁₈	C ₂₀	Oleic	Linoleic
<i>Anacardiaceae</i>							
<i>Anacardium occidentale</i> ^b	Cashew nut	—	6.4	11.3	—	73.77	7.7
<i>Pistacia vera</i>	Pistachio nut	0.6	8.2	1.6	—	69.0	20.0
<i>Burseraceae</i>							
<i>Canarium commune</i> ^c	Java almond (Canary tree)	—	29.5	15	—	43	12.5
<i>Mabaceae</i>							
<i>Gossypium arboreum</i> ^e	Cottonseed (tree)	3.3	19.9	1.3	0.6	29.6	45.3
<i>Gossypium herbaceum</i> ^e	Cottonseed (Levant)	2.0	19.6	2.7	0.7	24.6	50.4
<i>Gossypium barbadense</i> ^g	Cottonseed (Sea island)	0.3	20.2	2.0	0.6	35.2	41.7
<i>Gossypium hirsutum</i> ^h	Cottonseed (Upland)	0.5	21.9	1.9	0.1	30.5	44.8
<i>Bombacaceae</i>							
<i>Ceiba pentandra</i> , syn.							
<i>Eriodendron anfractuosum</i> ⁱ	Kapok seed	0.5	16.1	2.3	0.8	50.6	29.7
<i>Eriodendron anfractuosum</i> ^j	Kapok seed	—	10.2	8.4	1.2	45.2	32.9
<i>Lecythidaceae</i>							
<i>Bertholletia excelsa, nobilis</i> ^k	Brazil nut	1.9	13.55	2.58	—	55.64	21.65
<i>Solanaceae</i>							
<i>Nicotiana tabacum</i> ^l	Tobacco seed	—	3.1	4.8	—	16.2	70.4
<i>Nicotiana tabacum</i> ^m	Tobacco seed	—	9.8	5.9	—	28.0	56.3
<i>Lycopersicon esculentum</i> ⁿ	Tomato seed	—	12.47	5.89	0.4	45.0	34.2

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 172-174, 195.

^b C. K. Patel, J. J. Sudborough, and H. E. Watson, *J. Indian Inst. Sci.*, 6, Part 6, 111-129 (1923).

^c D. R. Dhingra and T. P. Hilditch, *J. Soc. Chem. Ind.*, 50, 9-12T (1931).

^d P. Pastrovich, *Chem. Ztg.*, 31, 781-782 (1907).

^e T. P. Hilditch and E. C. Jones, *J. Chem. Soc.*, 1932, 805-820.

^f T. P. Hilditch and A. J. Rhead, *J. Soc. Chem. Ind.*, 51, 198-202T (1932).

^g G. S. Jameson and W. F. Baughman, *J. Am. Chem. Soc.*, 42, 1197-1204 (1920).

^h G. S. Jameson and W. F. Baughman, *Oil & Fat Industries*, 4, 131-133 (1927).

ⁱ A. O. Cruz and A. P. West, *Philippine J. Sci.*, 46, 131-137 (1931).

^j G. S. Jameson and R. S. McKinney, *Oil & Soap*, 13, 233-234 (1936).

^k H. A. Schuette, R. W. Thomas, and M. Duthey, *J. Am. Chem. Soc.*, 52, 4114-4117 (1930).

^l W. L. Roberts and H. A. Schuette, *J. Am. Chem. Soc.*, 56, 207-209 (1934).

^m L. F. Salisbury, *J. Biol. Chem.*, 117, 21-25 (1937).

ⁿ F. Rabak, *U. S. Dept. Agr. Bull.*, No. 632, 1-15 (1917).

Petroselinic acid (6,7-octadecenoic acid), which is an isomer of oleic acid, is largely concentrated in the seed oils which belong to the family of *Umbelliferae*. It has been shown to make up 60% of the total fatty acids in fennel. It is found in such widely diverse umbelliferates as parsley,^{206,207} anise,²⁰⁸ carrot, celery, parsnip, chervil, caraway, fennel, and coriander.²⁰⁹

Erucic acid (13,14-docosenoic acid) is another unsaturated acid important in such specific food fats as rapeseed oil. It is present to the extent of 40 to 57%²¹⁰⁻²¹² in this oil. Large amounts are also present in mustard-seed oil (*Brassica* spp.), wallflower seed oil (*Cheiranthus* spp.),²¹³ and Jamba seed, or rocket salad oil (*Eruca sativa*),^{214,215} and in the seeds from the nasturtium (*Tropaeolaceae*). Nasturtium seed oil offers a good source of erucic acid, since this acid makes up 80-90% of the total.^{216,217} It is known, furthermore, that 40% of the neutral fat consists of the simple triglyceride, trierucin. Still another group of unsaturated fatty acids may be found in the seed fats of the *Flacourtiaceae*. These consist of such acids as hydrocarpic, chaulmoogric, and gorlic acids, all of which contain a cyclic penteno group. Because of the reputed activity of chaulmoogra oil in the treatment of leprosy, this group of acids has received considerable attention.

Table 12 includes data on the composition of the fats containing these unusual unsaturated fatty acids.

(c) *Fats Containing a Large Proportion of Saturated Short-Chain Acids.* In most cases the saturated acids comprise only about 10% of the fatty acids of the vegetable fats. However, a somewhat higher content (15-25%) of the saturated acids (chiefly palmitic) occurs in several plant families such as *Malvaceae* (cottonseed), *Solanaceae* (tomato and tobacco), *Anacardiaceae* (cashew and pistachio), and the *Bombaceae* (kapok). Such

²⁰⁶ T. P. Hilditch and E. E. Jones, *J. Soc. Chem. Ind.*, 46, 174-177T (1927).

²⁰⁷ E. Vongerichten and A. Köhler, *Ber.*, 42, 1638-1639 (1909).

²⁰⁸ Scherer, Dissertation, Strassburg, 1909. Quoted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 413.

²⁰⁹ B. C. Christian and T. P. Hilditch, *Biochem. J.*, 23, 327-338 (1929).

²¹⁰ T. P. Hilditch and H. Paul, *J. Soc. Chem. Ind.*, 54, 331-336T (1935).

²¹¹ T. P. Hilditch, T. Riley, and N. L. Vidyarthi, *J. Soc. Chem. Ind.*, 46, 457-462, 462-467T (1927).

²¹² J. J. Sudborough, H. E. Watson, P. R. Ayyar, and N. R. Damle, *J. Indian Inst. Sci.*, A9, 26-43 (1926).

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²¹⁴ H. P. Kaufmann and H. Fiedler, *Fette u. Seifen*, 45, 299-302 (1938); *Chem. Abst.*, 32 7756 (1938).

²¹⁵ J. J. Sudborough, H. E. Watson, P. R. Ayyar, and T. I. Mirchandani, *J. Indian Inst. Sci.*, A9, 52-64 (1926).

²¹⁶ T. P. Hilditch and M. L. Meara, *J. Chem. Soc.*, 1938, 1608-1610.

²¹⁷ J. J. Sudborough, H. E. Watson, P. R. Ayyar, and N. R. Damle, *J. Indian Inst. Sci.*, A9, 65-66 (1926).

TABLE 12
COMPOSITION OF SOME SEED OILS CONTAINING PRINCIPALLY OLEIC OR LINOLEIC ACIDS, OR BOTH, AND ELAEOSTEARIC, RICINOLEIC, PETROSELINIC, OR ERUCIC ACID^a

Family and species	Common name of oil	Saturated acids, weight per cent					Unsaturated acids, weight per cent				
		C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄	Oleic	Linoleic	Linolenic	Elaeostearic	
<i>Euphorbiaceae</i>											
<i>Aleurites cordata</i> ^b	Japanese tung seed	7	7	—	—	—	19.0	—	—	—	74.0
<i>Aleurites fordii, montana</i> ^c	Chinese tung seed	3.7	1.2	—	—	—	14.9	—	—	—	80.0 ^a
<i>Aleurites fordii, montana</i> ^d	Florida tung seed	4.6	4.6	—	—	—	1	—	—	—	95.0
<i>Ricinus communis</i> ^e	Castor seed	—	0.3 ^f	—	—	—	7.2	3.6	—	—	Ricinoleic
<i>Ricinus zanzibaricus</i> ^g	Castor seed	1.1	1.1	—	—	—	Trace	8.0	—	—	87.8
<i>Sapium sebiferum</i> ^h	Stillingia seed (Chinese tallow tree)	6.3	2.8	0.2	—	—	16.0	46	29	—	91.0
<i>Umbelliferae</i>											
<i>Angelica sylvestris</i> ⁱ	Woodland angelica	4	—	—	—	—	44	33	—	—	Petroselinic
<i>Anthriscus cerefolium</i> ^j	Salad chervil	5	—	—	—	—	0.5	53.5	—	—	41
<i>Apium graveolens</i> ^k	Celery	3	—	—	—	—	26	20	—	—	51
<i>Carum carvi</i> ^l	Caraway	3	—	—	—	—	40	31	—	—	26
<i>Coriandrum sativum</i> ^m	Coriander	8	—	—	—	—	32	7	—	—	53
<i>Daucus carota</i> ⁿ	Carrot	4	—	—	—	—	14	24	—	—	58
<i>Foeniculum capillaceum</i> ^o ..	Fennel	4	—	—	—	—	22	14	—	—	60
<i>Heracleum spondylium</i> ^p ..	Hogweed (cow parsnip)	4	—	—	—	—	52	25	—	—	19
<i>Pastinaca sativa</i> ^q	Parsnip	1	—	—	—	—	32	21	—	—	46
<i>Petroselinum sativum</i> ^r	Parsley	2	—	—	—	—	15	6	—	—	76
<i>Arabiaceae</i>											
<i>Hedera helix</i> ^s	English ivy	5	—	—	—	—	20	13	—	—	62
<i>Cruciferae</i>											
<i>Brassica alba</i> ^m	White mustard seed	2	—	1	—	—	28	14.5	1	—	Erucic
<i>Brassica (Sinapis) campestris</i> ⁿ	Rapeseed, Indian	—	1.6 ^o	—	0.5	2.4	20.2	14.5	2.1	—	57.2
<i>Brassica (Sinapis) campestris</i> ^m	Rapeseed, English	1	—	—	—	1	32	15	1	—	50
<i>Brassica (Sinapis) campestris</i> ^p	Rapeseed, German	0.8	—	—	—	—	39.4	11.0	3.6	—	45.3

Family and species	Common name of oil	Saturated acids, weight per cent					Unsaturated acids, weight per cent				
		C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄	Oleic	Linoleic	Linolenic	Elaeostearic	
<i>Brassica (Sinapis) campestris</i> ^a	Rapeseed, East Indian	2	—	—	—	1	17	29	—	51	
<i>Brassica (Sinapis) campestris</i> ^b	Rapeseed, Japanese	4	—	—	—	1	14	24	2	55	
<i>Brassica (Sinapis) campestris</i> ^c	Ravison seed	2	—	—	0.5	2	20.5	25.5	2	47.0	
<i>Brassica (Sinapis) juncea</i> ^d	Indian mustard	—	—	—	3.8	1.1	32.3	18.1	2.7	41.5	
<i>Brassica (Sinapis) nigra</i> ^e	Black mustard	2	—	—	—	—	24.5	19.5	2	50	
<i>Cheiranthus cheiri</i> ^f	Wallflower seed	3	—	—	—	—	12	42	4	38.5	
<i>Cheiranthus cheiri</i> ^g	Wallflower seed	5.2	—	—	—	—	4.9	24.7	19.4	40.6	
<i>Eruca sativa</i> ^h	Jamba seed (rocket salad)	—	4.2	—	4.5	1.8	28.7	12.4	2.1	46.3	

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, pp. 167, 180, 184.

^b R. S. McKinney and G. S. Jamieson, *Oil & Soap*, 14, 2-3 (1937).

^c A. Steger and J. Van Loon, *J. Soc. Chem. Ind.*, 47, 361-363T (1928).

^d R. S. McKinney and G. S. Jamieson, *Oil & Soap*, 12, 92-93 (1935); 15, 30-32 (1938).

^e P. Panjatin and M. Rapoport, *Chem. Umschau*, 37, 130-135 (1930); *Chem. Abst.*, 24, 3665 (1930).

^f Also contains 1.1% dihydroxystearic acid.

^g A. Steger, J. Van Loon, and C. Smelt, *J. Soc. Chem. Ind.*, 55, 41-42T (1936).

^h G. S. Jamieson and R. S. McKinney, *Oil & Soap*, 15, 295-296 (1938).

ⁱ T. P. Hilditch and E. E. Jones, *Biochem. J.*, 22, 326-330 (1928).

^j B. C. Christian and T. P. Hilditch, *Biochem. J.*, 23, 327-338 (1929).

^k T. P. Hilditch and E. E. Jones, *J. Soc. Chem. Ind.*, 46, 174-177T (1927).

^l A. Steger and J. Van Loon, *Rec. trav. chim.*, 47, 471-476 (1928).

^m T. P. Hilditch, T. Riley, and N. L. Vidyarthi, *J. Soc. Chem. Ind.*, 46, 457-462T (1927).

ⁿ J. J. Sudborough, H. E. Watson, P. R. Ayyar, and N. R. Damle, *J. Indian Inst. Sci.*, A9, 26-43 (1926).

^o Also contains 1.5% C₁₄.

^p K. Täufel and C. Bauschinger, *Z. Untersuch. Lebensm.*, 56, 253-264 (1928).

^q T. P. Hilditch and H. Paul, *J. Soc. Chem. Ind.*, 54, 331-336T (1935).

^r R. Yamasaki and K. Ichihari, *Bull. Chem. Soc. Japan*, 11, 114-117 (1936).

^s J. J. Sudborough, H. E. Watson, P. R. Ayyar, and U. M. Mascarenhas, *J. Indian Inst. Sci.*, A9, 43-51 (1926).

^t Also contains 0.5% C₁₄.

^u T. P. Hilditch and E. E. Jones, *J. Soc. Chem. Ind.*, 46, 467-469T (1927).

^v J. Van Loon, *Rec. trav. chim.*, 49, 745-753 (1930).

^w J. J. Sudborough, H. E. Watson, P. R. Ayyar, and T. J. Mirehandani, *J. Indian Inst. Sci.*, A9, 52-64 (1926).

Gramineae seed oils as barley,²¹⁸ corn,²¹⁹⁻²²¹ rice,²²² rye,²²³ and wheat²²⁴ likewise contain the higher proportion of saturated fats.

Special saturated acids are found in specific fats. Plants of the laurel family (*Lauraceae*), such as cinnamon²²⁵ and laurel,^{226,227} have a large proportion of lauric acid, while the *Myristicaceae* (nutmeg)²²⁶ as well as *Iringia barteri*²²⁷ (Dika nut) yield oils which contain considerable amounts of myristic acid. The *Palmae* are characterized by their high content of both lauric and myristic acids as well as of several saturated shorter chain acids (capric and caprylic). Coconut and palm oils are included in this group. The data on the composition of a number of the fats listed above are included in Table 13.

(d) *Fats Containing Large Amounts of Stearic Acid.* Most vegetable fats are characterized by their low stearic acid content, while animal fats, almost invariably, are relatively rich in this component. However, a number of varieties of tropical plants are largely devoid of linoleic acid and contain a considerable proportion of stearic acid in its place. Kokum butter and Njatno tallow contain 56.4 and 57.5% of stearic acid, respectively, while other representative fats in this group contain 30 to 50% of this saturated acid. The fats with the greatest proportion of stearic and oleic acids belong to the genera *Guttiferae* and *Sapotaceae*. Some of the more common fats in this group are included in Table 14.

(e) *Fats Containing Definite Amounts of the Saturated C₂₀₋₂₄ Acids.* The long-chain saturated acids, arachidic, behenic, and lignoceric, are found in the seed fats classed under the families *Leguminosae* and *Sapindaceae*. Arachidic acid, although named after the peanut (arachis nut), is found to the greatest extent in kusum oil.²²⁸ The total C₂₀₋₂₄ saturated acids in peanut oil^{94,228-232} amount to only 5-7%. It is interesting that palmi-

²¹⁸ K. Täufel and M. Rusch, *Z. Untersuch. Lebensm.*, *57*, 422-431 (1929).

²¹⁹ W. F. Baughman and G. S. Jamieson, *J. Am. Chem. Soc.*, *43*, 2696-2702 (1921).

²²⁰ H. E. Longenecker, *J. Biol. Chem.*, *129*, 13-22 (1939).

²²¹ K. F. Baur, Jr., and J. B. Brown, *J. Am. Chem. Soc.*, *67*, 1899-1900 (1945).

²²² G. S. Jamieson, *Oil & Fat Industries*, *3*, 256-261 (1926).

²²³ A. W. Stout and H. A. Schuette, *J. Am. Chem. Soc.*, *54*, 3298-3302 (1932).

²²⁴ B. Sullivan and C. H. Bailey, *J. Am. Chem. Soc.*, *58*, 383-390, 390-393 (1936).

²²⁵ S. V. Puntambekar, *J. Indian Chem. Soc., Ind. and News Ed.*, *1*, No. 1-2, 19-24 (1938).

²²⁶ G. Collin and T. P. Hilditch, *Biochem. J.*, *23*, 1273-1289 (1929).

²²⁷ G. Collin and T. P. Hilditch, *J. Soc. Chem. Ind.*, *49*, 138-139T (1930).

²²⁸ D. R. Dhingra, T. P. Hilditch, and J. R. Vickery, *J. Soc. Chem. Ind.*, *48*, 281-286T (1929).

²²⁹ T. P. Hilditch, M. B. Ichaporria, and H. Jasperson, *J. Soc. Chem. Ind.*, *57*, 363-368T (1938).

²³⁰ T. P. Hilditch and E. C. Jones, *J. Soc. Chem. Ind.*, *53*, 13-21T (1934).

²³¹ T. P. Hilditch and N. L. Vidyarthi, *J. Soc. Chem. Ind.*, *46*, 172-174T (1927).

²³² G. S. Jamieson, W. F. Baughman, and D. H. Brauns, *J. Am. Chem. Soc.*, *43*, 1372-1381 (1921).

TABLE 13
COMPOSITION OF SPECIES OF SEED FATS CONTAINING A PREDOMINANCE OF FATTY ACIDS WITH LESS THAN SIXTEEN CARBON ATOMS^a

Family and species	Common name	Saturated acids, weight per cent							Unsaturated acids, weight per cent			
		C ₆	C ₈	C ₉	C ₁₀	C ₁₂	C ₁₄	C ₁₅	C ₁₈	Oleic	Linoleic	
<i>Myristicaceae</i>												
<i>Myristica fragrans</i> ^b	Nutmeg	—	—	—	—	1.5	76.6	10.1	—	—	10.5	1.3
<i>M. officinalis (fragrans)</i> ^c	Nutmeg	—	—	—	—	—	60	32	—	—	8	—
<i>Viola (Myristica) otoba</i> ^d	Otoba nut	—	—	—	—	20.8	73.4	0.3	—	—	5.5	—
<i>Lauraceae</i>												
<i>Actinodaphne Hookeri</i> ^e	Daphne (Indian laurel)	—	—	—	—	96	—	—	—	—	4	—
<i>Cinnamomum camphora</i> ^f	Cinnamon	—	—	—	—	9.5	—	—	—	—	5	—
<i>Laurus nobilis</i> ^g	Laurel (Greekian)	—	—	—	—	35.0	—	9.7	—	—	36.6	18.7
<i>Neolitsea involucrata</i> ^h	Dawul-kurundu seed (Ceylon)	—	—	—	—	85.9	3.8	—	—	—	4.0	3.3
<i>Sabalporaceae</i>												
<i>Sabudora oboites</i> ^b	Khakan kernel	—	4.4	6.7	47.2	28.4	—	—	—	—	12.0	1.3
<i>Palmaceae</i>												
<i>Areca catechu</i> ⁱ	Betel nut	—	—	1.0	43.6	21.0	—	—	—	—	2.3	29.0
<i>Attalea cohune</i> ^j	Cohune nut	—	7.5	6.6	46.4	16.1	—	—	—	—	3.3	9.9
<i>Orbignya junifera</i> ^k	Babassu (coquilla nut)	0.1	6.5	2.7	45.8	19.9	—	—	—	—	18.1	0.9
<i>Cocos nucifera</i> ^l	Coconut	0.5	9.0	6.8	46.4	18.0	—	—	—	—	1.0	1.6
<i>Cocos nucifera</i> ^l	Coconut	Tr.	7.9	7.2	48.0	17.5	—	—	—	—	2.1	5.7
<i>Elaeis guineensis</i> ^m	Oil palm kernel (African)	—	2.7	7.0	46.9	14.1	—	—	—	—	1.3	18.5
<i>Manicaria saccifera</i> ⁿ	Tururu kernel (sleeve palm)	Tr.	5.3	6.6	47.5	18.9	—	—	—	—	2.4	9.7

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 200.^b G. Collin and T. P. Hilditch, *Biochem. J.*, **23**, 1273-1289 (1929); *J. Soc. Chem. Ind.*, **49**, 141-143T (1930).^c A. Heiduschka and H. Häbel, *Arch. Pharm.*, **271**, 56-63 (1933).^d W. F. Baughman, G. S. Jamieson, and D. H. Brauns, *J. Am. Chem. Soc.*, **43**, 199-204 (1921).^e S. V. Puntambekar and S. Krishna, *J. Indian Chem. Soc.*, **10**, 395-400 (1933).^f S. V. Puntambekar, *J. Indian Chem. Soc., Ind. and News Ed.*, **1**, No. 1-2, 19-24 (1938).^g B. G. Gunde and T. P. Hilditch, *J. Chem. Soc.*, **1938**, 1610-1614.^h C. K. Patel, S. N. Iyer, J. J. Sudborough, and H. E. Watson, *J. Indian Inst. Sci.*, **A9**, 117-132 (1926).ⁱ A. Rathje, *Arch. Pharm.*, **246**, 692-709 (1908).^j T. P. Hilditch and N. L. Vidyarthi, *J. Soc. Chem. Ind.*, **47**, 35-37 (1928).^k A. Heiduschka and R. Agsten, *J. prakt. Chem.*, **126**, **II**, 53-55 (1930).^l S. Lepkowsky, G. V. Feskov, and H. M. Evans, *J. Am. Chem. Soc.*, **53**, 978-981 (1931).^m G. Collin and T. P. Hilditch, *J. Soc. Chem. Ind.*, **47**, 261-269T (1928).ⁿ G. Collin, *Biochem. J.*, **27**, 1366-1372 (1933).

TABLE 14
 COMPOSITION (WEIGHT PER CENT) OF SOME SEED FATS UNUSUALLY RICH IN STEARIC ACID^a

Family and species	Common name	Saturated acids					Unsaturated acids	
		C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂	Oleic	Linoleic
<i>Sterculiaceae</i>								
<i>Theobroma cacao</i> ^b	Cacao butter	—	24.4	34.5	—	—	39.1	2.0
<i>Theobroma cacao</i> ^c	Cacao butter	—	24.4	35.4	—	—	38.1	2.1
<i>Guttiferae</i>								
<i>Garcinia indica</i> ^d	Kokum butter	—	2.5	56.4	—	—	39.4	1.7
<i>Garcinia morella</i> ^e	Gurgi nut	0.3	7.2	42.5	0.3	—	43.6	6.1
<i>Garcinia morella</i> ^f	Gurgi nut	—	0.7	46.4	2.5	—	49.5	0.9
<i>Mesua ferrea</i> ^g	Iron wood nut	1.6	8.5	10.4	1.8	—	66.5	11.2
<i>Pentadesma butyracea</i> ^h	Kanya butter	—	5.4	46.1	—	—	48.5	—
<i>Dipterocarpaceae</i>								
<i>Shorea aptera</i> ⁱ	Borneo tallow	1.4	21.5	39.0	—	—	38.1	—
<i>Shorea aptera</i> ^j	Borneo tallow	—	18.0	43.3	1.1	—	37.4	0.2
<i>Valeria indica</i> ^k	Dhupa or Malabar tallow	—	10.2	38.9	3.1	—	47.8	—
<i>Burseraceae</i>								
<i>Canarium commune</i> ^l	Java almond (Canary tree)	—	10.7	40.3	2.1	—	43.6	3.3
<i>Sapotaceae</i>								
<i>Butyrospermum parkii</i> ^m	Shea butter	—	8.5	35.9	—	—	49.9	5.3
<i>Madhuca butyracea</i> ⁿ	Phulwara butter	—	56.6	3.6	—	—	36.0	3.8
<i>Madhuca longifolia</i> ^o	Mowrah butter	—	23.7	19.3	—	—	43.3	13.7
<i>Palaquium oblongifolium</i> ^p	Njatno tallow (nato tree)	—	6.5	57.5	—	—	36.0	—

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, pp. 194-195.

^b C. H. Lea, *J. Soc. Chem. Ind.*, 48, 41-46T (1928).

^c T. P. Hilditch and W. J. Stainsby, *J. Soc. Chem. Ind.*, 55, 95-101T (1936).

^d T. P. Hilditch and K. S. Murti, *Unpublished observation*. Cited from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, p. 156.

^e D. R. Dhingra, G. L. Seth, and P. C. Speers, *J. Soc. Chem. Ind.*, 52, 116-118T (1933).

^f D. R. Dhingra and T. P. Hilditch, *J. Soc. Chem. Ind.*, 50, 9-12T (1931).

^g T. P. Hilditch and S. A. Saletore, *J. Soc. Chem. Ind.*, 50, 468-472T (1931).

^h T. P. Hilditch and J. Priestman, *J. Soc. Chem. Ind.*, 49, 197-200 T (1930).

ⁱ W. J. Bushell and T. P. Hilditch, *J. Soc. Chem. Ind.*, 57, 447-449T (1938).

^j T. P. Hilditch and W. J. Stainsby, *J. Soc. Chem. Ind.*, 53, 197-203T (1934).

^k Cited from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, p. 195.

^l W. J. Bushell and T. P. Hilditch, *J. Soc. Chem. Ind.*, 57, 48-49T (1938).

^m T. P. Hilditch and M. B. Ichaporria, *J. Soc. Chem. Ind.*, 57, 44-48T (1938).

ⁿ A. W. K. deJong and W. R. T. de Haas, *Chem.-Ztg.*, 23, 780 (1904).

toleic,^{87,233} caprylic, and lauric acids²³⁴ have recently been isolated from peanut oil. Small amounts of the higher saturated acids are present in soybean oil.²³⁵ The composition of representative fats in this group are reported in Table 15.

(2) *Glyceride Components of Natural Fats and Oils*

Although a considerable amount of information about the nature of a fat can be gleaned from an examination of its fatty acid composition, the arrangement of the fatty acids in the triglyceride molecule is of great importance in establishing its physical properties. Thus, it is possible, by the use of a catalyst, to cause a sample of an oil, such as cottonseed, to change from a clear limpid liquid to one which is partially turbid, by an alteration in the normal arrangement of the fatty acids to one of random distribution. In fact, by means of directed interesterification, a product can be obtained which, after thorough mixing, has a melting point well above room temperature. All of these three fats have the same saponification and iodine number and are composed of the same proportion of all the fatty acids. The variation in melting points is ascribable entirely to differences in the arrangement of the fatty acids in the triglyceride.

a. Specific Glycerides Isolated from Natural Fats and Oils. The first information available concerning the structure of fats was obtained by the separation and identification of individual triglycerides. However, such analyses prove to be extremely tedious and time-consuming, and the results can in no way be considered quantitative. Moreover, only a few of the individual triglycerides can be crystallized; in most cases it is impossible to separate the components of complicated mixtures.

(a) *Simple Triglycerides Isolated from Natural Fats and Oils.* The simple triglycerides are present in limited quantities in the fats and, in most cases, can be isolated in pure form only after many fractionations and crystallizations. Duffy,²³⁶ in 1853, was able to isolate 8 g. of tristearin from 2 kg. of mutton tallow, and this required 32 recrystallizations from ether at 16°C. The same investigator succeeded in preparing 2 g. of the same triglyceride from beef tallow. Bömer *et al.*^{237,238} separated 3% of pure tristearin from mutton tallow by systematic crystallizations of natural fats. In some

²³³ T. P. Hilditch, *Rec. trav. chim.*, 57, 501-508 (1938). T. P. Hilditch and H. Jasperon, *J. Soc. Chem. Ind.*, 57, 84-87T (1938).

²³⁴ H. L. Wykoff, J. M. Kaplan, and A. L. Berman, *J. Biol. Chem.*, 153, 227-235 (1944).

²³⁵ E. G. Dollear, P. Krauczunas, and K. S. Markley, *Oil & Soap*, 15, 263-264 (1938); 17, 120-121 (1940).

²³⁶ P. Duffy, *J. Chem. Soc.*, 5, 197-210 (1853).

²³⁷ A. Bömer, A. Schemm, and G. Heimsöth, *Z. Untersuch. Nahr. Genussm.*, 14, 90-117 (1907).

²³⁸ A. Bömer and G. Heimsöth, *Z. Untersuch. Nahr. Genussm.*, 17, 353-396 (1909).

TABLE 15
COMPOSITION OF SOME SEED FATS CONTAINING APPRECIABLE AMOUNTS OF SATURATED FATTY ACIDS WITH MORE THAN 18 CARBON ATOMS

Family name	Common name	Saturated acids, weight per cent					Unsaturated acids, weight per cent	
		C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄	Oleic	Linoleic
<i>Leguminosae mimosoideae</i>								
<i>Adenanthura pavonina</i>	Sandal bead tree nut ^b	9.0 ^c	1.1	—	—	25.5	49.3	14.7
<i>Parkia biglandulosa</i>	Parkia seed (mita tree) ^d	8.8	13.3	—	7.9	—	30.6	39.4
<i>L. caesalpinioideae</i>								
<i>L. caesalpinia Bonduc</i>	Bonducella nut ^e	10.0	5.79	—	—	—	21.6	61.4
<i>L. papilionatae</i>								
<i>Arachis hypogaea</i>	Virginia peanut ^f	6.3	4.9	—	5.9	—	61.1	21.8
<i>Arachis hypogaea</i>	Spanish peanut ^g	8.3	3.1	2.4	3.1	1.1	56.0	26.0
<i>Dipteris odorata</i>	Dutch tonka bean ^h	5.1	5.9	—	14.8	—	61.0	13.2
<i>Medicago sativa</i>	Alfalfa ⁱ	—	—	10.3	—	—	1.4	67.5
<i>Glycine soja</i>	Soybean ^j	6.8	4.4	0.7	—	0.1	33.7	52.0
<i>Sapindaceae</i>								
<i>Nephetium lappaceum</i>	Rambutan tallow ^a	2.0	13.8	34.7 ⁱ	—	—	45.3	^k 1
<i>Nephetium mutabile</i>	Pulasan tallow ^b	3.0	31.0	22.3	—	—	43.7	—
<i>Sapindus trifoliatus</i>	Three-leaf soapberry ⁱ	5.6	8.5	21.9	—	2.5	61.5	—
<i>Schleichera oleosa</i>	Malay lac tree ^m	8.7 ^a	1.7	22.6	—	2.2	59.2	4.5
(<i>trijuga</i>)	Kusam, macassar ^o	7.9 ^p	—	31.1	—	—	57.6	—

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, pp. 188-189.

^b S. M. Muddidri, P. R. Ayyar, and H. E. Watson, *J. Indian Inst. Sci.*, **A11**, 173-180 (1928).

^c Also contains 0.4% C₁₆.

^d D. R. Paranjpe, *J. Indian Chem. Soc.*, **8**, 767-772 (1931).

^e S. N. Godbole, D. R. Paranjpe, and J. G. Shrikhande, *J. Indian Chem. Soc.*, **6**, 295-302 (1929).

^f G. S. Jamieson, W. F. Baughman, and D. H. Brauns, *J. Am. Chem. Soc.*, **43**, 1372-1381 (1921).

^g T. P. Hilditch, M. B. Ichaporria, and H. Jasperson, *J. Soc. Chem. Ind.*, **57**, 363-368T (1938).

^h T. P. Hilditch and W. J. Stainsby, *J. Soc. Chem. Ind.*, **53**, 197-203T (1934).

ⁱ H. A. Schmette, H. A. Vogel, and C. H. Wartinbee, *Oil & Soap*, **15**, 35-36 (1938).

^j W. F. Baughman and G. S. Jamieson, *J. Am. Chem. Soc.*, **44**, 2947-2952 (1922).

^k Also contains 4.2% of eicosenoic acid (C₂₀H₃₈O₂).

^l D. R. Paranjpe and P. R. Ayyar, *J. Indian Inst. Sci.*, **A12**, 179-184 (1929).

^m D. R. Dhingra, T. P. Hilditch, and J. R. Vickery, *J. Soc. Chem. Ind.*, **48**, 281-286T (1929).

ⁿ Also contains 1.1% C₁₄.

^o S. M. Patel, *Thesis*, Bombay, 1930. Cited from T. P. Hilditch, *The Chemical Constitution of Natural Fats*, p. 153.

^p Also contains 1.1% C₁₀ and 2.3% C₁₂.

cases, many hundreds of crystallizations were involved in the study of a single fat. Klimont²³⁹ isolated another triglyceride, tripalmitin, from rabbit fat. The simple triglycerides of myristic and lauric acids have been separated from vegetable fats especially high in these acids. Thus, trimyristin was obtained in 40% yield by Bömer and Ebach²⁴⁰ from nutmeg butter, while trilaurin has been isolated by the same workers from the fat of the Grecian laurel (*Laurus nobilis*), to the amount of 30%. Krafft¹¹⁵ confirmed both of these results.

Some simple triglycerides of unsaturated fatty acids have likewise been shown to be natural products. Triolein has been isolated from duck and goose fats by two groups of workers.^{44,241} Hilditch and Meara²¹⁶ have prepared trierucin in a 40% yield from nasturtium seed oil. Amberger^{242,243} obtained evidence that butter fat contains about 2% of triolein, while it was indicated that this triglyceride is probably present to the extent of about 45% in goose fat.²⁴¹

Although the amount of the simple triglycerides is undoubtedly much higher than would be indicated by the quantities which have been isolated in pure form, the results nevertheless indicate that the major proportion of the fatty acids is probably present in the form of mixed triglycerides.

(b) *Mixed Triglycerides Isolated from Natural Fats and Oils.* For some time after Chevreul had demonstrated that fats were glycerol esters of palmitic, stearic, oleic, and other acids, it was more or less generally believed that they were composed entirely of simple triglycerides. However, after it was shown, in 1860, that glycerol is a triatomic alcohol, Berthelot⁴ suggested the possibility that mixed triglycerides might exist. Such a compound was first demonstrated by Heise,²⁴⁴ who separated a considerable quantity of oleodistearin from mkanyi fat from the East African tallow tree (*Allanblackia Stuhlmannii*),²⁴⁵ an observation which was later confirmed by Henriques and Künne.²⁴⁶ It is now realized that the mixed triglycerides are present to a much greater proportion in vegetable and animal fats than are the simple triglycerides.

Myristodilaurin has been found in coconut oil²⁴⁷ and in palm kernel fat,²⁴⁸ as well as in babassu fat.²⁴⁹ Laurodimyristin has been isolated from coco-

²³⁹ J. Klimont, *Monatsh.*, 33, 441-446 (1912).

²⁴⁰ A. Bömer and K. Ebach, *Z. Untersuch. Lebensm.*, 55, 501-524 (1928).

²⁴¹ C. Amberger and K. Bromig, *Z. Untersuch. Nahr. Genussm.*, 42, 193-218 (1921).

²⁴² C. Amberger, *Z. Untersuch. Nahr. Genussm.*, 26, 65-85 (1913).

²⁴³ C. Amberger, *Z. Untersuch. Nahr. Genussm.*, 35, 313-381 (1918).

²⁴⁴ R. Heise, cited in *Tropenpflanzer*, 3, 203-206 (1899).

²⁴⁵ Anonymous, *Tropenpflanzer*, 1, 10 (1897).

²⁴⁶ R. Henriques and H. Künne, *Ber.*, 32, 387-394 (1899).

²⁴⁷ A. Bömer and J. Baumann, *Z. Untersuch. Nahr. Genussm.*, 40, 97-151 (1920).

²⁴⁸ A. Bömer and K. Schneider, *Z. Untersuch. Nahr. Genussm.*, 47, 61-89 (1924).

²⁴⁹ A. Bömer and H. Hüttig, *Z. Untersuch. Lebensm.*, 75, 1-33 (1938).

nut²⁴⁷ and palm kernel fat,²⁴⁸ as is also the case with the palmitodimyristin.^{247,248} Myristodipalmitin has been reported as a component of palm kernel fat.²⁴⁸ Stearodipalmitin has been found to be widely distributed in natural products. It has been isolated from coconut oil,²⁴⁷ as well as from a number of animal fats, including goose fat^{241,250} and duck fat⁴⁴ (to the extent of 3–4%), lard (2%),^{251,252} and finally beef and mutton tallow.^{253,254} The oleodipalmitin is found both in such vegetable sources as cacao butter,^{39–42} Borneo tallow,⁴³ stillingia fat,⁴¹ and in such animal fats as butter^{242,243} and goose fat.²⁴¹

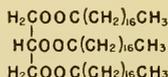
Palmitodistearins have been isolated only from animal sources, where they have been shown to be components of lard,^{251,252} butter,^{242,243} goose fat,²⁵⁰ and mutton and beef tallow.²⁵⁴ On the other hand, the oleodistearin has to date been reported only in vegetable fats (cacao butter,^{38,42} Borneo tallow,⁴³ and stillingia fat²⁵⁵).

Several dioleins have also been prepared from fats. These include the palmitodiolein in goose fat,²⁴¹ which Bömer²⁵¹ has indicated is present to the extent of 30% in lard, and stearodiolein, which has been reported in the same fat²⁵¹ to an amount of 5%.

Although much information concerning the structure of fats can be gleaned from the presence of one or another mixed triglyceride, the methods for their separation are exceedingly time-consuming and far from quantitative. Soluble mixed triglycerides cannot be separated, and the procedure is obviously not adapted to routine analysis.

b. Methods of Analysis for the General Class of Triglycerides. Although it is practically impossible to obtain anything but fragmentary data about the glyceride composition of fats by isolation procedures, Hilditch and his co-workers have developed routine laboratory technics which enable one to classify the fats according to the proportion of saturated and unsaturated fatty acids in the triglycerides. Thus, a natural fat containing only stearic and oleic acids would have four different types of triglycerides, which can be represented by the following formulae:

Trisaturated glyceride (I)



Tristearin

²⁵⁰ A. Bömer and H. Merten, *Z. Untersuch. Nahr. Genussm.*, **43**, 101–137 (1922).

²⁵¹ A. Bömer, *Z. Untersuch. Nahr. Genussm.*, **25**, 321–353 (1913).

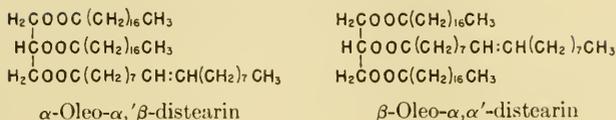
²⁵² C. Amberger and A. Wieseahn, *Z. Untersuch. Nahr. Genussm.*, **46**, 276–299 (1923).

²⁵³ W. Hansen, *Arch. Hyg.*, **42**, 1–15 (1902).

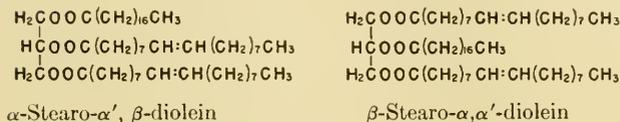
²⁵⁴ H. Kreis and A. Hafner, *Ber.*, **36**, 1123–1128 (1903).

²⁵⁵ J. Klimont, *Monatsh.*, **24**, 408–412 (1903).

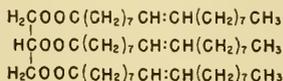
Monounsaturated disaturated triglyceride (II)



Monosaturated diunsaturated triglycerides (III)



Triunsaturated glyceride (IV)



Triolein

Hilditch and his associates have been able to classify the fats into the four general groups, *i.e.*, trisaturated or fully saturated, monounsaturated-disaturated, monosaturated-diunsaturated, and triunsaturated. It is not possible, by the routine procedures developed by Hilditch, to differentiate between the symmetrical and the unsymmetrical mixed triglycerides; special methods must be employed to determine the proportion of their isomers, and estimates have been made in only a few cases.

The most satisfactory terminology for describing the several classes of triglycerides is probably that employed by Hilditch.⁷⁹

Group I. Trisaturated, $\text{C}_3\text{H}_5(\text{O}\cdot\text{CO}\cdot\text{S})_3$ or GS_3 .

Group II. Monounsaturated-disaturated, $\text{C}_3\text{H}_5\begin{cases} (\text{O}\cdot\text{CO}\cdot\text{S})_2 \\ (\text{O}\cdot\text{CO}\cdot\text{U}) \end{cases}$ or GS_2U .

Group III. Monosaturated-diunsaturated, $\text{C}_3\text{H}_5\begin{cases} (\text{O}\cdot\text{CO}\cdot\text{S}) \\ (\text{O}\cdot\text{CO}\cdot\text{U})_2 \end{cases}$ or GSU_2 .

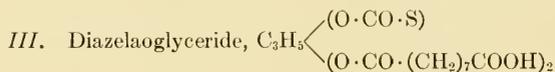
Group IV. Triunsaturated, $\text{C}_3\text{H}_5(\text{O}\cdot\text{CO}\cdot\text{U})_3$ or GU_3 .

(a) *Determination of Fully Saturated Glycerides (Hilditch).* The method of Hilditch and Lea²⁵⁶ offers an especially satisfactory and accurate system for the determination of GS_3 in the presence of GS_2U , GSU_2 , and GU_3 . This procedure is based upon the earlier observation of Armstrong and Hilditch²⁵⁷ that the saturated triglycerides possess extreme stability toward oxidation, in contrast to the ready susceptibility of the triglycerides

²⁵⁶ T. P. Hilditch and C. H. Lea, *J. Chem. Soc.*, 1927, 3106-3117.

²⁵⁷ E. F. Armstrong and T. P. Hilditch, *J. Soc. Chem. Ind.*, 44, 180-189T (1925).

containing one or more unsaturated fatty acid residues. When potassium permanganate is used for oxidation, the unsaturated acid residues are oxidized at the double bonds, without hydrolysis of the ester linkage with glycerol, to water-soluble azelaoglycerides. Thus, from the three types of glycerides containing unsaturated acids, the following products will result on oxidation:



According to the Hilditch and Lea technic, the neutralized fat mixture, dissolved in dry acetone, is treated with an excess of finely powdered potassium permanganate after the solution has been brought to the boiling point. Permanganate is added to the extent of about 4 times the weight of the fat sample used. After the permanganate has been gradually added to the acetone solution of the fat, the mixture is refluxed for several hours, after which the acetone is distilled off and the final traces are removed under suction. The dried residue is then mixed with sodium bisulfite in an amount 1.25 times the weight of permanganate employed (5 times the weight of the fat used), and the mixture is gradually and cautiously added to water. It is allowed to remain until the reaction has been completed. This is then acidified with 30% sulfuric acid until acid to Congo red paper, and the contents are heated until the sulfur dioxide set free by the reaction is completely removed and all the manganese oxides have been dissolved. After cooling of the aqueous solution, the organic compounds are removed by extraction with diethyl ether (Extract 1).

If a large portion of saturated triglycerides is present, the following procedure is adopted: The solid layer of saturated triglycerides and other organic material is removed and washed free from traces of mineral acids and salts by heating with water several times. The solid fat is then dissolved in about 10 volumes of diethyl ether and allowed to stand for several hours at 0°C. The fully saturated glycerides which precipitate are filtered and washed with cold ether, dried by heating at 100°C. under a vacuum, and weighed.

Additional traces of fully saturated triglycerides may be obtained by an ether extraction of the original aqueous extract (Extract 1a). This ether extract (1 or 1a) is washed to remove acidic products. It is advisable to treat the extract first with 10% potassium bicarbonate ($KHCO_3$) solution 2 or 3 times to remove nonanoic or hexanoic acid originating from the

oxidation of the unsaturated acid residues. Following this, the solution is washed alternately with the bicarbonate solution and water. Violent shaking should be avoided at first, but as the azelaoglycerides are removed, the ether extract should be vigorously agitated with the water. The combined potassium bicarbonate and water extracts are treated with ether to remove any neutral saturated triglyceride which may have been lost in the emulsion. The united ether extracts are dehydrated with anhydrous sodium sulfate and the ether is distilled off. The residual crude product is dried under a vacuum at 100°C., weighed, and combined with the earlier product which has been precipitated from the ether solution.

The crude saturated triglycerides may require further purification. If the quantity is too small for this step, the amount of free acid and the unsaponifiable residue can be determined; the weight can be corrected by this amount, assuming the acid product to be azelaopalmitostearin. If a considerable amount of crude saturated triglyceride is available, the latter should be tested for the presence of unsaturated triglycerides. This is readily achieved by determination of the iodine number. If the iodine number of the crude product exceeds one, the whole mass should be subjected to the entire oxidation process for a second time. If the original fat sample has an iodine number which exceeds 25 to 30, complete removal of the unsaturated triglycerides can seldom be effected in a single operation. In some cases, the oxidation procedure must be carried out a third time before pure saturated triglycerides can be isolated. A further purification suggested by Hilditch,⁷⁹ which can be employed if a considerable quantity of the crude saturated triglyceride is present, *i.e.*, 20 g. or more, involves boiling of the product with water in an open dish to which is added sufficient dilute potassium carbonate (K_2CO_3) to make the mixture alkaline to phenolphthalein. The aqueous layer is siphoned off and the triglyceride is boiled with water several times until the washings are neutral. By this procedure 80–90% of the triglycerides can be recovered in a purified form with a negligible acid value. An additional amount of somewhat lower purity can be recovered by ether extraction of the aqueous and potassium carbonate washings. The amount of free acidic compounds in the ether-extracted portion can be corrected for by isolation of the free acidic compounds from the extracted aqueous alkaline washings and by determination of their acid values.

Hilditch recommends the use of enough fat in the original sample to obtain at least 1 to 2 g. of saturated triglycerides. With fats of low saturated acid content, 50 or even 100 g. is the minimum quantity to be used. Where a determination of the proportion of the individual saturated fatty acids present is also required, considerably larger amounts of the original fat sample may be needed, as at least 20 g. of the saturated triglycerides are essential for this latter analysis. When it is necessary to work up such large

quantities of fat, it is advisable to carry out the oxidations on a series of small batches, using a maximum of 150 g. of fat for each run.

Steger and Van Loon²⁵⁸ have suggested an alternative procedure for purification of the crude saturated triglycerides. This involves the extraction with light petroleum ether (b.p. 40–60°C.) and removal of the acidic products from this extract by shaking with ammonia in 50% aqueous alcohol. Some ammonium salts of the acidic products remain in the petroleum ether. These can be partially removed by extraction with calcium chloride solution, followed by water. After drying of the petroleum ether, the solvent is removed by distillation. The residue is extracted with ethyl acetate, which dissolves the saturated triglycerides and leaves behind any impurities such as the calcium salts of the acidic products. The pure saturated triglycerides can be obtained by evaporation of the ethyl acetate.

(b) *Estimation of Partially or Completely Unsaturated Triglycerides (Hilditch)*. If the total fatty acid content of the fat is known, the limiting amount of fractions II and III (GS₂U and GSU₂) can be calculated by subtracting the saturated acid content (expressed in molar per cent) in the saturated triglyceride portion from the total saturated fatty acid content. The amount of any two groups of the unsaturated fractions (GS₂U, GSU₂, and GU₃) can readily be estimated if any one of the three groups is absent or its amount is known.

If such data are not available, one may readily calculate the limiting values of GU₃. The minimum quantity of triunsaturated glyceride can be estimated by assuming that the saturated acids present in the mixed triglyceride are associated with the maximum quantity of unsaturated acids, *i.e.*, as GSU₂. The smallest possible amount of GU₃ will obviously be determined by the difference between the total unsaturated acids and the unsaturated acids present in the mixed triglyceride.

On the other hand, the greatest possible amount of GU₃ which may be present can be calculated by assuming that the excess saturated acids are associated with a minimum of unsaturated acids in the mixed triglycerides, *i.e.*, as the GS₂U. Hilditch⁷⁹ states that, in most cases, the proportion of GU₃ tends to approximate the minimum rather than the maximum possible percentage.

Some idea of the total GU₃ content can be arrived at by the estimation of the tristearin content of completely hydrogenated fat, as well as by a study of the glyceride composition of the fat after partial hydrogenation.^{229, 230, 259–263} This procedure is of value because of the fact that the

²⁵⁸ A. Steger and J. Van Loon, *Rec. trav. chim.*, 54, 284–288 (1935).

²⁵⁹ T. P. Hilditch and E. C. Jones, *J. Chem. Soc.*, 1932, 805–820.

²⁶⁰ A. Banks, H. K. Dean, and T. P. Hilditch, *J. Soc. Chem. Ind.*, 54, 77–82T (1945).

²⁶¹ N. R. Dhingra, T. P. Hilditch, and A. J. Rhead, *J. Soc. Chem. Ind.*, 51, 195–198T (1932).

²⁶² T. P. Hilditch and W. J. Stainsby, *Biochem. J.*, 29, 90–99 (1935).

²⁶³ T. P. Hilditch and H. M. Thompson, *J. Soc. Chem. Ind.*, 56, 434–438T (1937).

disaturated-monounsaturated triglycerides (GS_2U), if present in small amounts, will become totally saturated before any of the monosaturated-diunsaturated (GSU_2) or triunsaturated triglycerides (GU_3) are completely hydrogenated. Hydrogenation follows the above pattern, especially if the so-called agitation process is employed.^{229,264,265} On the other hand, when the "drip" method is used (in which case the catalyst remains undisturbed instead of being constantly churned), the addition of hydrogen does not occur in a stepwise fashion but in a haphazard manner.

In his more recent work, Hilditch⁷⁹ has employed the procedure of Brown¹⁴¹ for the fractional crystallization of the whole fat from acetone at very low temperatures. Each fraction is analyzed for trisaturated glycerides, for the quantitative distribution of the fatty acids by fractional distillation of their esters, and for the unsaturated C_{18} glycerides after complete hydrogenation. From such information, when the solubility of specific triglycerides is known, the approximate glyceride structure can be deduced. The combination of these methods has proved much more satisfactory than the application of any single one.²⁶⁶⁻²⁷⁹

(c) *Estimation of Triglyceride Structure by Low-Temperature Crystallization (Brown)*. The separation of triglyceride fractions by crystallization from acetone at various low temperatures from 0° to $-60^\circ C$. as first worked out by Brown¹⁴¹ has become more and more widely used. The proportion of GS_2U and GSU_2 can be determined by this procedure. If the fractionations are properly carried out, they may contain: (1) a mixture of GS_3 , GS_2U , and GSU_2 , (2) GS_2U and GSU_2 , or (3) GSU_2 and GU_3 . If the total fatty acid composition has been determined and the composition of (1) or (2) is known, the amount of GU_3 can be calculated. Not only can the value of GSU_2 and GU_3 be deduced from the total fatty acid content and an analysis of (3), but also that of GS_2U . A comparison of the fractiona-

²⁶⁴ W. J. Bushell and T. P. Hilditch, *J. Chem. Soc.*, 1937, 1767-1774.

²⁶⁵ R. Escourrou, *Bull. soc. chim.*, 6, 360-367 (1939).

²⁶⁶ D. Atherton and M. L. Meara, *J. Soc. Chem. Ind.*, 58, 353-357T (1939); 59, 95-96T (1940).

²⁶⁷ W. J. Bushell and T. P. Hilditch, *J. Soc. Chem. Ind.*, 57, 48-49T, 447-449T (1938).

²⁶⁸ W. J. Bushell and T. P. Hilditch, *J. Soc. Chem. Ind.*, 58, 24-26T (1939).

²⁶⁹ T. G. Green and T. P. Hilditch, *J. Soc. Chem. Ind.*, 57, 49-53T (1938).

²⁷⁰ T. P. Hilditch and M. B. Ichaporia, *J. Soc. Chem. Ind.*, 57, 44-48T (1938).

²⁷¹ T. P. Hilditch and L. Maddison, *J. Soc. Chem. Ind.*, 59, 67-71T, 162-168T (1940).

²⁷² T. P. Hilditch, M. L. Meara, and W. H. Pedelty, *J. Soc. Chem. Ind.*, 58, 26-29T (1939).

²⁷³ T. P. Hilditch and K. S. Murti, *J. Soc. Chem. Ind.*, 58, 310-312T (1939).

²⁷⁴ T. P. Hilditch and K. S. Murti, *Biochem. J.*, 34, 1301-1311 (1940).

²⁷⁶ T. P. Hilditch and S. Paul, *Biochem. J.*, 32, 1775-1784 (1938).

²⁷⁶ T. P. Hilditch, S. Paul, B. G. Gunde, and L. Maddison, *J. Soc. Chem. Ind.*, 59, 138-144T (1940).

²⁷⁷ T. P. Hilditch and W. H. Pedelty, *Biochem. J.*, 34, 971-979 (1940).

²⁷⁸ T. P. Hilditch and W. J. Stainsby, *J. Soc. Chem. Ind.*, 55, 95-101T (1936).

²⁷⁹ M. L. Meara and Y. A. H. Zaky, *J. Soc. Chem. Ind.*, 59, 25-26T (1940).

tion of the triglycerides from liquid and solid fats in acetone at 0° to -10°C., or 0° to -60°C., is shown in Table 16.

TABLE 16
COMPOSITION OF FRACTIONS OF TRIGLYCERIDES AND OF NATURAL FATS BY ACETONE AT LOW TEMPERATURES

Components	Triglyceride fractions			
	I	II	III	IV
Triglycerides	GS ₃	GS ₂ U	GSU ₂	GU ₃
Compn.	{ Satd. Satd. Satd.	{ Satd. Satd. Unsatd.	{ Satd. Unsatd. Unsatd.	{ Unsatd. Unsatd. Unsatd.
Approx. m. p., °C.	45-60	30-45	0-10	Below 0
Typical components	Palmito- stearins	Oleopalmito- stearin	Palmito- diolein	Triolein, oleo- linoleins
Natural fats				
Liquid		++	++++	++++
Soft solid	++	++++	++++	++
Hard solid	++++	++++	++	
Soly. 0 to -10°C. (solid and semi- solid fats)				
Least sol.	++++	++++	+	
Intermed.	++	++++	++++	
Most sol.		++	++++	++++
Soly. 0 to -60°C. (liquid fats)				
Least sol.		++++	++++	
Intermed.			++++	++
Most sol.			++++	++++

Doerschuk and Daubert²⁸⁰ have markedly increased the usefulness of the low-temperature crystallization technic by increasing the number of fractions. In their study of the glyceride structure of corn oil, they collected 19 different precipitates from acetone at progressively decreasing temperatures. The individual samples seldom contain more than two different glycerides. By the use of the iodine number, saponification equivalents, melting points, and the refractive indices, the proportion of each component in the individual fractions could be deduced. The relative quantities of dienoic and trienoic acids were determined by spectrophotometric analysis in the ultraviolet region, coupled with the thiocyanogen values, while the oleic and the saturated acid contents were determined by the differences noted. The efficacy of their procedure is attested to by the fact that they were able to account for 982 g. in the fractions, out of the original total of 1000 g. of corn oil.

c. Theories Advanced to Explain the Glyceride Composition of Natural Fats and Oils. There has been a great deal of discussion during the last few years regarding the rules which govern the distribution of fatty acids in the triglyceride molecules. Up to recently, three theories have been

²⁸⁰ A. P. Doerschuk and B. F. Daubert, *J. Am. Oil Chemists' Soc.*, 25, 425-433 (1948).

proposed: the monoacid theory, that of "even" distribution, and that of random arrangement. However, Doerschuk and Daubert²⁵⁰ have recently proposed a new hypothesis, the "partial random theory," which seems to have excellent experimental evidence to support it.

(a) *Monoacid Theory.* This hypothesis predicates that like acids are associated with each other in the triglyceride molecule to give simple triglycerides. Although this suggestion is interesting historically inasmuch as it represents the first postulated structure for fats made by Chevreul²⁶ a century and a quarter ago, Berthelot³⁷ early disputed it and suggested that fats most probably contain mixed triglycerides. This theory has long since been abandoned by most fat chemists. The inability to isolate more than traces of simple triglycerides from relatively large amounts of natural fats cannot be harmonized with the monoacid theory. Moreover, the demonstration of the widespread distribution of mixed triglycerides in the natural fats has proved conclusively that triglycerides other than simple ones can occur.

(b) *Theory of "Even" Distribution.* The most widely accepted theory at the present time is the "even" distribution theory of Hilditch.⁷⁹ According to this hypothesis, there is a tendency for the unsaturated acids to be associated with a large proportion of the saturated acids and thus to have as wide a distribution in the triglyceride molecule as possible. Collin and Hilditch²²⁶ have named this tendency the "association ratio." This proportion is defined as the ratio of the moles of saturated fatty acids to the moles of unsaturated acids which occur in the mixed triglycerides. In fats in which the amount of saturated fatty acids exceeds 60% of the total, the figure for the association ratio is remarkably constant at between 1.3 and 1.4 to 1.

The general principle of "even" distribution was first pronounced by Bhattacharya and Hilditch,^{251, 252} in 1930, to be applicable to most vegetable fats. It was shown that no appreciable quantities of completely saturated triglycerides appear until the saturated fatty acid fraction makes up 60% or more of the total fatty acid content (expressed on a molar basis). When the saturated acids vary from 59 to 94% of the total acids, increasing proportions of simple triglycerides are present, but the ratio of saturated to unsaturated acids remains practically uniform at 1.4:1, instead of progressively rising as one might expect. Such an "association ratio" corresponds to a mixture of 3 moles of monounsaturated-disaturated triglycerides (GS₂U) and 1 mole of monosaturated-diunsaturated compound (GSU₂). These relationships are illustrated in the data assembled in Table 17, following, and are plotted in Figure 1 on page 220.

According to the "even" distribution scheme, when a saturated acid, A,

²⁵¹ R. Bhattacharya and T. P. Hilditch, *Proc. Roy. Soc. London*, A129, 468-476 (1930).

²⁵² R. Bhattacharya and T. P. Hilditch, *J. Chem. Soc.*, 1931, 901-907.

TABLE 17
RELATION BETWEEN SATURATED FATTY ACID CONTENT AND FULLY SATURATED TRIGLYCERIDES IN A NUMBER OF VEGETABLE FATS.
ASSOCIATION RATIO OF MIXED SATURATED, UNSATURATED TRIGLYCERIDES CONTAINED THEREIN^{a, b}

No. of fat	Name of species	Common name	Saturated acids in total, mole per cent	Fully saturated triglycerides, mole per cent		"Association ratio"
				Found	Calcd. ^c	
1	<i>Cocos nucifera</i>	Coconut oil	93.9	86	82.8	1.3-1.4
2	<i>Cocos nucifera</i>	Coconut oil	92.9	84	80.2	1.4
3	<i>Iringia barteri</i>	Dika fat	91.7	81	77.5	1.3
4	<i>Manicaria saccifera</i>	Turluru (sleeve-palm)	91.6	82	77.0	1.2
5	<i>Mysticaria fragrans</i>	Nutmeg butter	90.2	73	73.4	1.6
6	<i>Astrocarum lucuma</i>	Tueuna fat	88.0	73	68.0	1.25
7	<i>Acrocomia aculeata</i>	Gru-gru fat	86.3	69	64.3	1.3
8	<i>Elaeis guineensis</i>	Palm kernel oil	85.3	66	62.2	1.3-1.4
9	<i>Shorea stenoptera</i>	Borneo tallow	62.9	5.1	24.9	1.6
10	<i>Shorea stenoptera</i>	Borneo tallow	62.8	4.5	24.8	1.5
11	<i>Madhuca butyracea</i>	Phulwara butter	62.4	8	24.4	1.4
12	<i>Palaquium oblongifolium</i>	Taban merah fat (nato tree)	60.2	1.8	21.8	1.5
13	<i>Theobroma curaco</i>	Cacao butter	59.8	2.5	21.4	1.4
14	<i>Garcinia indica</i>	Kokum butter	59.0	1.5	20.5	1.4
15	<i>Myristica malibarica</i>	Nutmeg (Indian)	59.2	19	20.7	1.0
16	<i>Myristica malibarica</i>	Nutmeg (Indian)	56.2	16	17.8	1.0
17	<i>Laurus nobilis</i>	Laurel kernel fat	58.5	40.5	20.0	0.4
18	<i>Allanblackia Stuhlmannii</i>	Mkanyai fat (E. African tallow tree)	55.6	1.5	17.2	1.2
19	<i>Nephetium mutabile</i>	Pulasan fat	55.3	1.5	16.9	1.2
20	<i>Canarium commune (Dacryodes rostrata)</i>	Java almond fat (Canary tree)	53.4	1.8	15.2	1.1
21	<i>Caryocar villosum</i>	Piquia	53.1	2.5	15.0	1.1
22	<i>Pentadesma butyracea</i>	Gurgi nut butter	51.6	3.0	13.7	1.0
23	<i>Garcinia morella</i>	Gamboge butter	50.5	2.7	12.8	1.0
24	<i>Garcinia morella</i>	Gamboge butter	49.4	2.0	12.1	0.9
25	<i>Nephetium lappaceum</i>	Rambutan fat	49.0	1.4	11.8	0.9

No. of fat	Name of species	Common name	Saturated acids in total, mole per cent	Fully saturated triglycerides, mole per cent		"Association ratio,"
				Found	Calcd. ^c	
26	<i>Hodgsonia capitocarpa</i>	Kapayang (<i>Cucurbitaceae</i>)	47.8	2.7	10.9	0.9
27	<i>Butyrospermum parki</i>	Shea fat	46.3	4.5	9.9	0.8
28	<i>Butyrospermum parki</i>	Shea fat	45.1	2.5	9.2	0.8
29	<i>Madhuca longifolia</i>	Mowrah fat	43.4	1.2	8.2	0.8
30	<i>Schleichera oleosa</i> trijuga.....	Kusam fat (Malay lac tree)	34.6	1.2	4.1	0.6
31	<i>Melia azadirachta</i>	Neem oil (margosa)	32.0	0.6	3.3	0.6
32	<i>Gossypium hirsutum</i>	Cottonseed oil	27.3	<1	2.0	0.3
33	<i>Arachis hypogaea</i>	Peanut oil	15.5	<1	0.4	0.2
34	<i>Sesamum indicum</i>	Sesame oil	14.9	<1	0.3	0.2
35	<i>Thea sinensis</i>	Teaseed oil	10.0	<1	0.1	0.1
36	<i>Brassica campestris</i>	Rapeseed oil	3.6	<1	0.0	—
a	<i>Sapium sebiferum</i>	Stillingia tallow	72.5	28.4	38.1	1.6
b	<i>Sapium sebiferum</i>	Stillingia tallow	68.4	23.9	34.0	1.4
c	<i>Elaeis guineensis</i>	Palm oil, Belgian Congo	50.9	10.3	13.2	0.8
d	<i>Elaeis guineensis</i>	Palm oil, Sumatra	51.2	2.0	13.5	1.0
e	<i>Elaeis guineensis</i>	Palm oil, Belgian Congo	49.6	6.5	12.3	0.8
f	<i>Elaeis guineensis</i>	Palm oil, Malay	49.2	9.5	11.9	0.8
g	<i>Elaeis guineensis</i>	Palm oil, Cameroons	49.1	8.3	11.8	0.8
h	<i>Elaeis guineensis</i>	Palm oil, Drewin	46.6	7.4	10.2	0.7
i	<i>Elaeis guineensis</i>	Palm oil, Cape Palmas	41.5	3.4	7.2	0.7
j	<i>Caryocarp villosum</i>	Piquia pericarp	45.9	2.3	9.7	0.8
k	<i>Canarium commune</i>	Canary tree pericarp	38.7	1.0	5.8	0.6
l	<i>Laurus nobilis</i>	Laurel berry	25.4	3.0	1.6	0.3
m	<i>Olea europaea</i>	Olive oil	13.8	2.0	0.0	0.1

^a The fat numbers 1-36 represent seed fats, while those designated by letter (a-m) are fruit-coat fats.

^b Adapted from T. P. Hilditch, *The Chemical Constitution of Natural Fats*, Wiley, New York, 1947, p. 234.

^c Calculated for random distribution from (mole per cent saturated acids_{carb}).

is present to the extent of 33% or less, it will occur not more than once in the triglyceride molecules. If, on the other hand, it forms 33 to 67% of the

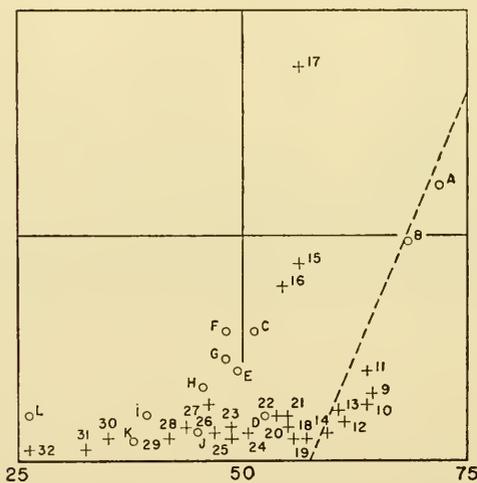
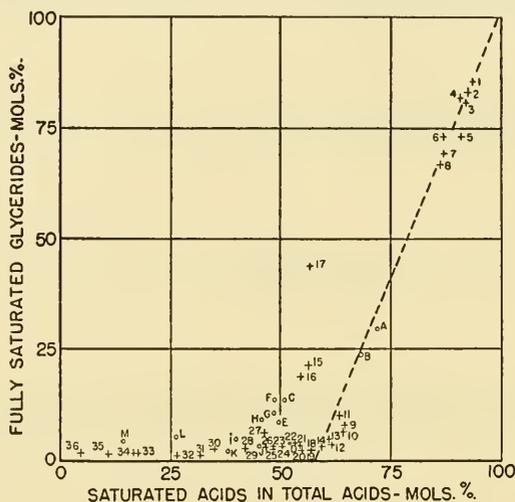


Fig. 1. Relationship between the mole per cent saturated acid and mole per cent fully saturated triglycerides.⁷⁹ The broken line represents the proportion between the fully saturated glyceride content and the proportion of saturated to unsaturated acids if these were distributed so that a maximum amount was in a 1.4:1 ratio. The lower figure represents an enlargement of the lower central area.

total fatty acid content, 2 molecules of A will be present in some glycerides; the frequency with which this will occur will increase as the proportion of the acid, A, approaches 67%. Finally, only after the con-

centration of *A* exceeds 67% will it make up an appreciable amount of simple triglyceride.

Although this rule of "even" distribution is not followed with mathematical rigidity, Hilditch points out that this generalization fits a vast

TABLE 18

RELATION BETWEEN SATURATED FATTY ACID CONTENT AND FULLY SATURATED TRIGLYCERIDES IN A NUMBER OF ANIMAL FATS, COMPARED WITH CALCULATED AMOUNTS OF SATURATED TRIGLYCERIDES BASED ON RANDOM DISTRIBUTION^a

Species	Source of fat	Satd. fatty acids, mole %				Fully satd. triglyc- erides, mole %	
		C ₁₄	C ₁₆	C ₁₈	Total	Found	Calcd. on random distrib.
Bird	Hen	0.1	27.1	6.8	34.0	2.5	3.9
Rodent	Wild rabbit	5.4	24.5	3.8	33.7	6.0	3.8
	Rat (low-fat diet)	6.0	25.5	3.0	34.5	2.5	4.1
		5.4	29.7	1.9	37.0	3.5	5.1
Herbivora							
Pig	Sow, back fat						
	outer	4.6	21.7	7.6	33.9	2.2	3.9
	inner	2.8	27.3	14.4	44.5	5.6	8.8
		4.6	27.7	10.6	42.9	6.7	7.9
	Sow, kidney fat	4.7	29.4	16.9	51.0	11.4	13.3
	Hog, kidney fat	2.0	27.4	17.5	46.9	13.2	10.3
		4.4	30.2	20.5	55.1	17.7	16.7
Ox	Beef tallow						
	N. Amer.	7.5	29.1	13.4	50.0	13.9	12.5
	S. Amer.	5.3	32.9	18.2	56.4	22.8	18.0
		9.5	29.2	23.2	61.9	25.8	23.7
		6.9	25.5	27.4	59.8	26.0	21.4
	Bullock, kidney fat	3.9	26.5	23.8 ^b	54.2	15.8	15.9
	Heifer, kidney fat	2.7	33.4	22.7 ^b	58.8	17.4	20.3
	Cow, kidney fat	3.5	31.0	20.4 ^b	54.9	18.4	16.5
Sheep	Mutton tallow	5.5	26.2	29.3	61.0	26.6	22.7
Goat	—	7.2 ^c	27.0	28.9 ^b	63.1	29.2	25.1
Buffalo,		3.9	33.4	32.2 ^b	69.5	32.5	33.6
Indian	—	8.8 ^d	45.6	19.5 ^b	73.9	37.2	40.4

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 298.

^b Includes small amounts (<1%) of arachidic acid.

^c Includes 4.7% lauric acid.

^d Includes 1.4% lauric acid.

majority of natural fats. However, Hilditch²⁸³ has noted that small concentrations of simple triglycerides do occur in seed fats, where the total acid, *A*, is present to the extent of less than 67%. Moreover, Hilditch

²⁸³ T. P. Hilditch, *Fortschr. chem. organ. Naturstoffe*, 1, 24-52 (1938).

and co-workers^{284,285} have also reported that 2 molecules of the same acid may be attached to a single triglyceride molecule when the concentration of

TABLE 19

RELATION BETWEEN SATURATED FATTY ACID CONTENT AND FULLY SATURATED TRIGLYCERIDES IN SEVERAL MILKS, COMPARED WITH CALCULATED AMOUNTS OF SATURATED TRIGLYCERIDES BASED ON RANDOM DISTRIBUTION^a

Species	Source of milk fat	Satd. fatty acids, mole %				Fully satd. triglycerides, mole %	
		C4-C14	C16	C18-C20	Total	Found	Calcd. on random distrib.
Cow	English						
	cod-liver oil diet	21.7	22.4	6.5	50.6	14.6	13.0
		22.9	22.8	7.8	53.5	17.2	15.3
	linseed oil diet	32.7	20.0	8.6	61.3	24.8	23.0
	rapeseed oil diet	30.3	17.0	12.1	59.4	25.3	21.0
	spring pasture	32.2	24.3	5.4 ^b	61.9	27.2	23.7
	autumn fed	28.8	27.1	7.1	63.0	29.1	25.0
	New Zealand, market sample	32.0	26.2	7.9	66.1	31.5	28.9
	Indian, pasture fed	35.6	26.8	5.5	67.9	33.7	31.3
	New Zealand, market sample	30.9	25.7	10.7	67.3	33.8	30.5
	English						
	stall fed	33.1	25.2	10.8	69.1	34.2	33.0
	soybean cake diet	38.7	23.7	7.6	70.0	38.2	34.3
	New Zealand, spring pasture	35.2	25.0	10.0	70.2	39.6	34.6
	English						
	stall fed	40.3	20.5	10.6	71.4	40.4	36.4
	coconut cake diet	44.4	24.1	3.9 ^b	72.4	41.3	38.0
Buffalo	Indian						
	pasture fed	31.4	28.7	10.0	70.1	34.3	34.4
		30.4	31.9	12.6	74.9	41.7	42.0
	cottonseed feed	17.7	25.1	20.1	62.9	24.3	24.9
Sheep	Indian, winter diet	47.5	20.4	6.7	74.6	36.8	41.5
Goat	Indian, winter diet	45.7	21.5	7.4	74.6	39.3	41.5
Camel	Indian, pasture	24.6	28.3	9.7	62.6	25.6	24.5
Human	English	18.8	23.6	7.4	49.8	9.1	12.4

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 299.

^b No arachidic acid reported.

the acid is as low as 25%. Such departures from the principle of "even" distribution were found in the case of cottonseed oil and in the oils of cer-

²⁸⁴ T. P. Hilditch and L. Maddison, *J. Soc. Chem. Ind.*, 59, 162-168T (1940); 61, 169-173T (1942).

²⁸⁵ H. C. Dunn, T. P. Hilditch, and J. P. Riley, *J. Soc. Chem. Ind.*, 67, 199-203 (1948).

tain citrus fruit seeds. However, allowing for these slight discrepancies, the principle of "even" distribution has been found to be applicable to most land vegetable fats, to the fats from aquatic flora and fauna (which include the larger fishes and marine animals) and to the fats of many land animals.

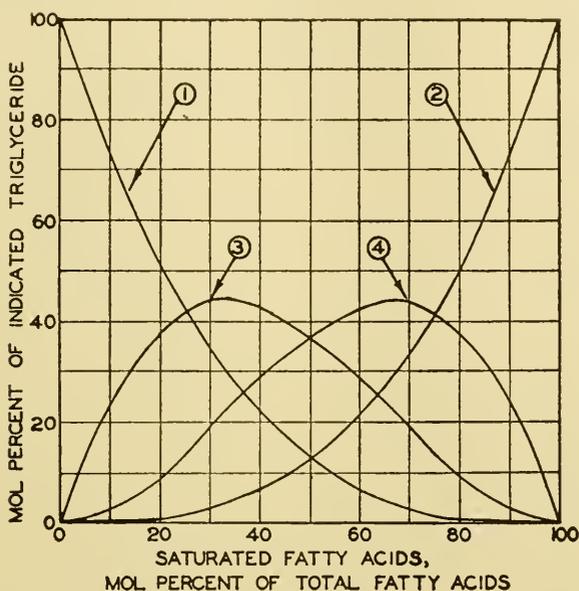


Fig. 2. Calculated composition of triglyceride mixtures containing saturated and unsaturated fatty acids distributed at random: (1) trisaturated glycerides; (2) trisaturated glycerides; (3) monosaturated-diunsaturated glycerides; (4) disaturated-monounsaturated glycerides.²⁸⁸

(c) *Theory of Random Distribution.* A third theory to explain triglyceride constitution, namely, that of random distribution, was originally suggested by Hilditch and his associates^{281, 286, 287} as applicable to certain animal fats which did not fit in with the scheme of even distribution. This is also known as chance or "indiscriminate" distribution. According to this theory, the fatty acids are believed to combine with glycerol in accordance with chance. The distribution of the fatty acids in the various types of glyceride molecules may readily be calculated. In the case of the simple triglycerides, the amount which should be present on a random basis amounts to S^3 (for GS_3) or U^3 (for GU_3).

There are two main classes of animal fats which seem to conform to this

²⁸⁶ A. Banks and T. P. Hilditch, *Biochem. J.*, **25**, 1168-1182 (1931).

²⁸⁷ A. Banks and T. P. Hilditch, *Biochem. J.*, **26**, 298-308 (1932).

²⁸⁸ E. W. Eckey, *Ind. Eng. Chem.*, **40**, 1183-1190 (1948).

triglyceride arrangement. These are animal depot fats rich in stearic acid, and milk fats. A summary of the saturated fatty acid content of some common animal fats is included in Table 18(p. 221), along with mole per cent of the fully saturated triglycerides calculated on the "indiscriminate"

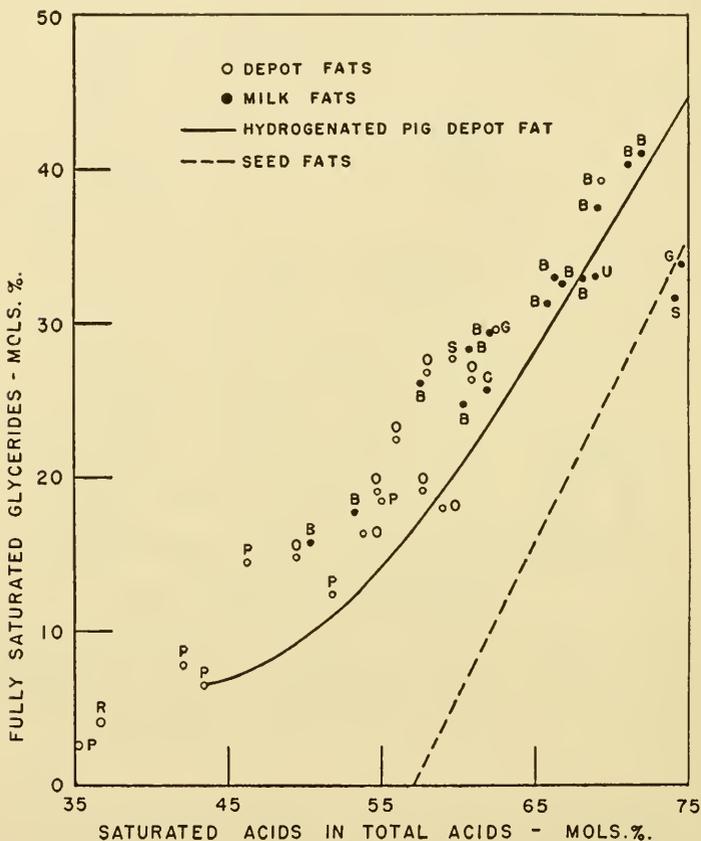


Fig. 3. Relationship between the mole per cent saturated acids and mole per cent fully saturated triglycerides.¹⁹ The solid line represents the mole per cent completely saturated triglycerides in samples of lard subjected to progressive hydrogenation, while the broken line represents the same relationship for fats based on the principle of "even" distribution of the fatty acids.

basis compared with those actually found. Table 19 (p. 222) gives the same data for the milk fats.

The correspondence of the body fats with a random arrangement is quite consistent. Of the fats listed in Table 18, 11 had a figure on an average of 1.8% lower than the estimated figure for GS_3 , while 10 of the fats were shown to have a higher value than calculated for S^3 , by a mean of

2.9%. In the case of the milk fats, which are summarized in Table 19, 6 values determined by analysis were lower by an average of 1.9% than the random value, while 15 samples gave 2.9% higher results, on an average. The variations on the positive or negative sides are not confined to any particular level of saturated fatty acids.

The relationship between the saturated fatty acid content and the proportion of completely saturated triglycerides is given in Figure 3. This graph shows a marked contrast to Figure 1, in which a similar relationship is plotted for some of the seed fats.

Longenecker,²⁸⁹ has also subscribed to the idea that the distribution of fatty acids in animal fats follows a random pattern. He believes that radically different principles govern the synthesis of many animal fats, as compared with those which are operative in the case of most vegetable fats.⁵⁰ This view is supported by Norris and Mattil,²⁹⁰ who have suggested that the enzymes responsible for the esterification may operate quite differently in the animal and in the plant systems.

In addition to the two groups of animal fats which seem to follow the random pattern, one series of vegetable fats likewise fits into the same category. These vegetable fats are composed of large proportions of saturated fatty acids other than stearic or palmitic acid. Thus, the fat of the *Myristica malibarica* (nutmeg), in which a high proportion of myristic acid is found, has a saturated triglyceride content very close to that calculated on the random basis (19% vs. 20.7%). On the other hand, laurel kernel oil, in which approximately 80% of the saturated acid is lauric acid, has about twice the content of saturated triglycerides which one would expect according to the chance distribution scheme (40.5% found vs. 20.0% calculated). The ability of the unsaturated acids to produce a large proportion of mixed triglycerides is apparently related to the type of saturated acids with which they are associated.

The fruit coat fats, likewise, may follow the random pattern more closely than they do that of "even" distribution. As an example, stillingia tallow, which has a saturated fatty acid content of 72.5 mole per cent, has been shown to have 28.4% of completely saturated triglyceride compared with a calculated amount of 38.1%. If the law of "even" distribution were rigidly followed, the trisaturated glyceride could amount to only 5.8 mole per cent.

In the case of the seed fats, no relationship can be shown between the saturated triglyceride content found and that which should occur if a chance distribution obtained. In the case of Borneo tallow (62.9% saturated acids), only 5.1% of GS₃ was found on analysis, while the theoretical figure for random arrangement based upon the value of S³ is 24.9%.

²⁸⁹ H. E. Longenecker, *Biol. Symposia*, 5, 99-115 (1941).

²⁹⁰ F. A. Norris and K. F. Mattil, *J. Am. Oil Chemists' Soc.*, 24, 274-275 (1947).

Gamboge butter, which contains 50.5% of saturated acids, has a saturated triglyceride content of only 2.7%, compared with a value of 12.8% calculated for random distribution. In the case of neem oil, which has a smaller amount of saturated acids (32%), the calculated value of GS_3 is 3.3% while that actually found amounts to only 0.6%.

Hilditch and his collaborators have recently reversed their earlier viewpoint that certain animal fats are assembled by an "indiscriminate" arrangement. In the first place, they note that the "stearic-rich" animal depot fats which fit into the random pattern quite consistently contain 20–30% of palmitic acid. The amount of totally saturated triglyceride is almost exactly proportional to the stearic acid content rather than to the sum of stearic acid and palmitic acid. As the proportion of total saturated acids decreases to 25% (the usual amount of palmitic acid), the quantity of completely saturated triglycerides approaches a figure of 0%.

However, the results obtained with Indian buffalo fat pose a striking contradiction to this conclusion. This fat contains only 19.5% of saturated C_{18} acid (stearic) and 45.6% of the C_{16} (palmitic) acid. Instead of containing under 20% of completely saturated triglycerides, as is the case with other animal fats having a similar proportion of stearic acid, buffalo fat was found actually to have 37.2% of GS_3 . This would lead us to be cautious in concluding that the completely saturated triglyceride content always follows the proportion of stearic acid. Moreover, it should be pointed out in support of the random theory that the quantities of completely saturated triglycerides become inappreciable when the saturated acid content is less than 25%. The calculated values for GS_3 are 2.7% for a 30% level, 1.6% for a 25% quantity, and only 0.8% for a 20% content of saturated acids. For this reason, one would expect an almost complete disappearance of completely saturated triglycerides below a 25% level of saturated acids, irrespective of whether palmitic or stearic acid is the acid in question.

Hilditch and co-workers^{79, 274, 275, 286, 287, 291–293} have proposed that the stearic-acid-rich animal fats and milk fats, also, represent "even" distribution, but are of a special nature. Certain changes are believed to be superimposed on the original fat formed from oleic and palmitic acids according to the general principles of "discriminate" or "even" distribution. In the case of the body fats rich in stearic acid, it is suggested that a "biohydrogenation" has occurred which changes the oleopalmitins into the completely saturated glycerides. The mammary glands also have the power to convert such original mixed triglycerides into the short-chain saturated and unsaturated glycerides characteristic of milk fat.

²⁹¹ T. P. Hilditch and Y. A. H. Zaky, *Biochem. J.*, **35**, 940–951 (1941).

²⁹² T. P. Hilditch and J. J. Sleightholme, *Biochem. J.*, **24**, 1098–1113 (1930).

²⁹³ T. P. Hilditch and J. J. Sleightholme, *Biochem. J.*, **25**, 507–522 (1931).

In further support of the theory that a biohydrogenation may take place *in vivo*, Hilditch and Stainsby²⁶² have shown that, on progressive hydrogenation of lard originally having a low stearic acid content, the proportions of fully saturated triglycerides correspond to those of fats originally having a correspondingly higher stearic acid content. The proportion of GS_3 to total saturated acids during the course of hydrogenation of the lard is indicated by the solid line in Figure 3.

In his criticism of the random theory, Hilditch⁷⁹ points out that even though it may appear to apply in some cases to the *saturated* acids, there is no evidence to indicate its applicability to the *unsaturated* acids either in the animal or in the vegetable kingdom. The highest amount of triolein, or completely unsaturated triglyceride (GU_3), reported in the above fats is 3%, whereas the proportion calculated on the basis of U^3 would be 2.7, 6.4, 12.5, 21.6 and 34.3%, respectively, when the unsaturated acids comprise 30, 40, 50, 60, or 70% of the total (or a saturated acid content of 70, 60, 50, 40 and 30%, respectively). In a recent study of glyceride structure based on ox, sheep, and pig body fats, Hilditch²⁹⁴ states that no correlation exists between the proportions of the di- and the triunsaturated glycerides with a random distribution, although the ratios of the trisaturated and monounsaturated triglycerides could be fitted into such a scheme. Hilditch²⁹⁴ sees no reason to postulate a different enzyme synthesis for animal and for vegetable fats.

There are a number of cases in which Hilditch agrees that the proportions of the four types of glycerides (GS_3 , GS_2U , GSU_2 , GU_3) agree with those calculated on a probability basis. This approximation to random distribution, however, may actually be accidental. For example, when the proportions of saturated or of unsaturated acids are relatively high, the values calculated for the trisaturated glycerides, either on the basis of "even" distribution or on the random theory, become almost identical. Experimental errors in the determinations are sufficient to render it impossible to state which of the two patterns is operative. This is the case with the triunsaturated glycerides of pig back fat²⁷⁷ and Indian sheep fats,²⁹⁵ as well as Indian cow fats.²⁷⁴

It is likewise impossible to draw conclusions from experimental values as to the pattern of assembling the triglycerides of seed fats in which the saturated acids comprise 80 to 85% of the total acids. Jackson and Longenecker²⁹⁶ have suggested that babassu fat, which is a seed fat belonging to the *Palmae* family, follows the random pattern. Hilditch,⁷⁹ on the other hand, points out that with a fat such as babassu, which contains 86.7% of saturated acids, one may as well describe the fatty acid arrangement as due

²⁹⁴ T. P. Hilditch, *J. Am. Oil Chemists' Soc.*, **26**, 41-45 (1949).

²⁹⁵ T. P. Hilditch and R. K. Shrivastava, *J. Am. Oil Chemists' Soc.*, **26**, 1-4 (1949).

²⁹⁶ F. L. Jackson and H. E. Longenecker, *Oil & Soap*, **21**, 73-75 (1944).

to "even" distribution. The proportion of completely saturated triglycerides calculated on the basis of "even" and random distribution are 70.0% and 65.2%, respectively. The experimentally determined figure for totally saturated triglycerides was 67.3%, which could be interpreted as agreeing with either school of thought.

Another instance in which a pattern of "even" distribution may be confused with a random one occurs when the major fatty acids exceed 4 in number. It is only in those fats having a maximum of 3 or 4 fatty acids in major amounts that a clear-cut example of "even" distribution may be observed. In those fats in which 6 or 7 principal acids occur, the calculation of their distribution is approximately similar, whether based upon the principle of "even" distribution or on one of chance arrangement. Hilditch and Maddison²⁹⁷ have shown that in certain marine oils in which palmitic, hexadecenoic, oleic, eicosenoic, eicosatetraenoic, eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids are present, the distribution of the acids agrees with the "even" scheme; however, the proportions of the several types of glycerides also show a fairly close correspondence to a random arrangement. Such types of evidence are obviously of little use in giving a satisfactory answer to the problem.

Another argument in support of the random method of assembling the fatty acids has been based upon the composition of fats synthesized *in vitro* from a mixture of fatty acids. Bhattacharya and Hilditch²⁸² found that, when diglycerides or triglycerides were produced artificially by heating diethylene glycol or glycerol at 145°C. under vacuum, in the presence of traces of an aromatic sulfonic acid, with various mixtures of lauric, palmitic, stearic, and oleic acids, the acids were arranged in a random manner. Norris and Mattil²⁹⁸ obtained similar results when interesterification tests were performed. Thus, when soybean oil, cottonseed oil, a mixture of tripalmitin and triolein, or one of lard and hydrogenated lard was heated with a small amount of $\text{Sn}(\text{OH})_2$ as a catalyst at 225°C. for short periods, a rearrangement occurred, with the newly formed triglycerides approximating the pattern indicative of chance arrangement. Naudet and Desnuelle²⁹⁹ have reported similar data with substrates consisting either of oleodistearin and steardiolein or of tristearin and triolein. For example, when an equimolecular mixture of tristearin and triolein was so treated, the products obtained at the completion of the reaction consisted of 11.3% of tristearin, 38.6% of oleodistearin, 39.2% of steardiolein, and 10.9% of triolein. Distribution on a random basis should have resulted in 12.5% of each of the simple triglycerides and 37.5% of each of the mixed triglycerides.

²⁹⁷ T. P. Hilditch and L. Maddison, *J. Soc. Chem. Ind.*, 67, 253-257 (1948).

²⁹⁸ F. A. Norris and K. F. Mattil, *Oil & Soap*, 23, 289-291 (1946).

²⁹⁹ M. Naudet and P. Desnuelle, *Bull. soc. chim.* [5], 14, 323-325 (1947).

Hilditch³⁰⁰ concludes that the results obtained by esterification of fatty acids with glycerol or from interesterification experiments do not contribute useful information explaining the behavior of enzymes in the living cells. The chemical reactions involved required high temperatures and other conditions incompatible with enzyme activity.

At the present time, one cannot give a decisive answer as to whether triglycerides are assembled exclusively according to a pattern of "even" distribution or to one of random arrangement, or whether the animal fats and vegetable fats are laid down in different patterns. The suggestion that biohydrogenation accounts for the apparent random arrangement of the fatty acids in some animal fats seems just as difficult to accept as the hypothesis that differences in enzyme action may account for the variations in arrangement of the fatty acids in some animal fats as compared with vegetable fats.

(d) *Theory of Partial Random Distribution.* The most recent analysis of the composition of corn oil by a modification of the Brown technic¹⁴¹ in which 19 different fractions of the triglycerides were separated at low temperatures from acetone has convinced Doerschuk and Daubert²⁸⁰ that neither the "even" distribution theory nor the straight random hypothesis can account for the composition of the mixed and simple triglycerides observed by them.

Corn oil is composed chiefly of only 4 acids, and might therefore be expected to follow the "even" pattern closely. From the original kilogram of corn oil analyzed, Doerschuk and Daubert²⁸⁰ were able to account for 979.6 g. of total glycerides. The proportions of the fatty acids found in the corn oil were 140.8 g. of saturated acids, 219.6 g. of oleic acid, 569.4 g. of linoleic acid, and 5.88 g. of linolenic acid, making a total of 935.7 g.

On the basis of a complete randomization, 40 different triglycerides should be present as calculated from the expression $(n^3 + n^2)/2$, in which n represents the number of kinds of fatty acids. However, since it is impossible to distinguish between the *positionally* different glycerides, the total number of *constitutionally* different triglycerides would be much less. According to the formula $(n^3 + 3n^2 + 2n)/6$, one should be able to separate 20 constitutionally different compounds without considering the isomeric forms.

If the "even" distribution pattern is the one followed in the case of corn oil, only 6 triglycerides should be present. The number of triglycerides would thus be limited, because oleic and linolenic acids, as well as a saturated acid, would not be expected to occur more than once, inasmuch as they are present in an amount of less than 33% of the total acids. Linoleic acid might appear to the extent of either 1 or 2 molecules, since it is present in an amount greater than 33% and less than 67% of the entire

³⁰⁰ T. P. Hilditch, *Fortschr. chem. organ. Naturstoffe*, 5, 72-100 (1948).

fatty acid content. The 6 possible mixed triglycerides would be as follows: *Lo, Lo, S* (2); *Lo, Lo, Ol* (2); *Lo, Lo, Ln* (2); *Ln, Lo, Ol* (3); *Ln, Lo, S* (3); *S, Ol, Lo* (3). The figures in parentheses represent the number of positionally different forms, while the symbols represent the following: *Lo*, linoleic acid; *Ln*, linolenic acid; *Ol*, oleic acid; and *S*, saturated acids.

Doerschuk and Daubert²⁵⁰ postulate that, according to the pattern of partial randomization, acids present in a concentration of less than 33% of the total acids may occasionally occur to the extent of 2 molecules in a single triglyceride. On the basis of the "even" distribution theory, this could not occur. Moreover, acids present in amounts of 33 to 67% which, according to the "even" distribution pattern, must occur in every molecule at least once, may actually be absent in some cases from triglycerides, on the basis of the new hypothesis. Under such circumstances, 14 constitutionally different simple and mixed triglycerides would occur in varying proportions.

TABLE 20
COMPARISON OF TRIGLYCERIDES FOUND IN CORN OIL WITH THOSE CALCULATED FROM FATTY ACID COMPOSITION ON BASIS OF DIFFERENT THEORIES FOR TRIGLYCERIDE STRUCTURE^a

Triglyceride structure	No. of positional isomers	Triglycerides, g./kg. corn oil calcd. on basis of several theories of structure				Triglycerides found (exptl.), g.
		Monoacid	"Random" distrib.	"Even" distrib.	"Partial random" distrib.	
<i>Ln, Ln, Ln</i>	1	6.15	0.003	0	0	0
<i>Lo, Lo, Lo</i>	1	596	223.00	0	8.0	8.9
<i>Ol, Ol, Ol</i>	1	230	12.5	0	0	0
<i>S, S, S</i>	1	147	3.24	0	0	0
<i>Ln, Ln, Lo</i>	2	0	0.07	0	0	0
<i>Ln, Ln, Ol</i>	2	0	0.03	0	0	0
<i>Ln, Ln, S</i>	2	0	0.02	0	0	0
<i>Lo, Lo, Ln</i>	2	0	6.96	0	0.5	0
<i>Lo, Lo, Ol</i>	2	0	257.00	524	485	482
<i>Lo, Lo, S</i>	2	0	164.00	291	332	335
<i>Ol, Ol, Ln</i>	2	0	1.02	0	0.50	0
<i>Ol, Ol, Lo</i>	2	0	98.0	0	70.0	72.7
<i>Ol, Ol, S</i>	2	0	23.9	0	1.69	3.6
<i>S, S, Ln</i>	2	0	0.41	0	3.20	4.1
<i>S, S, Lo</i>	2	0	39.9	0	17.5	16.8
<i>S, S, Ol</i>	2	0	15.3	0	4.16	0.18
<i>Ol, Lo, Ln</i>	3	0	5.34	15.5	1.00	0
<i>S, Lo, Ln</i>	3	0	3.40	3.0	2.94	3.19
<i>S, Ol, Ln</i>	3	0	1.30	0	10.5	11.8
<i>S, Ol, Lo</i>	3	0	125.00	146	40.8	41.5
<i>Total</i>	40	979.15	980.393	979.5	977.79	979.77

^a Adapted from A. P. Doerschuk and B. F. Daubert, *J. Am. Oil Chemists' Soc.*, 25, 425-433 (1948).

Ln = linolenic acid; *Lo* = linoleic acid; *Ol* = oleic acid; *S* = saturated acid.

The amount of each component which has been calculated by Doerschuk and Daubert,²⁵⁰ as well as the proportions actually found, are reported in Table 20.

The distribution of fatty acids in the triglycerides of corn oil agrees most remarkably with the values calculated on the basis of the partial random distribution theory. Only in several very minor instances were any appreciable deviations between the calculated and observed values noted. On the other hand, major discrepancies are noted between the triglycerides found and those postulated on the basis of the patterns of distribution which would result from the other theories. None of the 11 mixed triglycerides found is accounted for by the monoacid hypothesis. Major variations obtain between experimental values and those calculated on the random theory. The most striking deviations are those for Lo, Lo, Lo (223, theory; 8.9 found), Lo, Lo, Ol (257, theory; 482 found) and Lo, Lo, S (164, theory; 335 found).

In the case of "even" distribution, the discrepancies from the theory are almost as serious as in the random hypothesis. The triglyceride Lo, Lo, Ol is actually present to the extent of 482 instead of 535, while Lo, Lo, S is found in an amount of 335 instead of the hypothetical quantity of 291. The most devastating non-conformity is to be traced to the fact that, of the 9 triglycerides postulated in the partial random theory which are not accounted for by the "even" distribution theory, 7 have actually been found in amounts which correspond almost exactly to what would have been expected. The 2 triglycerides which were not observed were minor ones which should have been present to the extent of only 0.5 g. each. The brilliant research of Doerschuk and Daubert²⁵⁰ would seem to prove that neither random nor "even" distribution necessarily represents the pattern of fatty acid distribution in corn oil. It will be interesting to see whether or not the partial random pattern may apply to other vegetable fats and animal fats when the newer procedures for triglyceride resolution and analysis have been carried out. Until such data are available, one should probably keep an open mind as to the possibility that different fats may be assembled in several varying patterns.

d. Glyceride Composition of Fats. Appreciable amounts of the completely saturated triglycerides do not occur in the vegetable fats until the concentration of the saturated acids reaches 60%. As can be noted from Table 17, the ratio of unsaturated to saturated acids in the mixed triglycerides of seed fats having a higher proportion of saturated acids than 60% of the total remains remarkably constant at 1.3-1.4. This relationship is followed even in fats such as coconut oil, in which as much as 94% of the acids are saturated.

The proportions of various groups of glycerides of some common animal and vegetable fats are indicated in Table 21. The composition of some

other fats in which the proportions of palmitic and stearic acids have been determined is summarized in Table 22.

The composition of the fats which are listed in Table 22 gives no data as to the type of unsaturated fatty acids present. Thus, on hydrogenation,

TABLE 21
PROPORTIONS OF TRIGLYCERIDES PRESENT AS COMPLETELY SATURATED, MIXED SATURATED AND UNSATURATED, OR FULLY UNSATURATED TRIGLYCERIDES IN ANIMAL AND VEGETABLE FATS^a

Type of fat	Trisaturated glycerides GS ₃	Disaturated, monounsaturated glycerides GS ₂ U	Monosaturated, diunsaturated glycerides GSU ₂	Tri-unsaturated glycerides GU ₃
Animal fats				
Beef tallow ^b	25.8	33.7-53.9	40.5-0	0-20.3
Mutton tallow ^c	26	30-52	44-0	0-22
Goat (male) ^d	29.2	30.9-50.9	39.9-0	0-19.9
Goat (female) ^e	30.8	29.2-49.2	40.0-0	0-14.9
Butter ^f	33.7	36.5-51.4	29.8-0	0-14.9
Vegetable fats				
Pericarp fats:				
Palm oil ^{g,h}	3.5-7	16.5-29	66-58	0-14
Sterculia ⁱ	1	14	85	0
Piquia ^j	2	42	56	0
Stillingia tallow ^k	27.6	61-65	11.4-0	0-6
Seed fats:				
Coconut ^l	84	12	4	—
Palm kernel ^l	63	26	11	—
Cacao butter ^m	2.5	77	16	—
Borneo tallow ⁿ	4.5	85	6.5	4
Shea butter ^o	2.3	30.5-65	69.5-0	0-35
Bouandja ^o	1.5	68.5-82	36.5-0	0-18
Taban merah ⁱ	2	77	21	0
Cottonseed ^p	—	15	60	24

^a Adapted from H. E. Longenecker, *Chem. Revs.*, 29, 201-224 (1941).

^b A. Banks and T. P. Hilditch, *Biochem. J.*, 25, 1168-1182 (1931).

^c G. Collin, T. P. Hilditch, and C. H. Lea, *J. Soc. Chem. Ind.*, 48, 46-50T (1929).

^d D. R. Dhingra and D. N. Sharma, *J. Soc. Chem. Ind.*, 57, 369-370T (1938).

^e D. R. Dhingra and M. Haneef, *J. Soc. Chem. Ind.*, 58, 292-293T (1939).

^f T. P. Hilditch and J. Sleightholme, *Biochem. J.*, 25, 507-522 (1931).

^g A. Banks, H. K. Dean, and T. P. Hilditch, *J. Soc. Chem. Ind.*, 54, 77-82T (1935).

^h T. P. Hilditch and E. E. Jones, *J. Soc. Chem. Ind.*, 49, 363-368T, 369T (1930).

ⁱ T. P. Hilditch and W. J. Stainsby, *J. Soc. Chem. Ind.*, 53, 197-203T (1934).

^j T. P. Hilditch and J. G. Rigg, *J. Soc. Chem. Ind.*, 54, 109-111T (1935).

^k T. P. Hilditch and J. Priestman, *J. Soc. Chem. Ind.*, 49, 397-400T (1930).

^l G. Collin and T. P. Hilditch, *J. Soc. Chem. Ind.*, 47, 261-269T (1928).

^m T. P. Hilditch and C. H. Lea, *J. Soc. Chem. Ind.*, 48, 41-46T (1929).

ⁿ T. P. Hilditch and J. Priestman, *J. Soc. Chem. Ind.*, 49, 197-200T (1930).

^o T. P. Hilditch and S. A. Saletore, *J. Soc. Chem. Ind.*, 50, 468-472T (1931); 52, 101-105T (1933).

^p T. P. Hilditch and E. C. Jones, *J. Soc. Chem. Ind.*, 53, 13-21T (1934).

the same tristearin arises, irrespective of whether the parent triglyceride was steardiolein, steardilinolein, stearo-oleolinolein, oleodilinolein, triolein, or trilinolein. An attempt has been made to avoid the above difficulty by transforming the oleoglycerides into more solid fats by elaidinization, whereby they can be separated from the liquid fats. This can be ac-

TABLE 22. GLYCERIDES AND COMPONENT ACIDS IN SOME ANIMAL AND VEGETABLE FATS^a
P = palmitic; S = stearic; U = unsaturated acids (oleic + linoleic)

Fat	GS ₃					GS ₂ U			GSU ₂			GU ₁
	P ₃	P ₂ S	PS ₂	S ₃	P ₂ U	S ₂ U	PSU	PU ₂	SU ₂	GU ₁		
<i>Althabackia floribunda</i>	—	—	1.3	—	—	76.0	5.4	Trace	15.4	1.9		
<i>Althabackia parviflora</i>	—	—	1.3	—	—	60.0	9.3	Trace	26.1	3.3		
<i>Mimusca heckelii</i>	—	—	1.3	—	—	26.3	7.5	6.0	46.7	12.2		
<i>Storca stenoptera</i>	1.4	1.9	1.3	0.7	7.6	39.8	30.7	3.3	13.3	—		
<i>Theobroma cacao</i>	—	2.5	—	—	6.5	18.4	51.9	8.7	12.0	—		
<i>Hodgsonia caputicarpa</i>	2.1	0.6	—	—	33.1	—	27.3	24.1	—	12.8		
<i>Garcinia indica</i>	—	—	—	1.5	1.6	57.9	14.8	1.8	21.4	1.0		
<i>Melia azadirachta</i>	—	0.6	0.6	—	5.0	—	12.3	26.0	33.6	22.5		
<i>Madhuca longifolia</i>	—	1.2	—	—	0.9	—	26.9	41.3	29.7	—		
<i>Palmae</i> species	3.0	2.7	—	—	30.5	—	9.9	41.5	—	12.4		
<i>Palmae</i> species	5.1	3.3	—	—	42.7	—	10.8	31.5	—	6.6		
<i>Madhuca butyracea</i>	7.9	—	—	—	62.4	—	7.2	22.5	—	—		
<i>Achras zapota</i>	—	3.0	1.5	—	—	—	7.8	59.4 ^f	27.8	5.0		
<i>Butyrospermum parkii</i>	—	—	—	—	—	34.4	—	11.3	45.3	4.5		
Cow depot ^b	3.0	22.6	10.0	—	18.2	—	35.4	9.7	4.8	4.8		
Cow depot ^a	—	16.5	11.8	—	11.0	1.8	41.6	14.2	3.1	3.1		
Ox depot ^o	3.4	7.8	5.8	0.4	14.7	2.3	32.0	22.7	10.9	Trace		
Pig, outer back ^p	0.6	2.4	2.4	—	5.4	—	34.0	45.4	9.8	9.8		
Pig, perinephric ^p	0.1	4.3	4.7	—	9.0	—	39.2	35.2	7.5	7.5		

^a Adapted from H. E. Longenecker, *Chem. Revs.*, 29, 201-224 (1941), p. 215.^b M. L. Meara and Y. A. H. Zaky, *J. Soc. Chem. Ind.*, 59, 25-26T (1940).^c D. Atherton and M. L. Meara, *J. Soc. Chem. Ind.*, 59, 95-96T (1940).^d W. J. Bushell and T. P. Hilditch, *J. Soc. Chem. Ind.*, 57, 48-49T, 447-449T (1938).^e T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947.^f T. P. Hilditch, M. L. Meara, and W. H. Pedelty, *J. Soc. Chem. Ind.*, 58, 26-29T (1939).^g N. L. Vidyarthi and C. J. D. Rao, *J. Indian Chem. Soc.*, 16, 437-442 (1939).^h T. P. Hilditch and K. S. Murti, *J. Soc. Chem. Ind.*, 58, 310-312T (1939).ⁱ T. P. Hilditch and M. B. Ichhaporia, *J. Soc. Chem. Ind.*, 57, 44-48T (1938).^j T. P. Hilditch and L. Maddison, *J. Soc. Chem. Ind.*, 59, 67-71T (1940).^k N. L. Vidyarthi and M. V. Mallya, *J. Indian Chem. Soc.*, 16, 443-448 (1939).^l Includes 22.8% myristylidolein.^m T. G. Green and T. P. Hilditch, *J. Soc. Chem. Ind.*, 57, 49-53T (1938).ⁿ T. P. Hilditch and K. S. Murti, *Biochem. J.*, 34, 1301-1311 (1940).^o T. P. Hilditch and S. Paul, *Biochem. J.*, 32, 1775-1784 (1938).^p T. P. Hilditch and W. H. Pedelty, *Biochem. J.*, 34, 971-979 (1940).^q Traces.

complished either by using the oxides of nitrogen or sulfur,^{230,301-303} or by the action of selenium.^{304,305} The elaidoglycerides so formed have different solubilities in acetone, and may be resolved from the other triglycerides. Thus, by determinations of saturated, elaidic, and combined oleic and linoleic acids, it is possible to calculate the proportions of the different unsaturated acids in the fats. The accuracy is of a lower order than is possible for the solid fats having a larger proportion of saturated acids.

Hilditch⁷⁹ has suggested the distribution of unsaturated fatty acids as indicated in Table 23 for several vegetable fats. Such values are purely tentative and are based on the total C₁₅ unsaturated acids divided according to the "rule of even distribution," which has been shown to hold for liquid fats.

TABLE 23
CALCULATED PROPORTION (MOLE PER CENT) OF TRIGLYCERIDES IN SOME LIQUID FATS^a

Oil	Palmito- dioleins	Palmito- oleolein	Palmito- dilinoleins	Linolo- dioleins	Oleo- dilinolein	Tri- olein
Cottonseed	—	60	15	5	20	—
Olive	30	—	—	25	—	45
Peanut	30 ^b	25 ^b	—	45	—	—
Teaseed	35	—	—	20	—	45

^a Adapted from T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947, p. 219.

^b Stearic, arachidic, behenic, or lignoceric acid may be present in place of some of the palmitic acids.

Although much progress has been made in our knowledge of the composition of fat, little has as yet been accomplished insofar as the understanding of their specific configuration is concerned. As described earlier, a number of mixed triglycerides have been isolated from natural fats; however, these have given us only qualitative data rather than quantitative values. Moreover, by the technics available, only the least soluble have been separated. It is suggested by Longenecker⁵⁰ that the production of such a selective configuration may mean the operation of a specific biological process.

5. Constants Used for Identification of Fats and Oils

A number of physical and chemical constants are commonly employed to facilitate the identification of fats, as well as to establish their purity. Some of these values may be quite specific for the appraisal of the purity

³⁰¹ H. N. Griffiths and T. P. Hilditch, *J. Chem. Soc.*, 1932, 2315-2324.

³⁰² H. N. Griffiths and T. P. Hilditch, *Analyst*, 59, 312-318 (1934).

³⁰³ T. P. Hilditch and H. N. Griffiths, *J. Soc. Chem. Ind.*, 53, 75-81T (1934).

³⁰⁴ S. H. Bertram, *Chem. Weekblad*, 33, 3-5 (1936); *Öle, Fette, Wachse, Seife, Kosmetik*, 1938, No. 7, 1-4; *Chem. Abst.*, 32, 7755 (1938).

³⁰⁵ B. G. Gunde and T. P. Hilditch, *J. Soc. Chem. Ind.*, 59, 47-53T (1940).

or of the quality of a given fat. The most common constants which are of value include the melting point, refractive index, specific gravity, saponification number, iodine value, and the Reichert-Meissl number. Table 24 lists the commonly accepted constants for some animal and vegetable fats.

The figures for the various constants vary considerably for different samples of the same type of fat. They cannot be stated with the precision of chemically pure substances, since the natural fats are mixtures of a number of types of different triglycerides. However, certain arbitrary limits are defined within which such values fall. The commonly accepted values for some of the better known oils are included in Table 24.

Variations occur in the constants of vegetable oils obtained from the same species grown under different conditions, as well as in related species raised under the same environment. Although such differences are usually minor, they may become of sufficient magnitude to be of considerable commercial importance. Thus, the iodine numbers of linseed oils have been shown³⁰⁶⁻³⁰⁹ to vary from 128 to 209, while that prepared from the Bison variety of flaxseed raised in different localities had iodine values ranging from 155 to 196. Such factors as climatic conditions, high temperature, and insufficient moisture while the seed is ripening apparently produce linseed oils with extremely low iodine numbers. The variation in unsaturation of the linseed oils is considerably greater than is noted with other vegetable fats.

On the other hand, it has been widely recognized for many years that marked variations may occur in animal fats from the same species of animals. The diet of the animal seems to be the most important factor which causes an alteration in the composition of fat. As early as 1883, Lebedeff³¹⁰ demonstrated that the fat of a fasted dog could be profoundly affected by diet. When mutton tallow was fed, a very hard fat was deposited, while after the administration of linseed oil the fat was practically liquid, with an unsaturated fatty acid content much higher than normal. One year later, it was shown that erucic acid, which is ordinarily not present in dog fat, is laid down after the administration of rapeseed oil, a fat especially rich in this component.³¹¹ Although some polyunsaturated acids are present in the tissues of rats on fat-free diets, Rieckehoff and collaborators³¹² were able to find increased amounts of tetraenoic, pentaenoic, and

³⁰⁶ E. P. Painter, *Oil & Soap*, 21, 343-346 (1944).

³⁰⁷ E. P. Painter and L. L. Nesbitt, *Ind. Eng. Chem., Anal. Ed.*, 15, 123-128 (1943).

³⁰⁸ E. P. Painter and L. L. Nesbitt, *Oil & Soap*, 20, 208-211 (1943).

³⁰⁹ C. D. Hodgman, *Handbook of Chemistry and Physics*, 31st ed., Chem. Rubber Pub. Co., Cleveland, 1949, pp. 1267-1270.

³¹⁰ A. Lebedeff, *Arch. ges. Physiol. (Pflüger's)*, 31, 11-59 (1883).

³¹¹ I. Munk, *Arch. path. Anat. Physiol. (Virchow's)*, 95, 407-467 (1884).

³¹² I. G. Rieckehoff, R. T. Holman, and G. O. Burr, *Arch. Biochem.*, 20, 331-340 (1949).

TABLE 24
CONSTANTS OF SOME COMMON FATS AND OILS^a

Fat or Oil	Specific gravity (15.5°/15.5°C.)	Refractive index (15.5°C.)	Melting point, °C.	Saponification number	Iodine number	Reichert-Meissl number
Almond	0.914-0.920	1.472-1.475	—	188-195	93-100	0.5
Butter	0.930-0.940	1.445-1.449 ^b	28-35	221-233	26-38	25.0-33.0
Castor	0.960-0.967	1.479-1.481	—	175-185	82-90	1.0-2.0
Cacao butter	0.950-0.975	1.449-1.451 ^b	28-33	192-202	32-38	0.2-0.8
Coconut	0.926	1.439-1.443 ^b	22-27	246-260	8-10	6.6-8.4
Cod-liver	0.922-0.930	1.479-1.485	—	180-190	135-175	0.3-0.6
Corn	0.921-0.927	1.475-1.477	—	187-193	115-124	0.3-3.5
Cottonseed	0.920-0.925	1.473-1.476	—	191-196	105-115	0.7-0.9
Human ^c	0.918	1.459-1.461 ^d	35.5	193-199	64	0.25-0.5
Lard	0.934-0.938	1.450-1.454 ^b	36-45	195-200	50-65	0.2-0.6
Lard (oil)	0.915-0.916	1.469-1.472	—	193-198	67-82	0.0
Linseed	0.930-0.938	1.480	—	188-195	177-209	1.0
Menhaden	0.925-0.931	1.474-1.477	—	188-193	148-172	1.0
Mustard	0.915-0.919	1.479-1.482	—	171-176	94-113	—
Olive	0.915-0.918	1.470-1.472	—	185-196	79-90	0.6
Palm	0.921-0.924	1.450-1.452 ^b	27-43	196-204	53-57	0.8-1.9
Peanut	0.917-0.920	1.471-1.474	—	186-194	85-100	0.5
Poppyseed	0.924-0.926	1.476-1.478	—	190-196	133-139	0.0
Rapeseed	0.913-0.917	1.474-1.476	—	170-179	97-105	0.0-0.6
Sesame	0.921-0.925	1.474-1.476	—	188-193	103-112	1.2
Soybean	0.922-0.928	1.475-1.476	—	189-194	130-138	0.5-0.8
Sunflower	0.924-0.926	1.474-1.478	—	188-194	120-135	—
Tallow (beef)	0.860 ^e	1.449-1.452 ^b	43-48	193-198	35-45	0.2-0.5
Tallow (mutton)	0.858-0.860 ^e	1.451 ^b	44-47	192-195	32-45	0.3
Teaseed	0.915-0.919	1.470-1.473	—	190-195	80-87	0.3-1.0

^a A. G. Woodman, *Food Analysis*, McGraw-Hill, New York, 1941, p. 199.

^b At 60° C.

^c C. D. Hodgman, *Handbook of Chemistry and Physics*, 31st ed., Chemical Rubber Pub. Co., Cleveland, 1949, pp. 1267-1270.

^d Value at 40° C.

^e At 99°/15.5°.

hexaenoic acids, especially in the phosphatides, after the administration of cod-liver oil.

Although butyric, caproic,^{313,314} and caprylic³¹⁵ acids cannot be deposited by the rat in storage fat, even when the triglycerides of these acids are fed in large quantities, capric and lauric acids,^{315,316} as well as myristic acid,³¹³ are capable of being deposited in the storage fat when their triglycerides comprise an important part of the diet. It has recently been demonstrated in a most convincing manner that the triglyceride of an odd-chain acid, triundecylin, can be laid down in rat tissues when the unnatural fat is fed over a protracted period.³¹⁷ As much as 24% of the undecylic acid was found in the storage fats after a 6-week feeding period during which triundecylin was given.

A hard body fat with low iodine number results when a high carbohydrate or high protein diet is given. Anderson and Mendel³¹⁸ reported that, when cornstarch was substituted equicalorically for fat in the diet of rats, a progressive hardening of the body fat ensued. Great commercial importance may sometimes be attached to such variations in the type of fat produced. After hogs partook of a large proportion of peanuts, a "soft pork," in which the fat was partially liquid, resulted, giving a product unacceptable to the consumer.^{319,320} According to Ellis and Isbell,³²¹ fats having a high iodine number and a low melting point are produced in hogs when either peanuts or soybeans make up a large proportion of the diet; on the other hand, the ingestion of corn and skimmed milk or brewer's rice and tankage produces fats with a low iodine value and a relatively hard consistency. These examples of variations in the composition of natural fats emphasize the fact that wide ranges in the constants are perfectly normal.

(1) Melting Point

In some cases the nature of a fat may be determined from its melting point. This value is of little use in the case of the vegetable oils, since these are mostly liquid at ordinary temperatures. The same fat may exhibit several different melting points, depending upon the polymorphic state in which it exists—see discussion of polymorphism, Section 6(1). Be-

³¹³ H. C. Eckstein, *J. Biol. Chem.*, *81*, 613-628 (1929).

³¹⁴ H. C. Eckstein, *J. Biol. Chem.*, *84*, 353-357 (1929).

³¹⁵ M. Powell, *J. Biol. Chem.*, *89*, 547-552 (1930).

³¹⁶ M. Powell, *J. Biol. Chem.*, *95*, 43-45 (1932).

³¹⁷ F. E. Visscher, *J. Biol. Chem.*, *162*, 129-132 (1946).

³¹⁸ W. E. Anderson and L. B. Mendel, *J. Biol. Chem.*, *76*, 729-747 (1928).

³¹⁹ O. G. Hankins and N. R. Ellis, *U. S. Dept. Agric., Dept. Bull. No. 1407* (April, 1926).

³²⁰ O. G. Hankins, N. R. Ellis, and J. H. Zeller, *U. S. Dept. Agric., Dept. Bull. No. 1492* (Feb., 1928).

³²¹ N. R. Ellis and H. S. Isbell, *J. Biol. Chem.*, *69*, 239-248 (1926).

cause of the failure of many of the earlier investigators to realize this fact, many of the melting points reported in the literature are unreliable.

Although the melting points of the simple triglycerides composed of even-chain saturated acids are directly proportional to the chain length (and vary inversely with the saponification number), the presence of even minor amounts of the unsaturated fatty acids may lower the melting point profoundly. The effectiveness of such an unsaturated acid in decreasing the melting point must depend, not only on the degree of unsaturation, but also on its position in the mixed triglyceride molecule. The melting point can be caused to vary in a natural fat without altering its fatty acid composition, by rearrangement of the fatty acids. Thus, a limpid cottonseed oil can be changed to a semisolid form by interesterification, while a product solid at ordinary room temperature can be prepared by so-called "directed" interesterification. All of the natural fats listed in Table 24 with iodine numbers exceeding 65 are liquid at ordinary temperatures. However, unnatural products having considerably higher iodine numbers may be solid. Thus, the solid fat prepared from cottonseed oil by directed interesterification has the same iodine value as does limpid cottonseed oil, namely, 105 to 115.

(2) *Specific Gravity*

Rather wide variations in specific gravity obtain between the different fats. The values vary from a maximum of 0.960 for cacao butter to 0.915 for lard oil and rapeseed oil. The calculated values for beef and mutton tallow are much lower than those for the liquid fats, being 0.860. The differences in specific gravity are to be ascribed to the component fatty acids. In general, they increase with the increasing molecular weight of the combined acids; they also increase with larger proportions of unsaturated or hydroxy-acids. The specific gravity tends to increase when rancidity develops.

In the case of fats which are ordinarily solid at room temperature, the specific gravity is best determined at a point considerably above the melting point, usually at 40° or 50°C. The Sprengel tube is conveniently used for such a test.³²² The value for the specific gravity at 15.5°C. can be calculated by the following formula:

$$Sp_{15.5} = Sp_t + k(t - 15.5)$$

where Sp_t is the figure obtained at temperature t , and k is a constant. Some values for k for some ordinary fats are given by Woodman³²² as follows: butter fat, 0.000617; cacao butter, 0.000717; coconut oil, 0.000642; lard, 0.000650; palm oil, 0.000657; tallow, 0.000673.

³²² A. G. Woodman, *Food Analysis*, 4th ed., McGraw-Hill, New York, 1941.

(3) *Refractive Index*

The refractive index may be defined as the ratio of the sine of the angle of incidence to the sine of the angle of refraction. It is usually referred to by an italicized letter n , with the temperature at which the observation is made indicated. The standard temperature for reference in the case of oils is 25°C., while for fats the temperature is 40°C. A correction factor of 0.000365 (or 0.00038³²³) may be used to calculate the values at the reference temperature; however, the determination should be made as near as possible to the reference point. The refractive indices increase with a lowering of the temperature.

The refractive index of a fat is a measurement which can be made with considerable precision. Only an extremely small amount of sample is required. Thus, the determination can readily be made even when the quantity of a sample is limited. In general, the refractive index varies with the specific gravity. Highest values are also obtained with increasing degrees of unsaturation, as well as with larger molecular weights. Butter fat is an exception to this pattern, since it has a lower refractive index than other animal fats, although its specific gravity is higher. This discrepancy is apparently related to the high proportion of fatty acids of low molecular weight contained in this product.

The index of refraction is closely related to the iodine number of the fat. This is not surprising, since it is proportional to the unsaturation of the fat, the degree of which is measured by the iodine number. The relation of refractive index to iodine number is so constant that a simple refractometer graduated in iodine values is now available for field work. It is stated that the precision is such as to make it accurate to one-half of an iodine value unit. According to Peter and Kron,³²⁴ the iodine number of butter fat may be calculated from the refractive index by the following equation:

$$\text{Iodine Number} = (n_D^{40} - 1.45268)5700 + 26$$

The index of refraction is also related directly to the melting point of the fat. These relationships are illustrated in Figures 4 and 5, which are based on the data of Sudborough *et al.*³²⁵

The refractive index is also related to the proportion of short-chain acids. In general, it has been found that the higher the Reichert-Meissl value the lower the refractive index. However, the correlation in this case is quite poor. Soybean oil has the highest refractive index (1.475) of the common fats, and this is followed closely by poppyseed, corn, and mustard oils.

³²³ H. D. Richmond, *Analyst*, 32, 44-46 (1907).

³²⁴ A. Peter and S. Kron, *Milchw. Forsch.*, 14, 378-386 (1932).

³²⁵ J. J. Sudborough, H. E. Watson, and D. Y. Athawale, *J. Indian Inst. Sci.*, 5, 47-69 (1922).

Olive and lard oils are appreciably lower (1.467), while the high-melting fats have values from 1.452 (lard) to 1.441 (coconut oil) at 60°C.

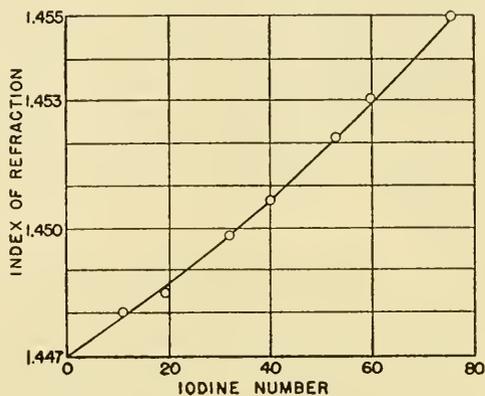


Fig. 4. The relationship between the refractive indices and iodine numbers of some fats.³²⁵

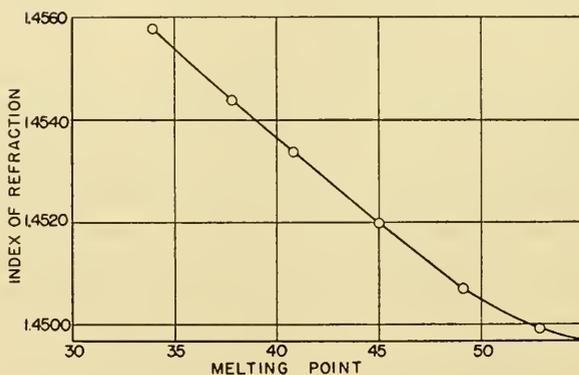


Fig. 5. The relationship between the melting points of fats and their refractive indices.³²⁵

(4) Saponification Number

The saponification value furnishes a measure of the average length of the fatty acid chains which make up any given fat. It is defined as the *number of milligrams of potassium hydroxide necessary to saponify one gram of fat or oil*. It is also sometimes referred to as the Koettstorfer number, after the scientist who first employed this constant.³²⁶

The saponification number is inversely proportional to the molecular

³²⁶ J. Koettstorfer, *Z. anal. Chem.*, 18, 199-207 (1879).

weight of the fat. The relationship of these values to those of some simple triglycerides is recorded in Table 25.

TABLE 25
SAPONIFICATION NUMBERS OF SOME SIMPLE TRIGLYCERIDES

Triglyceride	Molecular weight	Saponification value
Triacetin	218.1	770.3
Tributyryn	302.2	557.0
Tricaproin	386.3	434.9
Tricaprylin	470.4	357.1
Tricaprin	554.4	303.6
Trilaurin	638.5	263.5
Trimyristin	722.7	233.1
Tripalmitin	806.8	208.6
Tristearin	890.9	188.9
Triarachidin	975.0	172.5
Triolein	884.8	190.2
Trilinolein	878.8	191.5
Trilinolenin	872.7	192.9

In the case of the natural fats and oils which are largely mixed triglycerides containing several varieties of acids, the saponification values usually lie between 190 and 200. In a few cases in which an unusually large proportion of short-chain acids is present in the fat, high saponification values are the rule, *i.e.*, coconut oil, 253, butter fat, 227. In several instances in which longer chained acids make up a large proportion of the fatty acids (as erucic acid in rapeseed oil), saponification values markedly lower than those for the common fats are the rule, for example, mustard oil, 174, rapeseed oil, 173.

(5) Free Fatty Acids

Although refined oils are largely devoid of free fatty acids, considerable amounts of these constituents may be present in the crude oil. The presence of such acids may be an index of the purity of the oil. Such fatty acids may result from decomposition of the oil due to chemical treatment or to bacterial action. The rather high levels of free fatty acids ordinarily found in olive oils are a reflection of the lipases present in the pulp of the olive, which may occur as impurities in the oil. Palm oil is reported in some cases to contain as much as 75% in hydrolyzed form. The degree of edibility of a fat is generally considered to be inversely proportional to the total amount of free fatty acids.

The amount of free fatty acids is sometimes expressed as the acid number (or acid value), which is defined as the *number of milligrams of potassium*

hydroxide required to neutralize the free fatty acids in one gram of oil or fat. This value may also be expressed as the percentage of oleic acid. In the latter case, 1 ml. of 0.1 *N* alkali is considered equivalent to 0.0282 g. of oleic acid.

When the *acid number* is subtracted from the *saponification number*, the difference will equal the potassium hydroxide actually required for the saponification of the fat. This is sometimes referred to as the *ester number*. In the case of most refined oils, as well as of crude oils of high quality, the *saponification* and *ester* numbers are practically identical.

(6) Iodine Number

The iodine number is defined as the *number of grams of iodine absorbed by one hundred grams of fat or oil.* Under certain conditions, iodine is absorbed quantitatively by unsaturated acids or triglycerides at the point of unsaturation. Thus, each molecule of oleic acid will combine with 2 atoms of iodine, while one molecule of triolein will absorb 6 atoms of this halogen. Trilinolein reacts with 12 atoms and trilinolenin with 18 atoms of iodine. Whereas the reaction gives theoretical results with oleic or linoleic acids, this is not the case with linolenic acid, in which a value of only 223 has been found, in contrast to a theoretical result³²⁷ of 274. Much lower than theoretical values are obtained where a proximity exists between the double bond and the carboxyl group, as well as where the double bonds are conjugated.³²⁸

The iodine number is especially helpful in determining the ability of a fat to be hardened by hydrogenation. Coupled with the thiocyanogen number, it is an invaluable aid in ascertaining the usefulness of a fat to serve as a drying oil. Irrespective of these uses, it is also important in the identification of fats, as it is one of the constants less readily affected by the treatment of the oil than are some other constants.

Since free iodine reacts with fat very slowly, it is usually combined with other substances to facilitate the reaction. One of the earliest reagents for this use was the so-called *Hübl reagent*, which contained an alcoholic solution of iodine in the presence of mercuric chloride.³²⁹ Later modifications were the Wijs reagent^{330,331} in which iodine monochloride was employed in glacial acetic acid, and the Hanus method,³³² which involved the use of iodine monobromide in place of the iodine monochloride. The latter compound has the advantage of being more easily prepared than the chloride.

³²⁷ W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943.

³²⁸ B. P. Caldwell and F. A. Piontkowski, *J. Am. Chem. Soc.*, *56*, 2086-2089 (1934).

³²⁹ Baron Hübl, *Dinglers polytech. J.*, *253*, 281-295 (1884).

³³⁰ J. J. A. Wijs, *Ber.*, *31*, 750-752 (1898).

³³¹ J. J. A. Wijs, *Z. Untersuch. Nahr. Genussm.*, *1*, 561 (1898).

³³² J. Hanus, *Z. Untersuch. Nahr. Genussm.*, *4*, 913-920 (1901).

The Hanus reagent is also more stable. Bull³³³ states that, while the Wijs reagent lasts only one month, the Hanus solution remains satisfactory for at least one year.

Both the Hanus and the Wijs methods are in current use in the United States, but the latter procedure is employed much more in Great Britain and Europe. The results are 2 to 4% lower with the Hanus procedure than with the Wijs technic; Lewkowitsch³³⁴ states that the higher results are the correct ones. The discrepancies between the two procedures are especially marked where the oils have high iodine values. This is especially true with drying oils such as tung oil. Possibly the Wijs reagent brings

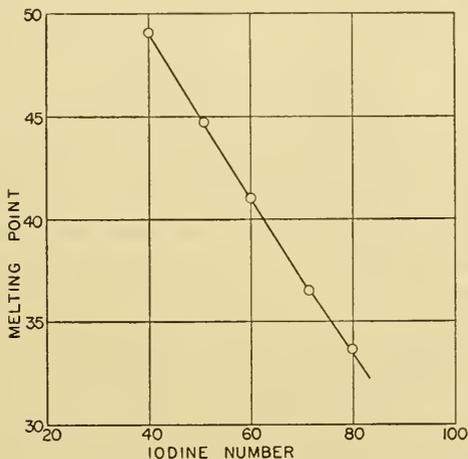


Fig. 6. The relationship between the melting point and the iodine number of cottonseed oil during hydrogenation.³³³

about a better addition of iodine to the conjugated double bonds present in the elaeostearic acid glycerides of tung oil. However, sterols will cause the results to be abnormally high when the Wijs procedure is used.

The Association of Official Agricultural Chemists has adopted the Hanus method as the official one. On the other hand, the Committee on Analysis of Commercial Fats and Oils of the Division of Industrial Chemists and Chemical Engineers of the American Chemical Society has accepted the Wijs procedure as the standard.³³⁵ A comparison of results obtained by the Hübl, Wijs, and Hanus methods has been reported by Hunt,³³⁶ as well as by Tolman and Munson.³³⁷

³³³ H. B. Bull, *The Biochemistry of the Lipids*, Burgess, Minneapolis, 1936, p. 87.

³³⁴ J. I. Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, and Waxes*, 6th ed., Vol. III, Macmillan, London, 1938 (reprint, original ed., 1921-1923).

³³⁵ "Standard Methods for the Sampling and Analysis of Commercial Fats and Oils," *Ind. Eng. Chem.*, 11, 1161-1168 (1919).

³³⁶ F. W. Hunt, *J. Soc. Chem. Ind.*, 21, 454-456 (1902).

³³⁷ L. M. Tolman and L. S. Munson, *J. Am. Chem. Soc.*, 25, 244-251 (1903).

The iodine numbers of the so-called drying oils exceed 130. Although the common figure for linseed oil approximates 180, extreme variations of 123 to 209 have been reported.^{306-309,338,339} The iodine values for many edible oils such as corn, cottonseed, peanut, and soybean oils³⁰⁹ are in the neighborhood of 100 (95-125). The latter fats are ordinarily not considered as drying oils, although soybean oil has found considerable application in the paint industry in recent years. On the other hand, the iodine number of coconut oil (8.4-8.8) approaches zero.

The relationship of the iodine number to the index of refraction has already been mentioned. The change in this constant which occurs during hydrogenation of cottonseed oil is given in Figure 6.

(7) Thiocyanogen Number

The thiocyanogen number is the amount of thiocyanogen, expressed as grams of iodine, absorbed by one hundred grams of fat or oil. This determination offers a useful adjunct in establishing the nature of the unsaturated fatty acids in a fat or oil. Kaufmann^{340,341} alone, and associated with Keller,³⁴² discovered that unsaturated fats react with thiocyanogen (CNS)₂ in a reproducible manner. However, instead of combining with all unsaturated linkages as is the case with iodine, thiocyanogen reacts with only 1 of the 2 unsaturated linkages in linoleic acid, and with 2 of the 3 double bonds in linolenic acid. The reaction between thiocyanogen and oleic acid or triolein is quantitative.^{343,344} Riemenschneider, Swift, and Sando³⁴⁵ have reported the thiocyanogen values of oleic acid, linoleic acid, and linolenic acid as 89.4, 93.9, and 162.0, respectively, when a 0.1 *N* thiocyanogen solution is employed. The sensitivity of the reaction to the experimental conditions which had been observed earlier^{346,347} is shown by the fact that the results on the 3 methyl esters tested were found to be 85.3, 92.7, and 159.6, respectively, when the thiocyanogen solution used had a concentration³⁴⁵ of 0.2 *N*. When used in connection with the iodine value, the thiocyanogen value can be used to estimate the quantity of each of the

³³⁸ J. B. McNair, *Botan. Rev.*, 11, 1-59 (1945).

³³⁹ S. L. Ivanov, *Biol. Generalis*, 5, 579-586 (1929).

³⁴⁰ H. P. Kaufmann, *Z. Unters. Lebensm.*, 51, 15-27 (1926).

³⁴¹ H. P. Kaufmann, *Analyst*, 51, 264-265 (1926).

³⁴² H. P. Kaufmann and M. Keller, *Analyst*, 54, 304 (1929).

³⁴³ D. H. Wheeler, R. W. Riemenschneider, and C. E. Sando, *J. Biol. Chem.*, 132, 687-699 (1940).

³⁴⁴ L. Zeleny and C. H. Bailey, *Ind. Eng. Chem.*, 24, 109-110 (1932).

³⁴⁵ R. W. Riemenschneider, C. E. Swift, and C. E. Sando, *Oil & Soap*, 18, 203-206 (1941).

³⁴⁶ P. J. Gay, *J. Soc. Chem. Ind.*, 51, 126-129T (1932).

³⁴⁷ J. P. Kass, W. O. Lundberg, and G. O. Burr, *Oil & Soap*, 17, 50-53 (1940).

types of unsaturated acids. The methods for performing the test, as well as the procedure for the calculation, are described elsewhere.³⁴⁸⁻³⁵⁰

(8) Reichert-Meissl Number

The Reichert-Meissl number is usually defined as the *number of milliliters of 0.1 N alkali required to neutralize the soluble volatile fatty acids distilled from five grams of fat*. In order to give the greatest amount of information, this constant should be considered in connection with the Polenske number, and also in some cases with the Kirschner value.

The Reichert-Meissl number is of especial value in the characterization of butter fat. This is the only edible fat containing appreciable amounts of volatile soluble fatty acids, although coconut oil contains a large proportion of volatile insoluble fatty acids. According to Jensen³⁵¹ the Reichert-Meissl value accounts for 85 to 88% of the butyric acid, 85 to 100% of the caproic acid, and 24 to 25% of the caprylic acid present in butter fat. The total volatile fatty acids in this product amount to 10 to 13.6% of the total acids. The failure to account for the full quota of butyric and caproic acids in the usual determination of the Reichert-Meissl value is to be traced to their incomplete removal by steam distillation. However, if certain arbitrary procedures are followed, comparable results may be obtained, although they do not necessarily give the absolute amounts of the several fractions. The low recovery of caprylic acid results, not only from the fact that all the acid cannot be removed by steam distillation, but also from the very low solubility of the acid, which precludes its determination in the Reichert-Meissl estimation.

Butyric, caproic, and to some extent caprylic acids are thus classed as the volatile fatty acids readily or slightly (caprylic) soluble in water which are included in the Reichert-Meissl figure. On the other hand, caprylic, capric, lauric, and also myristic acids belong in the category of volatile fatty acids which are almost incompletely soluble in water (with the exception of caprylic) and which are the basis of the value referred to as the Polenske number.

The Reichert-Meissl value of butter shows extreme variations between 12 and 40, but the figures for most samples fall between 24 and 34. However, slight variations in technic, such as altering the size of the sample employed, may result in markedly different results. With coconut oil,

³⁴⁸ W. H. Irwin, R. W. Bailey, T. C. Law, C. P. Long, H. J. Morrison, M. L. Sheely, L. M. Tolman, H. P. Trevithick, and J. J. Vollertsen, *Ind. Eng. Chem., Anal. Ed.*, **8**, 233-237 (1936).

³⁴⁹ Anonymous, *J. Assoc. Official Agr. Chem.*, **21**, 87-88 (1938).

³⁵⁰ G. S. Jamieson, *Vegetable Fats and Oils*, 2nd ed., Reinhold, New York, 1943, pp. 25, 395, 474.

³⁵¹ O. Jensen, *Z. Untersuch. Nahr. Genussm.*, **10**, 265-283 (1905).

the values are from 6 to 8, while in the case of other edible fats the Reichert-Meissl values are less than 1.0. However, the figures in the case of palm oil³⁰⁹ may slightly exceed 1 (0.9–1.9). Porpoise and dolphin oils give extremely high Reichert-Meissl numbers (40–90); this is to be traced to the isovaleric acid which makes up such a large proportion of the triglycerides of these marine fats. However, these are not classed as edible fats and their odor prevents their use for the adulteration of butter. Wool fat is reported³⁰⁹ as having a Reichert-Meissl value as high as 8.

The presence of animal fats or of coconut oil in butter in appreciable amounts can readily be detected by the application of this test. Lewkowitsch³³⁴ has stated that, although it is possible to prepare a fat mixture which duplicates butter insofar as refractive index, saponification value, iodine number, and melting point are concerned, such a mixture invariably falls short in simulating the Reichert-Meissl value of butter.

(9) *Polenske Number*^{352,353}

This constant is the number of milliliters of 0.1 N alkali required to neutralize the insoluble volatile fatty acid distilled from five grams of fat. Appreciable values for this constant are given only by those fats which have a sizable proportion of capric, lauric, and/or myristic acids in their triglycerides. Coconut oil gives the highest Polenske values (16.8–17.8), while palm oil is another edible fat which also gives a high figure (8.5–11.0); butter fats yield values which vary between 1.5 and 3.0. Woodman³²² indicates that the addition of coconut oil to butter fat, to the extent of 10%, will increase the Polenske number by about 1.0.

The so-called *Kirschner*^{354,355} *value* is sometimes employed to assist in the determination of the proportion of butter fat in a mixture. This value is obtained from the titrated Reichert-Meissl distillate by precipitation of the higher volatile soluble fatty acids as the silver salts and by a reestimation of the remaining soluble volatile fatty acids on the filtrate. The Kirschner values for butter fat are reported as 19 to 26, while coconut oil gives a figure of 1.9 and palm kernel oil one of 1.0. For the majority of fats and oils, the values range³²² between 0.1 and 0.2. The appreciable Kirschner values given by coconut oil and palm kernel oil, neither of which contains butyric acid, indicate that the method for separation of butyric from caproic and caprylic acids on the basis of solubility of the silver salts is not an absolute one.

(10) *Acetyl Number*

The acetyl value is the number of milligrams of potassium hydroxide required to neutralize the acetic acid liberated from one gram of acetylated fat or

³⁵² E. Polenske, *Arb. kaiserl. Gesundh.*, 20, 545–558 (1904).

³⁵³ H. Leffmann and W. Beam, *Analyst*, 16, 153 (1891).

³⁵⁴ A. Kirschner, *Z. Untersuch. Nahr. Genussm.*, 9, 65–70 (1905).

³⁵⁵ C. Revis and E. R. Bolton, *Analyst*, 36, 333–342 (1911).

oil. This constant is an index of the proportion of hydroxyl groups in a fat. When the fat under investigation is treated with acetic anhydride, an acetyl group is introduced wherever a free hydroxyl is present. After washing out of the excess acetic anhydride and the acetic acid liberated during the reaction from the acetylated fat, the latter can be dried and the acetic acid in combination set free from a weighed amount.³⁵⁶ As noted above, the weight of potassium hydroxide necessary to neutralize the acetic acid from one gram of acetylated fat is the unit in which this constant is expressed.

Although this constant is employed mainly to determine the amount of ricinoleic acid present in fats such as castor oil, it has some importance in the biological field, in view of the reputed nutritional value of some of the hydroxystearic acids. It is also of considerable value in assessing the amount of mono- and diglycerides in a mixture of fats. Any free hydroxyl groups of glycerol become acetylated in the same way as those on the fatty acid residue. By comparing the degree of acetylation of the unhydrolyzed fat with that of its component fatty acids, one obtains an index of the proportion of the glycerol combined with only one or two fatty acid residues. The acetyl value of castor oil is usually approximately 150, while that for most other fats varies from 28 to 13, with the exception of carnauba wax,³⁵⁶ for which the value is 55.

(11) *Unsaponifiable Matter*

The unsaponifiable fraction (also referred to as non-saponifiable residue or fraction, abbreviated as N.S.F.) makes up that part of the fat which cannot be changed to water-soluble products by the process of saponification. It can readily be separated by ether extraction from the soap solution resulting from saponification of the fat. More precise methods for its determination are outlined by the Fat Analysis Committee of the American Chemical Society.^{335, 357}

The products which are found in this fraction consist of the higher alcohols, including the sterols, the fat-soluble vitamins, A, D, and E, such provitamins as the carotenoids, and any hydrocarbons which are present, such as paraffin or mineral oil. Resinous substances may also be included if the oil has been prepared by ether or petroleum ether extraction of food products. Another interesting hydrocarbon found to the extent of 0.41 to 0.54% in olive oil is squalene,^{358, 359} which has the empirical formula,

³⁵⁶ E. G. Mahin, *Quantitative Analysis*, 3rd ed., McGraw-Hill, New York, 1924, pp. 382, 390-391.

³⁵⁷ Anonymous, *Official and Tentative Methods of Analysis of the Association of Official and Agricultural Chemists*, 4th ed., XXXI, "Oils, Fats and Waxes," pages 420-421, 1935.

³⁵⁸ T. Thorbjarnarson and J. C. Drummond, *Analyst*, 60, 23-29 (1935).

³⁵⁹ J. Grossfeld and H. Timm, *Z. Untersuch. Lebensm.*, 77, 249-253 (1939).

$C_{30}H_{56}$. This substance occurs only in extremely small amounts in other oil (peanut, 0.07%; rapeseed, 0.05%; apricot kernel, 0.02%). The type of sterol may also be of considerable assistance in the identification of a fat. The chemical behavior of the other compounds is discussed in later sections.

In most cases the total N.S.F. amounts to less than 1%. However, the results may be somewhat higher in cod-liver oil (0.54–2.68) and corn oil (1.5–2.8), while wool fat, which consists chiefly of cholesterol esters³ and triterpenes, is composed of 39 to 44% of unsaponifiable extract.³⁰⁹

6. Physical Properties of Fats and Oils

(1) Polymorphism and Melting Point

Since the classical work of Chevreul, it has been recognized that fats exhibit a peculiarity in melting point not shown by other organic compounds. Duffy,²³⁶ in 1853, noted that stearin prepared from mutton tallow had three melting points; this inspired the suggestion that isomeric stearins exist. For some years it has been known that tristearin which has been quickly chilled will melt at 55°C., after which it resolidifies as the temperature is increased, and then remelts at 71.5°C.²⁹ Smits and Bokhorst³⁶⁰ noted that, if the chilled sample was allowed to stand for some time at room temperature, it spontaneously changed to the higher melting modification. These workers attributed the existence of two melting points in the triglyceride to the presence of two different crystalline modifications which are interchangeable. That more polymorphic forms may exist in the case of mixed triglycerides is shown by the fact that β -lauro- α, α' -distearin is reported by various workers to have such widely divergent melting points as 36°, 52.5°, 56.5°, 58°, 59.8°, 60.5°, and 68.5°C.

The explanation for these results has become clearer as a result of the studies of Malkin and his collaborators.^{361–365} These workers have demonstrated that simple triglycerides can exist in three forms (γ , α , and β), while an additional modification (β') exists in the case of the mixed triglycerides and of the simple triglycerides composed of odd-chain acids. Each phase has its own melting point, so that it is necessary to know what particular modification is involved before the melting point can be used as a method for identification. These forms are to some extent interconvertible by the technic usually employed in determining the melting points. The

³⁶⁰ A. Smits and S. C. Bokhorst, *Proc. Akad. Wetenschappen, Amsterdam*, 15, 681–683 (1912); *J. Chem. Soc.*, 104, I, 157A (1913).

³⁶¹ M. G. R. Carter and T. L. Malkin, *J. Chem. Soc.*, 1939, 1518–1521.

³⁶² C. E. Clarkson and T. Malkin, *J. Chem. Soc.*, 1934, 666–671.

³⁶³ M. G. R. Carter and T. Malkin, *J. Chem. Soc.*, 1939, 577–581.

³⁶⁴ T. Malkin and M. L. Meara, *J. Chem. Soc.*, 1939, 103–108.

³⁶⁵ T. Malkin and M. L. Meara, *J. Chem. Soc.*, 1939, 1141–1144.

simplified method of Timmermans³⁶⁶ can be employed to ascertain the melting point of any phase without the sample undergoing a transformation during the course of the estimation. In the above procedure, the solid fat is placed in a thin-walled capillary tube without melting, and this is immersed in a bath of known temperature. If the sample melts, new unmelted samples are tested with baths of varying lower temperatures until the exact temperature at which melting just takes place is determined. If the sample fails to melt on the first trial, new samples are employed at each new higher temperature tested.

The different modifications of a triglyceride can be obtained by the following procedures. The most unstable form, called the glass form (or γ -modification) can be prepared by rapid cooling of the melted fat, but it cannot be produced from any of the solid forms without melting. Luton³⁶⁷ is of the opinion, however, that the amorphous glass form is an artifact and that only three polymorphic modifications exist. The α -polymorph may be formed by the slow cooling of the molten fat or by maintaining the γ -preparation near its melting point. The β -form, which is the stable modification, can be prepared, also, from the molten fat by very slow cooling, or from the α -form by maintaining it near its melting point. The fourth form, called the β' -isomer, occurs only in the case of the mixed triglycerides. It is thermodynamically unstable, and has a melting point between the α - and the stable β -compound.

That the variation in melting point is related to differences in crystalline structure can be readily demonstrated. The gross appearance of the γ -form is that of a vitreous translucent product, while the stable β -form is an opaque powdery substance. On microscopical examination with the polarizing nicol prisms, the γ -form appears as tiny uniaxial crosses which are indicative of spherulite formation. The α -form gives a similar picture except that the uniaxial crosses are much larger. When a conversion to the β -polymorph occurs, the uniaxial crosses disappear and a multitude of parallelograms of varying sizes and shapes appear.

The x-ray diffraction patterns also help to explain the divergency in properties between the different polymorphic forms. Such examinations are of value not only in establishing the polymorphic pattern which any given compound represents, but also in determining when one type of crystal has changed to another one. Although quantitative data on the x-ray spacings are not available, and thus the investigation of mixtures of phases cannot be interpreted accurately, the results of Malkin have been of great importance in establishing qualitative differences between the several forms.

In the γ -polymorph, it is believed that the hydrocarbon chains of the

³⁶⁶ J. Timmermans, *Chemical Species*, Chemical Pub. Co., New York, 1940, p. 26.

³⁶⁷ E. S. Luton, *J. Am. Chem. Soc.*, 67, 524-527 (1945).

triglyceride are probably in general alignment. Clarkson and Malkin³⁶² are of the opinion that the single diffuse band in the x-ray pattern (at 4.2 angstrom units) indicates that the long hydrocarbon chains are fixed at random degrees of rotation around the chain axis. The diffuseness of the band also suggests that the distances between the hydrocarbon chains are not very exactly defined. The lack of the long spacings in this polymorph probably indicates that the methyl and glyceryl groups at the ends of the long chains do not determine the planes. Clarkson and Malkin³⁶² have identified the γ -form with the glass polymorph.

In the α -form the hydrocarbon chains are also parallel and "frozen" around a central axis, although they are free to rotate within fixed limits. In contradistinction to the glass derivative, the sharpness of the single line which is believed to represent the side spacings indicates that the hydrocarbon chains are separated by uniform distances. These chains determine the planes, since the long spacings are present; apparently the hydrocarbon chains are perpendicular to the end-group plane because the long spacings are of considerable magnitude. Presumably, the structural unit of the crystal usually exists in a double chain length.

In the β -form of the triglycerides, the hydrocarbon chains are supposedly fixed in their position as to their relative degree of rotation around the central axis. Such a deduction is arrived at on the basis of the multiplicity of the short spacings. The other features, such as the fact that the end groups determine the plane and that the structural unit has a double plane length, are similar to those of the α -modification. However, because of the shorter long spaces in the β -series, it is believed that the hydrocarbon chains are tilted at an angle of about 65° to the end-group planes, in contradistinction to the 90° angle in the α -form. Malkin believes that this is the chief characteristic of the β -polymorph. This may well be the feature which causes the β -form to be the most stable. Malkin is of the opinion that an alternative structure, in which the three chains of a given molecule lie side by side, is probably incorrect, for mechanical reasons which appear in a three-dimensional mechanical model.

The β' -form which has been repeatedly observed with the mixed triglycerides resembles the γ - and α -forms in being thermodynamically unstable. The melting points are intermediate between the α - and the β -modifications. The hydrocarbon chains are usually intermediate between the 90° tilt of the α -polymorph and the 65° angle of the stable β -form, insofar as the relation to the end-group planes is concerned. A summary of these differences exhibited by the γ -, α -, and β -forms is included in Table 26.

Bailey and collaborators³⁶⁸ and Lutton³⁶⁷ have come to the conclusion

³⁶⁸ A. E. Bailey, M. E. Jefferson, F. B. Kreeger, and S. T. Bauer, *Oil & Soap*, 22, 10-13 (1945).

TABLE 26
CHARACTERISTICS OF POLYMORPHIC FORMS OF A TYPICAL SIMPLE TRIGLYCERIDE (TRISTEARIN)^a

Property	Polymorphic form		
	γ	α	β
Relative stability	Least	Intermediate	Most; the only thermodynamically stable form
Melting point	Lowest; non-alternating for odd and even chain	Intermediate; non-alternating	Highest; alternating
Gross appearance	Vitreous, translucent	Intermediate	Opaque, powdery
Microscopic appearance	Tiny black and white uniaxial crosses; mosaic between crossed nicols	Much larger uniaxial crosses, less brilliant	Small parallelograms; best, no nicols (can form spherulites)
X-ray evidence: Short spacings ^b	One, diffuse, 4.2 A.	One, fairly sharp, 4.2 A.	Multiple, sharp; 3.7, 3.9, 4.6 A., strong; 5.3 A., weak
Long spacings ^c	None found	Longer; 2 chain lengths (perpendicular chains)	Shorter; more than one, less than 2 chain lengths (tilted chains)
Crystal structure	Possibly glass or liquid crystalline	Possibly orthorhombic (mesomorphic?)	Monoclinic
Density	Least	Intermediate	Greatest
Heat of crystallization	44 cal./g.	Undetermined; probably intermediate	62 cal./g.
Dielectric constant	Near liquid value	Undetermined	Lowest
Obtained from: liquid other solid	Rapid cooling	Slow cooling	Very slow cooling
Existence of type form in other long-chain compounds	Apparently <i>uniquely</i> in triglycerides	From γ -form near γ melting point	From α -form near α melting point

^a R. H. Ferguson and E. S. Lutton, *Chem. Revs.*, 29, 355-384 (1941), p. 363.

^b Short spacings refer to distances of separation of hydrocarbon chains.

^c Long spacings refer to lengths of hydrocarbon chains.

and fatty acids

not in saturated fatty acids

that all three polymorphic forms of tristearin are crystalline, and that the γ - or glassy form does not exist. These polymorphic modifications are designated as follows by Lutton: α (lowest melting and identical with the α -form of Clarkson and Malkin³⁶²); β' (intermediate melting point and not observed by Clarkson and Malkin³⁶² in the case of simple triglycerides, but reported by them for mixed triglycerides); β (highest melting and stable form, identical with the product similarly designated by Clarkson and Malkin³⁶²).

a. Simple Triglycerides. The data on the melting points and the x-ray spacings of the polymorphic forms of the simple glycerides composed of the common saturated and unsaturated fatty acids are summarized in Table 27.

The melting point data on the α - and β -forms of tristearin of Clarkson and Malkin,³⁶² Lutton,³⁶⁹ and Filer and co-workers³⁷⁰ are in excellent agreement. The same is true for the data of Clarkson and Malkin³⁶² and of Filer *et al.*³⁷⁰ on trielaidin.

Alternation of melting points is observed only with the stable modification (β -form), while the values for the melting points of the less stable forms are straight-line functions (Fig. 7). On the other hand, the long x-ray spacings do not exhibit alternation with any of the crystalline modifications (Fig. 8).

The structure of the three polymorphic forms of simple triglycerides of the saturated acids apparently agrees with the "tuning fork" arrangement suggested by Malkin *et al.*,³⁶² which is illustrated in Figure 9.

This conception is likewise in harmony with the data of Lutton³⁷¹ obtained by x-ray diffraction studies. Such structures are termed DCL (double chain length), in contrast to TCL (triple chain length) which occurs with some mixed triglycerides, and QCL (quadruple chain length), which has also been noted.

Ferguson and Lutton³⁷² have confirmed the earlier finding of Wheeler *et al.*³⁴³ that triolein may exist in three polymorphic forms each with distinct melting points. The long spacings for triolein is somewhat less than for tristearin; the shortening is especially evident in the case of the α -modification, which is unusual. For this reason, Ferguson and Lutton³⁷² suggest that it must involve a tilted form.

The short spacings for the triglycerides made up of even-chain saturated acids are identical at 3.7, 3.9, 4.6, and 5.3 A., while the pattern varies slightly for those composed of odd-chain acids, in which case the spacings

³⁶⁹ E. S. Lutton, *J. Am. Chem. Soc.*, **68**, 676-679 (1946).

³⁷⁰ L. J. Filer, Jr., S. S. Sidhu, B. F. Daubert, and H. E. Longenecker, *J. Am. Chem. Soc.*, **68**, 167-171 (1946).

³⁷¹ E. S. Lutton, *J. Am. Chem. Soc.*, **70**, 248-254 (1948).

³⁷² R. H. Ferguson and E. S. Lutton, *J. Am. Chem. Soc.*, **69**, 1445-1448 (1947).

TABLE 27
MELTING POINTS AND X-RAY SPACINGS FOR POLYMORPHIC FORMS OF SIMPLE TRIGLYCERIDES

Triglycerides	Number of C atoms	Melting point, °C.		Long spacings, Å.			Short spacings of β -form, Å. ^e
		α	β^f	α	β'	β	
Saturated							
Tricaproin ^a	6	—	—	—	—	—	—
Tricaprylin ^b	8	—	—	9.8-10.1 ^c	—	—	—
Trimonolin ^d	9	—	—	8.7 ^e	—	—	—
Tricaprin ^e	10	18	—	31.5	—	—	—
Triundecylin ^e	11	1	—	30.5	—	26.8	3.7, 3.9, 4.6, 5.3
Triaurin ^f	14	26.5	—	43.9	—	29.6	—
Tritridecylin ^e	13	41	34	44.0	37.7	31.15	3.7, 3.9, 4.6, 5.3
Trimyrstin ^f	14	32	44	55.5	41.4	34.1	3.65, 4.0, 4.6, 5.3
Tripentadecylin ^e	15	51.5	—	54.0	42.9	35.45	3.7, 3.9, 4.6, 5.3
Tripalmitin ^f	16	44	55.5	65.5	46.3	38.9	3.65, 4.0, 4.6, 5.3
Trimargarin ^e	17	61	—	63.5	48.5	40.9	3.7, 3.9, 4.6, 5.3
Tristearin ^f	18	54	64	73.1	50.6	43.5	3.65, 4.0, 4.6, 5.3
Trinonadecylin ^g	19	60	66.8	70.5	—	45.15	3.7, 3.9, 4.6, 5.3
Unsaturated							
Triolein (cis) ^h	18	—32	-12	4.9	45.2 ⁱ	43.3	3.71, 3.84, 3.97, 5.28 ^k
Trielaidin (trans) ^h	18	15.5	—	42	—	44.1	3.75, 3.95, 4.95, 5.35 ^k
Trilinolein ^h	18	-43	—	-12.9	—	—	—
Trilinolenin ^l	18	—	—	-23 ^j	—	—	—
Traerucin (cis) ^j	22	6	25	30	—	51.1	3.70, 3.84, 4.03, 4.60, 5.24 ^k
Tribrassidin (trans) ^j	22	43	—	59	59.3	53.6	3.75, 3.95, 4.6, 5.35 ^k

^a A. Eisenstein, *Chem. Umschau*, 27, 3-4 (1920); *Chem. Abstr.*, 14, 1050 (1920).

^b E. B. Hershberg, *J. Am. Chem. Soc.*, 61, 3587-3588 (1939).

^c Freezing point.

^d P. E. Verksade, J. van der Lee, and W. Meerburg, *Rec. trav. chim.*, 51, 850-852 (1932).

^e C. E. Clarkson and T. Malkin, *J. Chem. Soc.*, 1934, 660-671.

^f E. S. Lutton, *J. Am. Chem. Soc.*, 67, 524-527 (1945).

^g D. W. Woolley and R. B. Sandin, *J. Am. Chem. Soc.*, 57, 1078-1079 (1935).

^h D. H. Wheeler, R. W. Riemschneider, and C. E. Sando, *J. Biol. Chem.*, 132, 687-699 (1940).

ⁱ R. H. Ferguson and E. S. Lutton, *J. Am. Chem. Soc.*, 69, 1445-1448 (1947).

^j M. G. R. Carler and T. Malkin, *J. Chem. Soc.*, 1947, 554-558.

^k R. H. Ferguson and E. S. Lutton, *Chem. Revs.*, 29, 355-384 (1941), p. 371.

^l B. F. Daubert and H. R. Baldwin, *J. Am. Chem. Soc.*, 66, 997-1000 (1944).

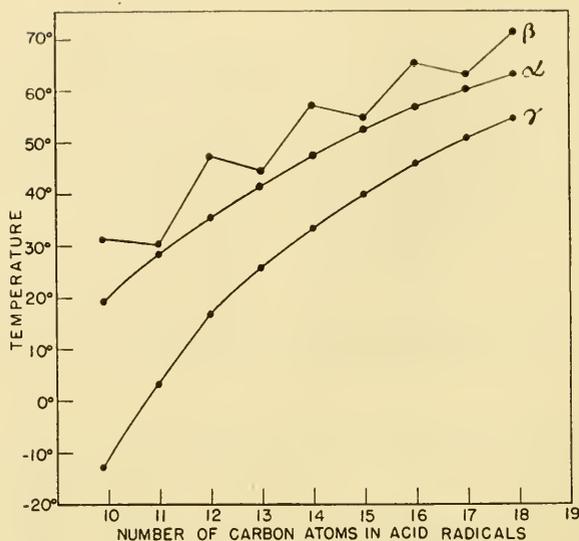


Fig. 7. The melting points of the polymorphic forms of the simple glycerides.³⁶²

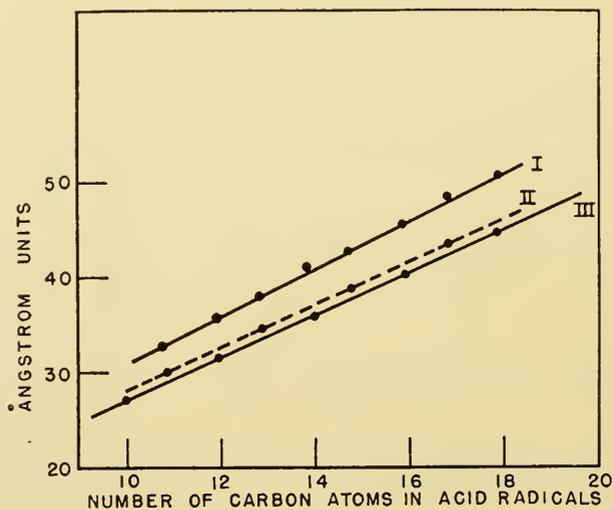


Fig. 8. The long x-ray spacings of the β (I)-, α (II)-, and γ (III)-forms of triglycerides.³⁶²

are at 3.65, 4.0, 4.6, and 5.3 Å. Considerable variations obtain in the short spacings of the triglycerides of the unsaturated acids.

b. Symmetrical Mixed Triglycerides. The pioneer work on the symmetrical mixed triglycerides was carried out by Malkin and Meara,^{364,365} who conducted a comprehensive study of 20 different synthetic compounds.

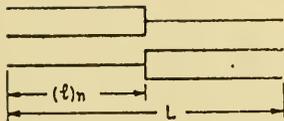


Fig. 9. The double chain length structures or "tuning fork" type of configuration for simple triglycerides proposed by Malkin *et al.*³⁶²

These triglycerides were found to crystallize in four different forms: γ , α , β' , and β . The γ -polymorphs have the lowest melting points and the β -forms melt at the highest temperatures. The symmetrical mixed triglycerides undergo transformation from one modification to another much more readily than is the case with the simple triglycerides. Consequently, data on melting points obtained by the capillary tube method may be unsatisfactory. Therefore, heating and cooling curves are employed by preference.³⁶² The symmetrical mixed triglycerides exhibit spherulite formation, but their appearance differs from that of the simple triglycerides in showing a striking rippled effect which can be seen microscopically.

A close relationship in x-ray diffraction pattern has been shown to exist between the several members of the groups when they are arranged according to differences in chain length of the component acids. The following groups may be listed which are based upon the difference in length of the single component acid compared with the 2-component acid (expressed in number of carbons): 1, 2 less; 2, 2 more; 3, 4 less; 4, 4 more; 5, 6 less; 6, 6 more; 7, 8 less; 8, 8 more.

When the chain lengths of the two acids vary by only two carbons (Groups 1 and 2), the length of the unit cell is twice that of the longer fatty acid molecule. The symmetrical mixed triglyceride has the tuning fork arrangement of the simple triglycerides. Adjacent molecules are packed side-by-side in reversed positions, which accounts for the length being twice that of the longer chain acid. The stable β -modification in Group 1 has the chain tilted at an angle of $69^{\circ}33'$, while in Group 2 the inclination amounts to only $65^{\circ}30'$. Carbon-to-carbon distances also vary, being 1.18 and 1.147 Å., respectively, for the two groups. The greater value for the long spacings in the α - and β' -form of Group 1 is attributed to the fact that they possess vertical chains. In the case of Group 2, the α -polymorph likewise possesses vertical chains, while in the β' type they are inclined at an angle of $70^{\circ}55'$. All members in Group 1 except caprodilaurin have been shown to solidify as the γ -form, while the mixed triglycerides in Group 2 (except steardipalmitin) crystallize in the α -modification. The β -type of polymorphs may be obtained directly by solvent crystallization. The corresponding members of Groups 1 and 2, *i.e.*, caprodilaurin and laurodicaprin, possess essentially similar melting points in each of their polymorphic forms.

In the remaining six groups, in which the chain lengths of the component acids differ from each other by 4, 6, or 8 carbon atoms, the triglycerides

TABLE 28
MELTING POINTS AND X-RAY SPACINGS FOR POLYMORPHIC FORMS OF SYMMETRICAL MIXED TRIGLYCERIDES^a

2-Acyl- diglyceride	Group No.	C atoms in single vs. 2-acid component	Melting point, °C.			Long spacings, Å.			Refractive indices (n_D^{20}) b, c, d
			γ	α	β	β'	α	β'	
Capryldilaurin.....	1	-2	8	23	33	38.5	—	30.0	1.43706
Laurodimyristin.....	1	-2	24	35	45	50	36.7	34.7	1.43901
Myristodipalmitin.....	1	-2	37	46	55	60	42.4	39.0	—
Palmitodistearin.....	1	-2	50	56	64	68	47.5	44.2	1.44374
Laurodicaprin.....	2	+2	6	25	34	37.5	—	29.0	—
Myristodilaurin.....	2	+2	24	37	44	48	34.5	33.6	1.43907
Palmitodimyristin.....	2	+2	38	49	55	58.5	39.7	38.1	—
Stearodipalmitin.....	2	+2	49	59	65	68	44.7	43.2	1.44325
Capryldimyristin.....	3	-4	16	37	43.5	40.0	—	52.5	—
Laurodipalmitin.....	3	-4	34	47	53.5	50.0	77.0	59.0	1.44044
Myristodistearin.....	3	-4	47	56	62.5	59.0	44.7	65.8	1.44300
Myristodicaprin.....	4	+4	3	21	34	30	30.3	46.5	—
Palmitodilaurin.....	4	+4	19	35	45.5	42.5	36.6	35.5	1.43980
Stearodimyristin.....	4	+4	33	47	55.5	53.0	41.0	40.0	—
Capryldipalmitin.....	5	-6	20	42	51.5	48.0	74.0	56.5	—
Laurodistearin.....	5	-6	36	52	60.5	58.0	42.4	63.7	1.44222
Palmitodicaprin.....	6	+6	6	27	40	36	—	49.5	—
Stearodilaurin.....	6	+6	21	38	47	43	37.5	56.8	1.44031
Capryldistearin.....	7	-8	30	47	57	53	76.3	61.2	1.44153
Stearodicaprin.....	8	+8	5	34	44.5	40.0	—	51.6	—

^a T. P. Mallin and M. L. Meara, *J. Chem. Soc.*, 1939, 103-108, 1141-1144.

^b O. E. McElroy and C. G. King, *J. Am. Chem. Soc.*, 56, 1191-1192 (1934).

^c H. E. Robinson, J. N. Roche, and C. G. King, *J. Am. Chem. Soc.*, 54, 705-710 (1932).

^d H. P. Averill, J. N. Roche, and C. G. King, *J. Am. Chem. Soc.*, 52, 365-367 (1930).

were reported by Malkin and Meara^{364,365} to exist in four modifications. In each group, the rate of transition of the different crystal forms decreases with increasing chain length. In the presence of the shorter chain acids, a linear relation between the chain length and the long spacings usually cannot be observed for the α - or β' -forms. In the case of the β -members, the long spacings usually correspond to two molecular lengths. Strangely enough, palmitodilaurin and stearodimyristin possess a much shorter long spacing. Malkin and Meara^{364,365} attribute this discrepancy to an acute angle tilt of $41^\circ 18'$.

Data on the melting points, the long spacings, and the refractive indices are summarized in Table 28.

2-Caproyldistearin and 2-caprylyldistearin have likewise been studied and shown to melt at 47.2° and 51.8°C ., respectively.⁵⁶ The refractive indices for these two compounds are reported as 1.44019 and 1.44140, respectively, at 70°C .

Daubert and his co-workers have synthesized a series of 2-oleo-1,3-diacyl glycerides³⁷³ which were shown to have the following melting points: dicapryl, $5\text{--}6^\circ\text{C}$.; dilauryl, $14.5\text{--}15^\circ\text{C}$.; dimyristyl, $26\text{--}27^\circ\text{C}$.; dipalmityl, $35.5\text{--}36^\circ\text{C}$.; and distearyl, $42.5\text{--}43^\circ\text{C}$. The usual four polymorphic forms were reported by Daubert and Clarke,³⁷⁴ and their transformation temperatures were determined. The 2-oleo compounds showed crystal spacings approximately three times the length of the longer acid.^{92,369,370,375} This fact precludes the tuning fork arrangement originally noted with the completely saturated symmetrical triglycerides and the simple triglycerides. 2-Oleodistearin, isolated from kokum butter, has been shown to be identical with the synthetic product of Filer *et al.*³⁷⁰ as regards both the thermal and the x-ray data on the 3-polymorphic forms. Lutton³⁶⁹ reported melting points of 22.4 , 36.2 , and 44.3°C . for the α , $\alpha\text{-}3$, and β_3 forms. The intermediate compound possesses a pattern not hitherto encountered.

The symmetrical triglycerides can be assumed to have a modified tuning fork arrangement. The projected and tilt forms of this structure for the double chain length structures is shown in Figure 10 and for the triple chain length structures in Figure 11. A special case of the TCL structure as applied to symmetrical oleodistearin is given in Figure 12. A new "chair" arrangement for unsymmetrical diacid triglycerides recently proposed by Lutton³⁷¹ is pictured in Figure 13.

In several instances, the β' -forms of the symmetrical mixed triglycerides of $C_nC_{n-4}C_n$, $C_nC_{n-6}C_n$ and mixed $C_{10}\text{-}C_8$, were shown to have long x-ray

³⁷³ F. L. Jackson, B. F. Daubert, C. G. King, and H. E. Longenecker, *J. Am. Chem. Soc.*, **66**, 289-290 (1944).

³⁷⁴ B. F. Daubert and T. H. Clarke, *J. Am. Chem. Soc.*, **66**, 690-691 (1944).

³⁷⁵ S. S. Sidhu and B. F. Daubert, *J. Am. Chem. Soc.*, **69**, 1451-1453 (1947).

patterns indicative of a chain length equivalent to that of four fatty acids. Lutton³⁷¹ has no explanation for this interesting phenomenon.

A comprehensive study has recently been reported by Lutton, Jackson, and Quimby³⁷⁶ of the various mixed triglycerides of palmitic (P) and stearic

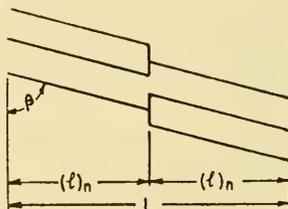


Fig. 10. The tilt form of symmetrical diacid triglycerides composed of saturated acids having a "tuning fork" arrangement and a double chain length structure.³⁷¹

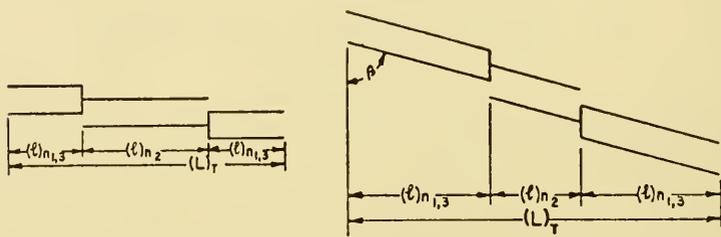


Fig. 11. The projected and tilt forms of symmetrical diacid triglycerides composed of saturated acids having a "tuning-fork" arrangement and a triple chain length structure.³⁷¹

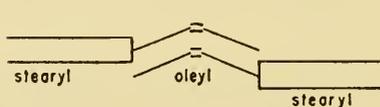


Fig. 12. The projected form of symmetrical oleodistearin.³⁷¹

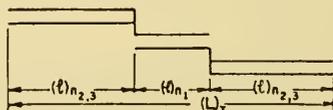


Fig. 13. The unsymmetrical or "chair" arrangement of unsymmetrical diacid triglycerides.³⁷¹

(S) acids. Marked differences were observed in the properties of the four compounds, two of which were symmetrical, *i.e.*, PSP and SPS, and two of which were unsymmetrical, namely, SPP and PSS. The results of Lutton and co-workers³⁷⁶ based on these triglycerides differed in a number of respects from the earlier report of Clarkson and Malkin.³⁶² Thus, the more

³⁷⁶ E. S. Lutton, F. L. Jackson, and O. T. Quimby, *J. Am. Chem. Soc.*, **70**, 2441-2445 (1948).

recent work reports a maximum of three polymorphic forms instead of the four noted earlier. The glassy or γ -polymorph has not been found. The symmetrical isomers showed a high degree of crystallinity, while the unsymmetrical forms were microcrystalline. The α -forms were the lowest melting in all cases; this polymorph was unusually stable in the case of 2-palmitodistearin and quite labile in the case of 2-stearodipalmitin. PSP gave rise to the β' - but not to the β -form, while SPS was found only as the β -polymorph. In the case of 1-palmitodistearin, only the β' -form obtained from the melt and the β -type from the solvent; both were equally stable. The results on 1-stearodipalmitin were normal and both the β' - and the β -crystals were stable. The melting points for a given form of triglyceride were shown to vary by several degrees, depending upon the degree of stabilization. Table 29 summarizes the data on the long spacing.

TABLE 29
LONG SPACINGS (ANGSTROM UNITS) OF MIXED TRIGLYCERIDES CONTAINING PALMITIC
AND STEARIC ACIDS

Triglyceride	Data of Lutton <i>et al.</i> ^a			Data of Carter and Malkin ^{b, c}		
	α	β'	β	α	β'	β
2-Palmito-1,3-distearin	49.2	—	43.1	50.5	47.5	44.2
2-Stearo-1,3-dipalmitin	46.65	42.75	—	50.2	44.7	43.2
1-Palmito-2,3-distearin	48.5	45.1	44.7	48.8	44.7	46.5
1-Stearo-2,3-dipalmitin	47.6	43.8	42.1	47.8	43.9	42.5

^a E. S. Lutton, F. L. Jackson, and O. T. Quimby, *J. Am. Chem. Soc.*, 70, 2441-2445 (1948).

^b T. Malkin and M. L. Meara, *J. Chem. Soc.*, 1939, 103-108.

^c M. G. R. Carter and T. Malkin, *J. Chem. Soc.*, 1939, 577-581.

Lutton,³⁷¹ in a recent study of saturated diacid triglycerides, has demonstrated that the α -forms, in all cases, and the β' -forms had the double chain length structure (DCL). On the other hand, where one of the acids was four or more carbons shorter than the second acid component, a triple length structure (TCL) was invariably found for the β -polymorph. This phenomenon was reported for the following saturated mixed triglycerides: $C_nC_{n-4}C_n$; $C_nC_{n-6}C_n$; $C_{n-6}C_nC_{n-6}$; and mixed $C_{10}-C_8$. It is also probable that the TCL is the form for $C_{n-4}C_nC_{n-4}$.

c. Unsymmetrical Mixed Triglycerides. A large number of the 1-acyl-2,3-diglycerides have been synthesized by Carter and Malkin.^{361, 363} This series of triglycerides was shown to exist in the usual four common polymorphic forms. The transition from one type of polymorph to another proceeds more sluggishly than is the case with their counterparts in the symmetrical mixed triglyceride series. The change from β' to β takes place quite slowly. The melting points of the unsymmetrical mixed triglycerides are in general slightly lower than those of their symmetrical isomers. The

long crystal spacings and the side spacings of the β -form as demonstrated by the x-ray diffraction patterns show close similarity, in most cases, to those of the simple triglycerides. A summary of the data on melting points, long spacings, and refractive indices is included in Table 30.

As is the case with the symmetrical diacid triglycerides, all the α - and β' -modifications (with one possible exception) have the double chain length structure. However, the triple chain length pattern obtains in a number of the β -compounds where the chain lengths of the 2-acid components differ by 4 or more carbon atoms. These include $C_nC_{n-4}C_{n-4}$, $C_{n-6}C_nC_n$, and $C_nC_{n-6}C_{n-6}$.

Daubert, Fricke, and Longenecker³⁷⁷ have succeeded in overcoming the difficulties inherent in the synthesis of unsymmetrical mixed triglycerides with an unsaturated acid component. The monooleoglycerides were found to have melting points approximately 35°C. lower than the corresponding saturated compounds; the 1-elaido-2,3-diacyl glycerides melt approximately 15° higher than the corresponding oleo compounds, but 20° lower than the stearo derivatives. Some of the data on these are summarized in Table 31.

The introduction of a linoleic acid residue in place of the oleo group brings about a further lowering in melting points of the several derivatives. The series of 1-acyl-2,3-dioleins studied possessed somewhat lower melting points than the 1-oleo derivatives, while the dilinolein series was found to have the lowest melting points of any of the samples investigated.

d. Triacid Triglycerides. A considerable number of triglycerides containing three separate acids are known to occur naturally. An extensive series has also recently been synthesized by Chen and Daubert,³⁷⁸ using a reaction first described by Verkade and van der Lee²⁴ in which a 1-monoglyceride is first converted to 1-acyl-3-tritylglycerol. A second different acid residue can be placed in the β -position; on hydrolysis, a 1,3-diglyceride results, into which a third acid can then be introduced into position 2. If the trityl group is removed from the 1,2-diacyl-3-tritylglycerol by hydrogenolysis, the 1,2-diglyceride results, instead of the 1,3-isomer, and the third acid residue can then be directed into position 3.^{379,380}

The triacid glycerides exist in the usual three forms (α , β' , β). In some cases, the β' -variety with the intermediate melting point has been shown to have the greatest stability. Frequently, the β' -form results on crystallization of the triglyceride from solvents, instead of the β -variety of crystals which are usually formed under such circumstances. Sidhu and

³⁷⁷ B. F. Daubert, H. H. Fricke, and H. E. Longenecker, *J. Am. Chem. Soc.*, **65**, 2142-2144 (1943).

³⁷⁸ C. Chen and B. F. Daubert, *J. Am. Chem. Soc.*, **67**, 1256-1258 (1945).

³⁷⁹ P. E. Verkade, F. D. Tollenaar, and T. A. P. Posthumus, *Rec. trav. chim.*, **61**, 373-382 (1942); *Chem. Zentr.*, 1942, II, 1339.

³⁸⁰ P. Verkade, *Rec. trav. chim.*, **62**, 393-397 (1943).

TABLE 30
MELTING POINTS AND X-RAY SPACINGS FOR POLYMORPHIC FORMS OF UNSYMMETRICAL MIXED TRIGLYCERIDES^a

1-Acyl- diglyceride	Group No.	C atoms in single rs. 2-acid component	Melting point, °C.			Long spacings, Å.			Refractive indices (n_D^{20}) ^b	
			γ	α	β'	β	α	β'		β
Capryldilaurin.....	1	-2	5	26	31	35.5	—	30.4	31.8	1.43705
Laurodimyristin.....	1	-2	22	37	42	46.5	—	35.3	36.5	—
Myristodipalmitin.....	1	-2	36	47.5	52	57	43.9	40.3	41.5	—
Palmitodistearin.....	1	-2	50	57	61	65	48.8	44.7	46.5	—
Laurodicaprin.....	2	+2	0	17.5	26	30	—	—	28.4	—
Myristodilaurin.....	2	+2	19	33.5	39	43.5	—	34.5	33.0	1.43878
Palmitodimyristin.....	2	+2	34	45.5	50.5	54	42.8	39.5	37.7	—
Stearodipalmitin.....	2	+2	46.5	55	59.5	62.5	47.8	43.9	42.5	—
Capryldimyristin.....	3	-4	15	32	38	43.5	—	33.8	35.2	—
Laurodipalmitin.....	3	-4	32	45	49.5	54	43.4	38.5	39.8	—
Myristodistearin.....	3	-4	44	54	57.5	62	48.5	43.4	45.0	—
Myristodicaprin.....	4	+4	3	20	31	34.5	—	31.3	47.5	—
Palmitodilaurin.....	4	+4	20	33	43	46.5	—	36.2	54.6	1.43965
Stearodimyristin.....	4	+4	36	46	52	56	46.4	41.7	61.4	—
Capryldipalmitin.....	5	-6	23	37	41	45.5	—	74.1	56.2	—
Laurodistearin.....	5	-6	36	47	52	—	47.4	42.8	—	—
Palmitodicaprin.....	6	+6	2	24	32	35	—	—	49.7	—
Stearodilaurin.....	6	+6	20	31	41.5	45	—	38.7	57.0	—
Capryldistearin.....	7	-8	33	42.5	46	49	73.7	—	60.0	1.44058
Stearodicaprin.....	8	+8	13	32	38	41	—	51.0	52.6	—

^a Data of M. G. R. Carter and T. Malkin, *J. Chem. Soc.*, 1939, 577-581, 1518-1521.

^b A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948.

TABLE 31
MELTING POINTS AND REFRACTIVE INDICES FOR SOME UNSYMMETRICAL MIXED TRIGLYCERIDES CONTAINING AN UNSATURATED ACID COMPONENT

Mixed triglyceride	Melting point, °C.	Refractive index (n_D^{20})	Mixed triglyceride	Melting point, °C.	Refractive index (n_D^{20})
1-Oleyl-2,3- ^a					
dicaprin.....	3-4.....	1.45185	dicaprylin.....	3.0.....	1.44786
dilaurin.....	20.0.....	1.45322	dicaprin.....	15.0.....	1.44895
dimyristin.....	25.0.....	1.45458	dilaurin.....	27.0.....	1.45023
dipalmitin.....	34.5.....	1.45562	dimyristin.....	39.5.....	1.45136
distearin.....	38.5.....	1.45700	1-Acyl-2,3-diolamins ^b		
1-Acyl-2,3-dioleins ^c					
Capryl.....	-11.0 to -10.0.....	1.46114 ^d	1-Acyl-2,3-dilaidins ^b		
Caprylyl.....	-6.6 to -5.6.....	1.45998	Capryl.....		
Capryl.....	-0.5 to 0.5.....	1.45941	Laural.....		
Laural.....	5.5-6.5.....	1.45932	Myristal.....		
Myristal.....	12.5-13.5.....	1.45995	Palmital.....		
Palmital.....	18.0-19.0.....	1.46060	Stearal.....		
Stearal.....	22.5-23.5.....	1.46190	1-Acyl-2,3-dilinoleins ^e		
1-Linoleyl-2,3- ^e					
dicaprylin.....	-13 to -12.....	1.45183 ^f	Laural.....		
dicaprin.....	-1.0-0.0.....	1.45226	Myristal.....		
dilaurin.....	15-16.....	1.45287	Palmital.....		
dimyristin.....	20-21.....	1.45287	Stearal.....		
dipalmitin.....	26-27.....	1.45385	Lauro.....		
distearin.....	32-33.....	1.45462	Myristo.....		
1-Acyl-2,3-dilaidins ^b					
Lauro.....					
Myristo.....					
Palmito.....					
Stearo.....					

^a B. F. Daubert, H. H. Fricke, and H. E. Longenecker, *J. Am. Chem. Soc.*, 65, 2142-2144 (1943).

^b B. F. Daubert, *J. Am. Chem. Soc.*, 66, 290-292 (1944).

^c B. F. Daubert, C. J. Spiegl, and H. E. Longenecker, *J. Am. Chem. Soc.*, 65, 2144-2145 (1943).

^d $t = 35^\circ\text{C}$. in this series.

^e B. F. Daubert and A. R. Baldwin, *J. Am. Chem. Soc.*, 66, 1507-1509 (1944).

^f $t = 50^\circ\text{C}$. in this series.

Daubert³⁷⁵ have shown that this phenomenon obtains with synthetic 1-myristo-2-lauro-3-caprin.

However, some triacid glycerides correspond with the simple triglycerides in having double chain length structure. This is true when the acids vary from each other by 2 carbons as, for example, 1-stearo-2-palmito-3-myristin, 1-palmito-2-myristo-3-laurin, and 1-myristo-2-lauro-3-caprin.³⁷⁵ On the other hand, according to Lutton,³⁷¹ the β -forms of other triacid glycerides have a triple chain length structure. Even the isomeric forms of the compounds prepared by Sidhu and Daubert, namely, $C_{18}C_{14}C_{16}$ and $C_{16}C_{12}C_{14}$, have been shown to exist in TCL structure.

Sidhu and Daubert³⁷⁵ have reported the following constants on the triacid glycerides synthesized by them: 1-stearo-2-palmito-3-myristin, long spacing 40.7 A., transition temperatures, 43.5–44°, 54°, and 59°C.; 1-palmito-2-myristo-3-laurin, 35.7 A., 36–37°, 44°, and 48.5–49°C., respectively, and 1-myristo-2-lauro-3-caprin, 33.4 A., 22°, 33–34°, and 36.5–37°C., respectively.

e. Diglycerides. A large number of diglycerides have been prepared in which the 2-acid components are the same. Two types of such diglycerides are recognized, namely, *symmetrical* (α , α' or 1,3-) and *unsymmetrical* (α , β or 1,2-). Many of the diglycerides described in the earlier literature as unsymmetrical were in reality the symmetrical isomers, due to the ease in the shift of the acyl group from the 2- to the 3-position during the removal of the protective grouping from the 3-position.

In addition to those diglycerides containing a single fatty acid component, considerable information has recently become available on diglycerides which contain two different fatty acid components, as, for example, 1-myristo-3-palmitin or 1-lauro-2-stearin. In both cases (symmetrical and unsymmetrical diglycerides), carbon atom 2 (β) is asymmetric, which is a prerequisite for the existence of the diglycerides in enantiomorphic forms (see discussion under optical activity of fats).

(a) *Symmetrical Diglycerides.* Malkin and his co-workers³⁸¹ have reported thermal data and x-ray diffraction patterns on the symmetrical diglycerides from 1,3-dicaprin to 1,3-distearin. A number of such compounds have likewise been synthesized by Averill, Roche, and King.²⁶

Grün and Theimer²⁰ reported, as early as 1907, that symmetrical diglycerides exhibit the phenomenon of two melting points. This finding has since been confirmed and extended by the demonstration that, in many cases, three polymorphic forms exist.^{26,381} The x-ray studies seem to indicate that the diglycerides are built up of double molecules with two acid chains lying parallel on the same side of the molecule and the two free hydroxyl groups facing each other as indicated in Figure 14.

³⁸¹ T. Malkin, M. R. el Shurbagy, and M. L. Meara, *J. Chem. Soc.*, 1937, 1409–1413.

TABLE 32
MELTING POINTS, LONG SPACINGS, AND REFRACTIVE INDICES OF SYMMETRICAL DIGLYCERIDES COMPOSED OF A SINGLE ACID

Diacyl diglycerides	Melting points, °C.	Melting points, °C. ^c			Long spacings, Å.			Refractive index (n_D^t)
		α	β'	β	α	β'	β	
1,3-Dicaprin.....	—	37	42	44.5	—	—	—	—
1,3-Diundecanoin.....	—	43.5	47	49	—	—	—	—
1,3-Dilaurin.....	56.6 ^a	49.5	54	56.5	—	—	—	—
1,3-Ditridecanoin.....	—	54.5	57	59.5	—	—	—	—
1,3-Dimyristin.....	63.8-64.4 ^a	60	63	65.5	—	—	—	—
1,3-Dipentadecanoin.....	—	63.5	65.5	68.5	—	—	—	—
1,3-Dipalmitin.....	69.5 ^a	68	—	72.5	—	—	—	—
1,3-Dimargarin.....	—	71.5	—	74.5	—	—	—	—
1,3-Distearin.....	79.1 ^b	74	—	78	—	—	—	—
1,3-Diarachidin.....	75 ^d	—	—	75 ^d	—	—	—	—
1,3-Diolein.....	25 ^e	—	—	21.5 ^f	—	—	39.7 ^g ; 39.3 ^f	1.44690 ^{7b}
1,3-Dilinolein.....	—	—	—	—	—	—	45.2 ^f	1.46416 ^{3a}
1,3-Dilinolenin.....	—	—	—	—	—	—	40.3	1.47610 ^{3a}
1,3-Dielaidin.....	—	49 ^e	53 ^e	55 ^e	—	—	49.8 ^e	—
1,3-Dierucin.....	47 ^g	41 ^e	44.5 ^e	46.5 ^e	—	—	47.8 ^e	—
1,3-Dibrassinin.....	65 ^g	63.5 ^e	66.5 ^e	68.5 ^e	—	—	58.8 ^e	—
							63.1 ^e	1.48781 ^{3a}

^a H. P. Averill, J. N. Roche, and C. G. King, *J. Am. Chem. Soc.*, **51**, 866-872 (1929).

^b B. F. Daubert and H. E. Longenecker, *J. Am. Chem. Soc.*, **66**, 53-55 (1944).

^c T. Malkin, M. L. el Shurbagy, and M. L. Mears, *J. Chem. Soc.*, **1937**, 1409-1413.

^d A. W. Ralston, *Fatty Acids and Their Derivatives*, 2nd ed., Wiley, New York, 1948, p. 560.

^e M. G. R. Carter and T. Malkin, *J. Chem. Soc.*, **1947**, 554-558.

^f B. F. Daubert and E. S. Lutton, *J. Am. Chem. Soc.*, **69**, 1449-1451 (1947).

^g C. L. Reimer and W. Will, *Ber.*, **19**, 3322-3327 (1886).



Fig. 14. The molecular structure of symmetrical diglycerides as postulated by Malkin *et al.*³⁸¹

Data on the melting points and x-ray spacings are summarized in Table 32.

Baur, Jackson, Kolp, and Lutton³⁸² have recently made a reinvestigation of the saturated 1,3-diglycerides from dilaurin through distearin. They disagree with Malkin *et al.*³⁸¹ as to the presence of a polymorphic α -form.

TABLE 33
LONG SPACINGS, TRANSITION TEMPERATURES, AND REFRACTIVE INDICES OF SOME
DIACID SYMMETRICAL DIGLYCERIDES

Diglyceride	Long spacing of β -form, A.	Transition temperature, °C.			Refractive index (n_D^{70})
		α	β'	β	
1-Myristo-3-caprin ^a	38.0	39.0	44.0	48.0	—
1-Palmito-3-laurin ^a	42.7	51.0	56.0	59.5	—
1-Stearo-3-myristin ^a	47.6	56.0	63.0	67.0	—
1-Lauro-3-stearin ^b	—	—	—	62.0	—
1-Myristo-3-stearin ^b	—	—	—	65.5–66.5	—
1-Palmito-3-stearin ^b	—	—	—	71.0–71.5	—
1-Lauro-3-olein ^b	—	—	—	32.0	1.44335
1-Myristo-3-olein ^b	—	—	—	41.0	1.44455
1-Palmito-3-olein ^b	—	—	—	46.0	1.44574
1-Stearo-3-olein ^b	—	—	—	54.0	1.44690

^a S. S. Sidhu and B. F. Daubert, *J. Am. Chem. Soc.*, 68, 2603–2605 (1946).

^b B. F. Daubert and H. E. Longenecker, *J. Am. Chem. Soc.*, 66, 53–55 (1944).

Judging on the basis of the strong 4.6 A. short spacings, only two varieties of crystals occur, both of which are believed to be of the β -type. In conformity with the suggestion of Malkin these are referred to as β -a and β -b. The long spacings for β -b exceed those of β -a by about 2 A. β -a is invariably obtained from the melted diglyceride and sometimes from solvent crystallization; it is highly stable at room temperature and changes to the β -b variety only when near its melting point. The β -b form is thermodynamically the only stable type; not only is it obtained by transformation from β -a, but also it is the form ordinarily resulting on crystallization from solvents. The melting points and long x-ray spacing data indicate that the 1,3-diglycerides are tilted at angles of $72^\circ 12'$ and $66^\circ 30'$, respectively. They exhibit spherulite structure when solidified from the melted form.

A number of symmetrical diglycerides composed of different acids have

³⁸² F. J. Baur, F. L. Jackson, D. G. Kolp, and E. S. Lutton, *J. Am. Chem. Soc.*, 71, 3363–3366 (1949).

been prepared by Sidhu and Daubert.³⁵³ These exist in double chain length as postulated by Malkin.³⁶² Three different polymorphic modifications are indicated by the transition temperature. Table 33 lists the recent data on the symmetrical diacid diglycerides.

(b) *Unsymmetrical Diglycerides*. Because of the ready shift of the acyl group from the 2 to the 3 position, the unsymmetrical (1,2) isomers are more difficult to prepare than are the symmetrical (1,3) compounds. Several 1,2-diglycerides composed of the same and of different acids which have been synthesized are listed in Table 34, together with their melting points. Sowden and Fischer³⁵⁴ have prepared the optically active forms of the 1,2-dipalmitin and 1,2-distearin.

TABLE 34
MELTING POINTS OF SOME UNSYMMETRICAL DIGLYCERIDES

Unsymmetrical diglyceride	Melting point, °C.	$[\alpha]_D$ (chloroform)
1,2-Dimyristin ^a	59.0	—
1,2-Dipalmitin ^a	64.0	—
1,2-Dipalmitin ^b	70-73	—
1,2-Distearin ^c	68.5-69	—
1-Palmityl-2-stearin ^c	60.5-61	—
1-Stearo-2-palmitin ^b	73.4	—
1-Stearo-2-palmitin ^c	68.5-69.5	—
D-1,2-Dipalmitin ^d	67-67.5	-2.3°
D-1,2-Distearin ^d	74.5-75	-2.7°

^a B. F. Daubert and C. G. King, *J. Am. Chem. Soc.*, **61**, 3328-3330 (1939).

^b P. Golendeev, *J. Gen. Chem. U. S. S. R.*, **6**, 1841-1846 (1936); *Chem. Abst.*, **31**, 4274 (1937).

^c P. E. Verkade, W. D. Cohen, and A. K. Vroege, *Rec. trav. chim.*, **59**, 1123-1140 (1940).

^d J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **63**, 3244-3248 (1941).

The melting points of the same compounds prepared by the several investigators do not show close agreement. In one case³⁴ this may have been due to confusion with the 1,3-isomer, since precautions to prevent the shift of the acyl group were not taken.

f. Monoglycerides. Two types of monoglycerides have been investigated, namely, the symmetrical (β or 2) and the unsymmetrical (α or 1). The unsymmetrical form is optically active, since the β -carbon of glycerol is asymmetrical. On the other hand, the symmetrical (β) monoglyceride is optically inactive, since no asymmetric carbon exists in the glycerol moiety. Although the α -monoglycerides are readily prepared by the method of Fischer, Bergmann, and Bärwind,³ the β -form is quite difficult to synthesize.

³⁵³ S. S. Sidhu and B. F. Daubert, *J. Am. Chem. Soc.*, **68**, 2603-2605 (1946).

³⁵⁴ J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **63**, 3244-3248 (1941).

(a) α -Monoglycerides. Fischer *et al.*³ first observed that 1-monopalmitin and 1-monostearin each occurred in two solid modifications which were shown later³⁸⁵ to have specific melting points. Rewadikar and Watson³⁸⁵ studied the polymorphism of the monoglycerides from 1-monocaprylin through 1-monostearin, without the help of x-ray diffraction data. The classical work of Malkin and el Shurbagy³⁸⁶ demonstrated the presence of three polymorphic forms: a low-melting type (α), possessing vertically rotating chains, an intermediate-melting (β') type, and a high-melting (β) variety, each characterized by tilting chains. The α -forms do not show alternation, but the β' - and β -types do exhibit this phenomenon.

Recently, Lutton and Jackson,³⁸⁷ while confirming most of the earlier data of Malkin and el Shurbagy,³⁸⁶ have come to the conclusion that an additional polymorphic form exists in the case of the 1-monoglycerides. This new form undergoes a reversible transformation to α at about 25° below the α melting point, and therefore has no characteristic melting point of its own. The α -form originates on cooling of the melted monoglyceride, and it is changed to the β -modification on heating. The β -variety is likewise obtained by slow crystallization from the solvent or by transformation from the β' -variety, but it does not originate directly from the melted monoglyceride. It is the only thermodynamically stable type. The β' -polymorph has been obtained only from the solvent.

All forms of the monoglyceride from the same acid have approximately the same long spacings and appear to be tilted double chain length structures. The short spacings for the several polymorphic forms show marked variations. The sub- α -modification has a single line at 4.15 A., with other medium lines at 3.9, 3.75, and 3.55. In the case of the α -polymorph, there is a single line at 4.15 A., and other weaker lines; the β' -type has a strong line at 4.15 A., and a medium one at 3.65 A. Finally, in the β -monoglyceride, the strongest line is at 4.55 A. Data on the melting points, long spacings, and the refractive indices for the 1-monoglycerides are included in Table 35.

Optically active L-1-monoglycerides containing the saturated fatty acids from 2 to 18 carbons (except 13, 15, and 17 carbon acids) have been prepared by Baer and H. O. L. Fischer.³⁸⁸ Only a relatively small optical rotation was observed. This is discussed further in the section on optical activity.

(b) β -Monoglycerides. Bergmann and Carter¹⁷ are credited with having prepared the first authentic sample of a β -monoglyceride. These workers synthesized 2-monopalmitin by catalytic reduction of the 1,3-benzylidene-

³⁸⁵ R. S. Rewadikar and H. E. Watson, *J. Indian Inst. Sci.*, **A13**, 128-140 (1930); *Chem. Abst.*, **25**, 613 (1931).

³⁸⁶ T. Malkin and M. R. el Shurbagy, *J. Chem. Soc.*, 1936, 1628-1634.

³⁸⁷ E. S. Lutton and F. L. Jackson, *J. Am. Chem. Soc.*, **70**, 2445-2449 (1948).

³⁸⁸ E. Baer and H. O. L. Fischer, *J. Am. Chem. Soc.*, **67**, 2031-2037 (1945).

TABLE 35
MELTING POINTS, LONG SPACINGS, AND REFRACTIVE INDICES OF SOME UNSYMMETRICAL (α) AND SYMMETRICAL (β) MONOGLYCERIDES

Monoglyceride	Melting point, °C.			β	Long spacing of β -form, Å.	Refractive index, $(n_D^t)^a$
	Sub- α transformation point	α	β'			
1-Monocaprin ^b	—	27	49	53	32.9 ^b , 32.6 ^d	—
1-Monoundecanoin ^b	—	36.5	52	56.5	35.2 ^b	—
1-Monolaurin ^b	—	44	59.5	63	37.3 ^b , 36.9 ^d	—
1-Monotridecanoin ^b	—	50	61	65	39.6 ^b	—
1-Monomyristin ^b	—	56	67.5	70.5	41.5 ^b , 41.6 ^d	—
1-Monopentadecanoin ^b	—	62	69	72	43.8 ^b	—
1-Monopalmitin ^b	34, 39 ^c	66.5, 66.9 ^c	74, 74.6 ^c	77, 77 ^c	45.8 ^b , 45.7 ^d	—
1-Monomargarin ^b	—	74.5	74.5	77	48.2 ^b	—
1-Monostearin ^b	42, 47.5, 49 ^c	74, 74 ^c	79, 78 ^c	81.5, 81.5 ^c	40, 49.9 ^d	—
1-Monoarachidin ^d	—	77	81.5	83.5	51.4 ^d	—
1-Monoolein	(12.5) ^e	25 ^e	32 ^e	35 ^e , 35.5 ^f	—	—
1-Monoelaidin	(29.5) ^e	42 ^e , 44.5 ^g	56 ^e	58.5 ^e , 58.5 ^g	—	1.46262 ⁴⁰
1-Monolinolein ^h	—	—	-22.8	12.3	—	—
1-Monolinolein ^h	—	—	-13.5	15.7	—	—
1-Monoerucin ^e	(15)	—	47	50	—	—
1-Monoerassin ^e	(37)	—	68.5	71	—	—
2-Monocaprin ⁱ	—	—	—	40.4	—	1.44045 ⁷⁰
2-Monolaurin ⁱ	—	—	—	51.1	—	1.44240 ⁷⁰
2-Monomyristin ⁱ	—	—	—	61.0	—	1.44420 ⁷⁰
2-Monopalmitin ⁱ	—	—	—	68.5, 69 ^j	—	1.44605 ⁷⁰
2-Monostearin ⁱ	—	—	—	74.4	—	1.44770 ⁷⁰

^a Superscript figures represent the values for *t*.

^b T. Malkin and M. R. el Shurbagy, *J. Chem. Soc.*, 1628-1634.

^c E. S. Lutton and F. L. Jackson, *J. Am. Chem. Soc.*, 70, 2445-2449 (1948).

^d S. S. Sidhu and B. F. Daubert, *J. Am. Chem. Soc.*, 68, 1975-1976 (1946).

^e M. G. R. Carter and T. R. Malkin, *J. Chem. Soc.*, 1947, 554-558.

^f B. F. Daubert, H. H. Fricke and H. E. Longenecker, *J. Am. Chem. Soc.*, 65, 2142-2144 (1943).

^g B. F. Daubert, *J. Am. Chem. Soc.*, 66, 290-292 (1944).

^h B. F. Daubert and A. R. Baldwin, *J. Am. Chem. Soc.*, 66, 997-1000 (1944).

ⁱ B. F. Stimmel and C. G. King, *J. Am. Chem. Soc.*, 56, 1724-1725 (1934).

^j M. Bergmann and N. M. Carter, *Z. physiol. Chem.*, 191, 211-221 (1930).

2-palmitylglycerol. The same procedure was employed later by Stimmel and King¹⁸ for the preparation of a number of other symmetrical diglycerides. Daubert¹⁹ synthesized 2-monobutyryn, which boils at 140–141°C. at 4 mm. Hg.

Although the β -monoglycerides do not rearrange to produce the α -ester on melting, such a shift takes place readily when hydrogen or hydroxyl ions are present as catalytic agents. King and associates^{6,18} have reported that β -monopalmitin completely changes to the α -isomer within 24 hours when it stands in an alcoholic solution containing 0.05 *N* hydrochloric acid or 0.1 *N* ammonium hydroxide. The aromatic monoglycerides are distinctly more stable than are the related aliphatic esters.

The melting points of the 1-monoglycerides are consistently higher than those of the 2-monoglycerides. The latter compounds are more soluble in organic solvents than are the 1-monoglycerides. Thus, symmetrical monopalmitin dissolves in alcohol at 25°C. to the extent of 4.61 g. per 100 ml., compared with a value of 4.09 g. for the unsymmetrical compound. The difference in ether is much greater, however, being 11.15 and 2.75 g. per 100 ml., respectively, for the two types of monopalmitin.⁶

Although no thermal data are available on the polymorphic forms of 2-monoglycerides, the results from x-ray diffraction studies indicate that the glycerol residues are similarly oriented in the two forms.³⁸⁹ However, the angle of tilt of the β -form is greater in the 1-monoglyceride than in the 2-monoglyceride, being 58°41' and 45°19', respectively. This produces a difference in long spacing corresponding to that of 2 carbons. The data on melting point and refractive indices for the 2-monoglycerides are summarized in Table 35.

(2) Solubility

Most of the glycerides are soluble in all of the so-called "fat solvents." These include petroleum ether, hexane, diethyl ether, chloroform, benzene, ethanol (hot), acetone, carbon disulfide, ligroin, gasoline, cyclohexane, carbon tetrachloride, and other similar solvents. None of the glycerides is soluble in water.

The unsymmetrical mixed triglycerides are more soluble than are the corresponding symmetrical compounds. Thus the following variations have been reported^{55,364,365} in the solubility of the unsymmetrical acyl dilaurins and of the symmetrical acyl dilaurins expressed in g./100 ml. in ethanol at 25°C.: caprylyl, 16.31 and 14.34; capryl, 9.00 and 3.02; myristyl, 1.21 and 0.25; and palmityl, 0.54 and 0.38. Similar discrepancies in solubility of acyl distearins have been reported. Thus, α -acyl (unsym-

³⁸⁹ L. J. Filer, S. S. Sidhu, B. F. Daubert, and H. E. Longenecker, *J. Am. Chem. Soc.*, **66**, 1333–1337 (1944).

metrical) and β -acyl distearins (symmetrical) were found to have the following solubilities expressed in g./100 ml. in acetone: capryl, 39.45 and 2.57 at 29°C.; and palmityl, 1.82 and 0.61 at 27.5°C. Variations of solubility in alcohol are similar, being 0.43 and 0.06 at 23°C. for the acetyl derivatives, 0.22 and 0.14 at 27.5°C. for capryl esters, 0.59 and 0.47 at 29°C. for the myristodistearins and 0.42 and 0.10 at 27.5°C. for α -palmito- α' , β -distearin and β -palmito- α , α' -distearin, respectively. Even in such an excellent solvent as petroleum ether, α -lauro- α' , β -distearin dissolves to the extent of 38.41 g./100 ml. at 27.5°C., compared with a value of 11.42 g. for β -lauro- α , α' -distearin.⁵⁶ On the other hand, the symmetrical (β) monoglycerides have been shown to have a somewhat higher solubility than the unsymmetrical (α) members.³⁹⁰

Hixson and Bockelmann³⁹¹ have investigated the solubility of tricaprylin, tripalmitin, tristearin, and some of the common fats in liquid propane. Data on these tests are summarized in Table 36.

TABLE 36
CRITICAL SOLUTION TEMPERATURE AND PER CENT SOLUTE PER WEIGHT OF FATS IN LIQUID PROPANE^a

Fat	Critical solution temperature	Per cent solute by weight
Tricaprylin.....	100.5°	30.0
Tripalmitin.....	73.5°	30.0
Tristearin.....	69.2°	28.0
Cottonseed oil.....	66.3°	29.5
Crisco.....	70.0°	13.8
Crude palm oil.....	71.1°	30.0
Coconut oil.....	86.5°	7.0
Soybean oil.....	63.8°	7.0

^a A. W. Hixson and J. B. Bockelmann, *Trans. Am. Inst. Chem. Engrs.*, **38**, 891-930 (1942).

Variations in solubility of the triglycerides occur in acetone at very low temperatures. In general, the triunsaturated triglycerides (GU₃) are most soluble, followed by the monosaturated-diunsaturated (GSU₂), then by the monounsaturated-disaturated (GS₂U) triglycerides. Hilditch⁷⁹ has used this procedure for isolation of the several fractions of natural fats.

(3) Optical Isomerism

Since glycerol is capable of asymmetric substitution, many of the glycerides can exist in enantiomorphic forms. This is true of the un-

³⁹⁰ H. P. Averill, J. N. Roche, and C. G. King, *J. Am. Chem. Soc.*, **52**, 365-367 (1930).

³⁹¹ A. W. Hixson and J. B. Bockelmann, *Trans. Am. Inst. Chem. Engrs.*, **38**, 891-930 (1942).

symmetrical (α -) monoglycerides, the unsymmetrical (α,β -) diglycerides, as well as the symmetrical (α,α' -) diglycerides containing different fatty acids. It may also result in the case of the triglycerides where two different acyl groups are introduced into the α - and α' -positions.

Because of the large proportion of mixed triglycerides which exist in natural fats and oils, it would appear highly probable that many optically active triglycerides are present. One would expect this to be of considerable importance in explaining the activity of the lipases, since their action is highly selective insofar as optical isomers are concerned.³⁹² Although optically active α -glycerol phosphate and phospholipids have been known for a long period, optically active enantiomorphs have not been observed among the triglycerides composed of the longer fatty acid chains. The difficulties in isolation are considerable, since the procedures employed in such isolation might be expected to destroy any asymmetry that was present, by such changes as migration of the acyl groups. Moreover, because of the low order of rotation which has been observed for known asymmetric glycerides, one might expect quite low values for natural products, which are mixed with considerable amounts of inactive material. Most of our information about optically active glycerides has therefore been derived from synthesis of representative compounds rather than from examination of products isolated from natural sources.

The original terminology of the glycerides employed by Abderhalden and Eichwald³⁹³ was to prefix the name of the compound with *d*- or *l*- according to its rotation. However, the nomenclature adopted more recently by Fischer and Baer³⁹⁴ is to relate them to the corresponding *d*- and *l*-glyceraldehydes, similar to the current nomenclature for the sugars.

a. Synthesis of Optically Active Glycerides. The earlier procedures for preparation of the optically active antipodes involved resolution of the enantiomorphs from racemic mixtures. Care had to be taken at all stages to avoid racemization by the acid or basic residues which were introduced to render such separations possible. Abderhalden and Eichwald³⁹³ prepared optically active α -monoglycerides and α,β -diglycerides^{395,396} from the optically active forms of 1-amino-2,3-dibromopropane which were resolved by means of *d*-tartaric acid. Bergmann and Sabetay³⁹⁷ carried out the resolution of the α -acyl esters of γ -aminopropylene glycol with saccharic acid, followed by treatment with nitrous acid to prepare the optically active monoglyceride. The α,β -diacyl esters of γ -aminopropylene glycol were employed to prepare the enantiomorphs of α,β -

³⁹² E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 145, 61-68 (1942).

³⁹³ E. Abderhalden and E. Eichwald, *Ber.*, 47, 1856-1866 (1914).

³⁹⁴ H. O. L. Fischer and E. Baer, *Chem. Revs.*, 29, 287-316 (1941).

³⁹⁵ E. Abderhalden and E. Eichwald, *Ber.*, 48, 113-117 (1915).

³⁹⁶ E. Abderhalden and E. Eichwald, *Ber.*, 48, 1847-1865 (1915).

³⁹⁷ M. Bergmann and S. Sabetay, *Z. physiol. Chem.*, 137, 47-61 (1924).

TABLE 37
MELTING POINTS AND SPECIFIC ROTATIONS OF SOME OPTICALLY ACTIVE (α)
MONOGLYCERIDES^a

Monoglyceride	M.p., °C.	$[\alpha]_D$	Solvent
<i>l</i> (-)- α -Monobutyrim	<i>b</i>	-6.0°	Pyridine
		-2.2°	H.S. ^c
<i>d</i> (+)- α -Monobutyrim	<i>b</i>	+2.2°	H.S. ^c
<i>l</i> (-)- α -Monolaurin	53-54	-3.76°	Pyridine
<i>l</i> (-)- α -Monopalmitin	71-72	-4.37°	Pyridine
<i>l</i> (-)- α -Monostearin	76-77	-3.58°	Pyridine

^a Adapted from H. O. L. Fischer and E. Baer, *Chem. Revs.*, 29, 287-316 (1941).

^b Liquid. ^c H. S. = homogeneous substance.

TABLE 38
BOILING OR MELTING POINTS, REFRACTIVE INDICES, AND SPECIFIC ROTATIONS OF SOME
 α -MONOGLYCERIDE DERIVATIVES^a

α -Monoglyceride deriv.	B.p. or M.p., °C.	<i>n</i> ^b	$[\alpha]_D$ ^b	Solvent ^c
Acetone compd. of: <i>l</i> (-)- α -acetyl glycerol	85-86 (10-11 mm.)	1.4288 ^{15°}	+3.24	H. S.
<i>l</i> (-)- α -propionyl glycerol	88-89 (7 mm.)	1.4260 ^{26°}	+3.6	H. S.
<i>d</i> (+)- α -propionyl glycerol	88-89 (7 mm.)	1.4269 ^{24°}	-3.6	H. S.
<i>l</i> (-)- α -butyryl glycerol	97-98.5 (7 mm.)	1.4270 ^{25°}	+4.92	H. S.
<i>d</i> (+)- α -butyryl glycerol	97-99 (7 mm.)	1.4280 ^{23°}	-4.9	H. S.
<i>l</i> (-)- α -caproyl glycerol	118-120 (7 mm.)	1.4322 ^{25.5°}	+4.5	H. S.
<i>d</i> (+)- α -caproyl glycerol	118-120 (7 mm.)	1.4349 ^{20°}	-4.5	H. S.
<i>l</i> (-)- α -lauryl glycerol	130-131 (0.002 mm.)	1.4448 ^{20°}	+3.42 ^{21°}	H. S.
<i>l</i> (-)- α -palmityl glycerol	33-35	—	+2.48	P
<i>l</i> (-)- α -stearyl glycerol	43.5	—	+1.9	P
<i>l</i> (-)- α -(<i>p</i> -toluene sulfonyl)- glycerol	63-64	—	-7.3	P
<i>l</i> (-)- α -(<i>p</i> -nitrobenzoyl) glycerol	88-89	—	-17.1	Et

^a Adapted from H. O. L. Fischer and E. Baer, *Chem. Revs.*, 29, 287-316 (1941).

^b Superscript figures represent temperature (°C.).

^c H. S. = homogeneous substance; P = pyridine; Et = ethanol.

diglycerides.³⁹⁸ Finally, Grün and co-workers³⁹⁹ obtained highly active strychnine salts of the sulfuric acid esters, but were unable to obtain any optically active product after removal of the sulfuric acid.

An entirely different principle for the preparation of the optical antip-

³⁹⁸ M. Bergmann, E. Brand, and F. Dreyer, *Ber.*, 54, 936-965 (1921).

³⁹⁹ A. Grün and R. Limpächer, *Ber.*, 60, 255-265 (1927).

odes has been used by Baer and Fischer.^{12,400-402} This involves the use of the optical activity of *d*(+)- and *l*(-)-mannitols for preparing the enantiomorphs of acetoneglycerols. Such *d*(+)- and *l*(-)-acetoneglycerols can be used for preparing the corresponding α -monoglycerides by acylation according to the usual procedure.³ These methods have been reviewed by Fischer and Baer.³⁹⁴

b. Properties of Known Optically Active Glycerides. The specific rotation and melting points of pure monoglycerides are included in Table 37, while these constants for some closely related compounds are given in Table 38. Data on a diglyceride and several triglycerides are summarized in Table 39.

TABLE 39
MELTING POINTS AND SPECIFIC ROTATIONS OF SOME OPTICALLY ACTIVE DI- AND TRI-GLYCERIDES^a

Glyceride	M.p., °C.	$[\alpha]_D$	Solvent
<i>d</i> (-)- α,β -distearin	—	- 2.7	CHCl ₃
α -Palmityl- α',β -dilaurin.....	44	0.0	CHCl ₃ , C ₅ H ₅ N
α -Stearyl- α',β -dipalmitin.....	48.5	0.0	C ₅ H ₅ N
α -Lauryl- α',β -distearin.....	62.5	0.0	C ₅ H ₅ N
α -(<i>p</i> -Nitrobenzoyl)- α',β -distearin.....	67-67.5	- 1.4	CHCl ₃
α -(<i>p</i> -Nitrobenzoyl)- α',β -dibenzoylglycerol ...	87-88	-19.9	(CHCl ₂) ₂

^a Adapted from H. O. L. Fischer and E. Baer, *Chem. Revs.*, 29, 287-316 (1941).

Fischer and Baer³⁹⁴ reported a considerably higher specific rotation for the α -monobutyryns ($\pm 2.2^\circ$) than was found by Abderhalden and Eichwald^{393,395} ($\pm 0.84^\circ$). Similar discrepancies were noted between the values for *d*(-)- α,β -distearin obtained by the method of Grün and Limpächer³⁹⁹ (0.0°) and of Fischer and Baer³⁹⁴ (-2.7°), as well as for other compounds. This would indicate the superiority of using *d*(+)- and *l*(-)-acetoneglycerol as starting materials rather than attempting to resolve the racemic mixtures.

The aliphatic α -monoglycerides are quite unstable and show a gradual decrease in rotation.⁷ In fact, one-third to one-half of the original rotation of crystalline monoglycerides has been shown to disappear within a year. On the other hand, the aromatic α -monoglycerides are extremely stable. According to Jackson and King,⁷ no change resulted from one year's standing.

The failure of the unsymmetrical mixed triglycerides to show any observable rotation, although they were prepared from optically active α monoglycerides, is quite perplexing. Fischer and Baer³⁹⁴ have suggested

⁴⁰⁰ E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 128, 475-489 (1939).

⁴⁰¹ E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 128, 491-500 (1939).

⁴⁰² H. O. L. Fischer and E. Baer, *Naturwissenschaften*, 25, 588-589 (1937).

that such asymmetry without measurable optical activity may well be a characteristic of triglycerides made up of fatty acid residues. When aromatic nuclei such as occur in α -(*p*-nitrobenzoyl)- α' , β -dibenzoglycerol are present, an intense optical rotation is observed. It is further suggested that, although the natural asymmetric triglycerides do not show a rotation, they may not be racemic but might be present in either of the two possible enantiomorphic forms. The optical isomerism observed by Suzuki and Inoue^{403,404} in freshly isolated natural fats may possibly be attributed to α -monoglycerides, since these compounds show a gradual decrease in rotation on standing at room temperature.

7. Chemical Properties of Fats and Oils

(1) Hydrolysis

The most vulnerable spots in the triglyceride molecule are the ester linkages. In the digestion and absorption of fat, the first change which, it is generally agreed, takes place, is the hydrolysis of the ester linkage, with the resultant formation of glycerol and three free fatty acid molecules. Not until this primary rupture has taken place can any profound alteration occur in a *saturated* fat molecule; however, it is possible for changes to be brought about on *unsaturated* triglycerides without the breaking down of the ester linkage, although this does not take place in the alimentary tract. Such reactions as occur at the double bonds may completely change the physical properties of the fat.

There are a number of ways in which hydrolysis of the triglycerides may be produced. These methods are all ordinarily referred to as saponification (soap-making), since most frequently employed procedures require alkali, with the formation of soap as one of the end products. Soaps are not produced where saponification results from acid or enzyme hydrolysis.

Four methods for alkaline hydrolysis are in general use. The commonest procedure involves the heating of the fat with a solution of sodium or potassium hydroxide at a boiling temperature. Aqueous solutions of the alkalis may be employed, but more rapid results obtain with alcoholic solutions (such as alcoholic potash), where the reaction can proceed in a one-phase system. A slight modification of this procedure involves the use of sodium ethylate. Hydrolysis is completed when the fat and sodium ethylate are refluxed for at least 30 minutes. Saponification can be readily produced by autoclaving, in which the fats are treated with superheated steam in the presence of small amounts of calcium hydroxide.

The cold saponification method is the mildest one which employs alkali.

⁴⁰³ B. Suzuki, *Proc. Imp. Acad. Tokyo*, 7, 230-233 (1931); *Chem. Zentr.*, 1931, II, 2345.

⁴⁰⁴ B. Suzuki and Y. Inoue, *Proc. Imp. Acad. Tokyo*, 6, 71-74 (1930); *Chem. Abst.*, 24, 4265 (1930).

Concentrated alkali is mixed with the fat and is allowed to stand for several days. This procedure is especially useful where the application of heat would bring about changes in some component in the unsaponifiable residue which is to be purified by removal of the triglyceride.

There are two saponification procedures in general use which employ acid. One involves the heating of the fat with dilute hydrochloric or sulfuric acid under pressure at a temperature which exceeds 100°C. The other method, discovered by Twitchell,^{405,406} makes use of a 1% solution of sulfobenzene stearic acid (Twichell reagent) which acts catalytically to produce hydrolysis. As the result of each of these reactions, glycerol and the free fatty acids (rather than the soaps) are the end products.

Another method makes use of the lipases or lipolytic enzymes. Fatty acids and glycerol are the end products of this procedure. It is obvious that a minimum alteration in the fat will occur, since the reaction proceeds best near neutrality at about 37°C. Enzyme preparations are available from animal tissues (pancreas and liver), as well as from such sources as the castor bean. Although such enzymes are believed to be on the whole non-specific, in that they act on the various fats equally well, this is not always the case. For example, Longenecker and Haley⁴⁰⁷ have shown that markedly different rates of hydrolysis obtain when ricinus lipase of the castor bean acts on different fats. The rate of hydrolysis of the fats, in descending order, was as follows: peanut, castor, corn, cottonseed, soybean, rapeseed, olive, linseed, neat's foot, peach kernel, coconut, whale, fish, and sperm. After a period of 10 hours, 97.2% of the peanut oil was hydrolyzed, while the sperm oil was split to the extent of only 19.5%.

(2) Hydrogenation

The process of hydrogenation of fats has developed into one of tremendous commercial importance. By such a procedure, liquid fats can be partially or completely saturated with hydrogen to yield solid fats which have possibilities for wide variations in melting points. Hydrogenation has been especially widely employed in the preparation of vegetable shortenings and margarines, in the hardening of soap stock, and more recently in improving lard from the standpoint of its hardness, plasticity, and keeping qualities. The methods employed in hydrogenation have been discussed in Chapter II.

(3) Interesterification

Several types of reaction are included under the category of interesterification. The interchange between an alkoxy group of an ester and the

⁴⁰⁵ E. Twitchell, *J. Am. Chem. Soc.*, 28, 196-200 (1906).

⁴⁰⁶ E. Twitchell, *J. Am. Chem. Soc.*, 29, 566-571 (1907).

⁴⁰⁷ H. E. Longenecker and D. E. Haley, *J. Am. Chem. Soc.*, 57, 2019-2021 (1935).

alkoxy group of an alcohol is one type, known as *alcoholysis*. A second reaction involves interchange of alkoxy groups between different esters. Such a process is most properly spoken of as an *interesterification*. The third class of reactions included under this category is *acidolysis*. This last interchange involves the replacement of an acid group of an ester with another acid.

a. Alcoholysis. This type of reaction is the one which has been known for the longest time. Duffy⁴⁰⁸ reported, almost one hundred years ago, that ethyl stearate originates when ethyl alcohol and glyceryl stearate are caused to react.

Several types of reactions can be classified under alcoholysis. These include the reactions between: (1) monoesters and monohydric alcohols, (2) monoesters and polyhydric alcohols, (3) polyesters and monohydric alcohols, and finally (4) polyesters and polyhydric alcohols. When the reaction is carried out with a specific alcohol, it is frequently identified by the name of that alcohol. Thus, *methanolysis*, *ethanolysis*, and *propanolysis* refer to alcoholysis reactions carried out with methyl, ethyl, or propyl alcohol, respectively.

Alcoholysis is usually carried out by heating an excess of the alcohol with the ester in the presence of a catalyst. The catalysts usually employed are the mineral acids, such as hydrochloric or sulfuric acid, in 1-2% concentration. Benzenesulfonic acid¹¹⁷ may also be used in that capacity, while alkaline hydroxides likewise exert a catalytic action.⁴⁰⁹ All glycerides react with alcohols, but those of low molecular weight are the most reactive. Substrates soluble in alcohol are attacked most readily, while the use of alkali solvents for the fat also improves the rate of reaction. An alcohol group of lower weight usually replaces one of higher weight.⁴¹⁰

Alcoholysis, when employed with triglycerides, is a stepwise process. Thus, when tristearin and ethyl alcohol react, not only is ethyl stearate formed, but di- and monoglycerides as well. Thus, when 3000 g. of tristearin was treated with an excess of ethanol in the presence of hydrochloric acid, Grün *et al.*⁴¹¹ isolated, in addition to 1200 g. of ethyl stearate, 300 g. of distearin and 200 g. of monostearin, while 400 g. of the original tristearin were found at the end of the reaction. A similar stepwise process occurs when a hydroxide is used as the catalytic agent.⁴⁰⁹

The interaction of most triglycerides and alcohol takes place slowly, due to the insolubility of the fat in alcohol. As the reaction proceeds, an increasing solubility results. When an homogenous solution is obtained, it is complete. Due to the ready solubility of castor oil in alcohol resulting from

⁴⁰⁸ P. Duffy, *J. Chem. Soc.*, 5, 303-316 (1853).

⁴⁰⁹ Y. Toyama and T. Tsuchiya, *J. Soc. Chem. Ind. Japan*, 36, suppl., 232-233B (1933).

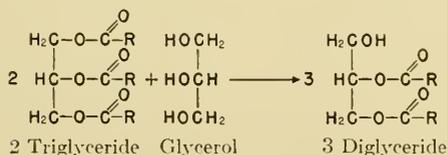
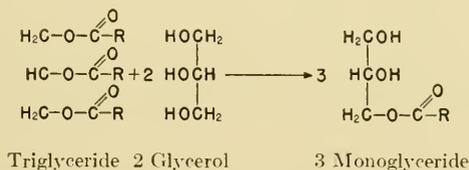
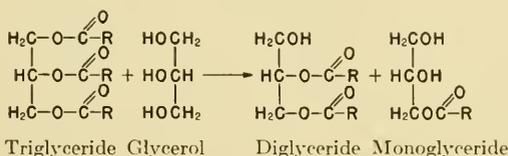
⁴¹⁰ E. M. Bellet, *Compt. rend.*, 193, 1020-1023 (1931).

⁴¹¹ A. Grün, F. Wittka, and E. Kunze, *Chem. Umschau*, 24, 15-16 (1917); *Chem. Abst.*, 13, 2768-2769 (1919).

the large proportion of the hydroxyl-containing triglyceride triricinolein, this fat reacts rapidly with alcohols.⁴¹² A methanolysis of coconut oil in the presence of hydrochloric acid has been used by Elsdon⁴¹³ for the estimation of the component acids.

(a) *Glycerolysis*. A special type of alcoholysis is exemplified by the reaction between the triatomic alcohol glycerol, and fat. This reaction is widely employed commercially for the production of partially esterified products.

According to Markley,⁴¹⁴ several different reactions are possible between glycerol and triglyceride molecules. According to these schemes, one mole of triglyceride can react with $\frac{1}{2}$, 1, or 2 moles of glycerol to produce varying proportions of either mono- or diglycerides or both. These reactions are illustrated below:



Eckey and Formo²³ have recently described a procedure for the syntheses of mono- and diglycerides in which the so-called "directed" interesterification is employed. Previously dried c.p. glycerol is mixed with cottonseed oil to which the catalyst, consisting of sodium methoxide in xylene, has previously been added; precautions are taken to exclude air during the mixing of the catalyst and the cottonseed oil. Various proportions of glycerol may be employed. The mixture is continuously agitated over the desired period at a constant temperature. When the proportion of free hydroxyl groups to ester groups is between 1 to 5 and 1 to 2, the reaction mixture consists chiefly of diglycerides, provided that a favorable time-

⁴¹² A. Haller, *Compt. rend.*, 144, 462-466 (1907).

⁴¹³ G. D. Elsdon, *Analyst*, 38, 8-11 (1913).

⁴¹⁴ K. S. Markley, *Fatty Acids*, Interscience, New York, 1947.

temperature cycle is employed; with a ratio of 1 to 1, nearly pure monoglyceride is precipitated. The glycerides prepared by alcoholysis of cottonseed oil were shown to be 1-monopalmitin and 1,3-dipalmitin, respectively. The relation of reaction mixture to the products present at equilibrium are shown in Figure 15.

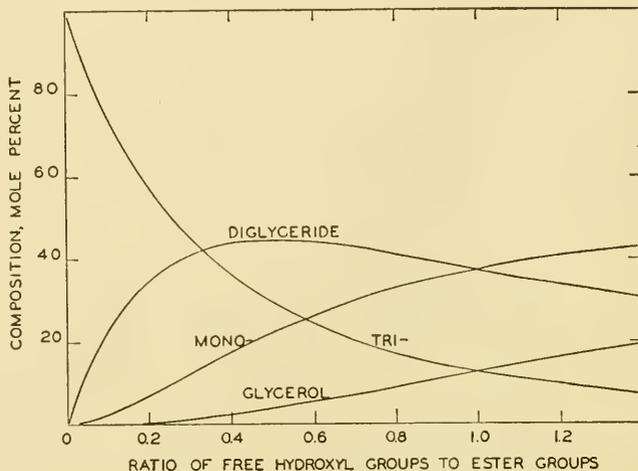


Fig. 15. Calculated composition produced at equilibrium by single-phase alcoholysis of triglycerides with glycerol.²³

Many workers have studied glycerolysis as a method of preparing monoglycerides. Most of the procedures^{89,415} involve heating glycerol and the triglyceride fat at 170–250°C. Various catalysts such as xylene, caustic soda, caustic potash, and sodium alcoholates are suggested in amounts of 0.05 to 0.20% of the fat used. However, in the procedure of Ecey and Formo,²³ a maximum temperature of 32.2°C. (90°F.) was employed. The procedure has been patented by Ecey.⁴¹⁶ A number of other patents have been issued for the commercial production of di- and monoglycerides, including those of Edeler and Richardson,⁴¹⁷ Ecey and Clark,⁴¹⁸ Richardson and Ecey,⁴¹⁹ Christensen,⁴²⁰ and Hilditch and Rigg.⁴²¹

⁴¹⁵ S. Kawai and S. Yamamoto, *J. Soc. Chem. Ind. Japan*, 43, suppl., 219–220B(1940); *Chem. Abst.*, 35, 1255 (1941).

⁴¹⁶ E. W. Ecey, *U. S. Patent No. 2,442,534* (June 1, 1948).

⁴¹⁷ A. Edeler and A. S. Richardson (to Procter & Gamble), *Canadian Patents Nos. 340,803 to 340,805* (April 10, 1934).

⁴¹⁸ E. W. Ecey and C. C. Clark (to Procter & Gamble), *U. S. Patent No. 2,065,520* (Dec. 29, 1936).

⁴¹⁹ A. S. Richardson and E. W. Ecey (to Procter & Gamble), *U. S. Patent No. 2,132,437* (Oct. 11, 1938).

⁴²⁰ C. W. Christensen (to Armour), *U. S. Patent No. 2,022,493* (Nov. 26, 1935).

⁴²¹ T. P. Hilditch and J. G. Rigg (to Imperial Chemical Industries), *U. S. Patent No. 2,073,797* (March 16, 1937).

b. Ester-Ester Interchange. Rearrangements of the acid components in esters may take place when several esters are mixed and the conditions are adjusted to render such interchange possible. There are a number of procedures which may be used.

(a) *Undirected Interesterification.* The usual method may be referred to as undirected esterification which, as carried out in the past, involves the use of high temperatures without a catalyst to accomplish the change. Under these conditions all reactants are molten and in a single phase. This method is slow, and the yields are poor. In 1865, Friedel and Crafts⁴²² demonstrated that, when ethyl benzoate and amyl acetate were heated, the best production of amyl benzoate and ethyl acetate occurred at 300°C. Normann⁴²³ reported that a mixture of 10 parts tristearin and 90 parts soybean oil having a melting point of 130.1°F. was changed to one melting at 110.7°F., after heating for 17 hours at 250°C.; after 24 hours of such treatment the melting point of the interesterified mixture was reduced to 93.7°F. Van Loon⁴²⁴ reported that the melting point of a mixture of 20 parts of beef stearin and 80 parts of soybean oil dropped from 108.5°F. to 97.0°F. after heating for 16 hours at 275°C. It is therefore evident that a rearrangement of the fatty acids in the triglycerides occurs during esterification, with the result that the melting point is lowered in the new mixed triglycerides. Under conditions of undirected esterification, the fatty acids are arranged entirely in a random distribution in the triglycerides.

A second procedure for carrying out ester-ester interchange involves the use of a catalyst. Under such conditions the reaction may take place at room temperature; the high degree of heating required to bring about the ester interchange in the absence of a catalyst is not needed, nor is it desirable. Alkali metal alkoxides have been shown to be extremely effective catalysts. Sodium ethylate can be used at room temperature satisfactorily,²⁸⁸ when prepared and employed under favorable conditions. Although sodium ethylate or methylate has previously been used as a catalyst where the reaction mixture was heated at 275–400°F.,^{424–426} reactions other than ester interchange are brought about, and these may be undesirable.

Sodium ethylate can be employed in amounts as low as 0.1% at temperatures below 120°C., provided the medium is dry, peroxide-free, and the catalyst is immediately dispersed in the oil mixture. It is necessary to exclude air and moisture rigidly. Sodium methoxide may also be used in a

⁴²² C. Friedel and J. R. Crafts, *Ann.*, 133, 207–211 (1865).

⁴²³ Firma Oelwerke Germania G.M.B.H. and W. Normann, *German Patent* No. 417,215 (June 26, 1920).

⁴²⁴ C. Van Loon, *U. S. Patent* No. 1,873,513 (Aug. 23, 1932).

⁴²⁵ E. W. Eckey (to Procter & Gamble), *U. S. Patent* No. 2,378,005 (June 12, 1945).

⁴²⁶ M. Naudet and P. Desnuelle, *Bull. soc. chim.* [5], 13, 595–598 (1946).

methanol solution, or as a fine powder, especially when suspended in xylene. Eckey²⁸⁸ has reported that alkoxides of ethanol, isopropanol, *tert*-butanol, ethylene glycol, laurool and other alcohols, when suspended in xylene, have approximately the same catalytic activity on a molecular basis as sodium methoxide. The action of the catalyst can be immediately stopped by the addition of water or of dilute acetic acid.

(b) *Directed Interesterification.* Under the usual conditions of performing an ester-ester interchange, either at high temperatures without a catalytic agent or at lower temperatures with a catalyst, the net result of the rearrangement is to change the fatty acid distribution in the mixed triglycerides to a random one. However, when the reaction is performed at a temperature sufficiently low so that one of the products formed can crystallize from the molten mixture, the end product no longer involves a random mixture but an extremely oriented product. Such a reaction is termed by Eckey²⁸⁸ a "directed interesterification."

Such directed ester-ester interchange can likewise be carried out somewhat more satisfactorily when inert solvents are used than with the molten mixture. Eckey²⁸⁸ has reported that pentane, hexane, benzene, and toluene may serve well in that capacity. Lower temperatures are required when the reaction occurs in a solvent than when it takes place in the melted fat. Since directed interesterification, to be effective, requires the separation of a crystalline product, the volatile solvents are unsatisfactory because the high-melting glycerides are more soluble in them. Alcohol cannot be used, since the presence of this substance will cause an alcoholysis.

Eckey²⁸⁸ found that, when cottonseed oil was subjected to undirected interesterification at 120°F., the cloud point of the oil was increased only from 27° to 56°F. On the other hand, when the directed procedure was employed, the melting points of the resulting fat mixtures were much higher. When the reactions were carried out at 70°, 60°, or 50°F. for varying periods, maximum clouding temperatures were found to be 89°, 92°, and 89°F., respectively. The melting point of the solid phase which crystallized out was 141°F., and its iodine number approached zero.

By the application of directed interesterification, Eckey²⁸⁸ has shown that oils like cottonseed can be converted to a semi-solid type of shortening without hydrogenation. Such a treated cottonseed fat has an iodine number and saponification value identical with that of the oil from which it originated. Only the melting point and related properties are altered. Under some conditions a shortening of this type may have an advantage over the conventionally prepared hydrogenated fats in having a higher unsaturated fatty acid content. However, this product does have the disadvantage of becoming rancid more readily, due to the presence of highly unsaturated acids.

Palm oil can be improved for use in blended shortenings by direct in-

teresterification. Thus, while untreated palm oil does not have a good plastic range for use as a shortening, it is markedly improved after directed interesterification. Tallow, also, can be improved for use in shortenings by ester-ester interchange. Coconut oils and others of this class become harder on directed interesterification, which renders them more satisfactory for confectioners' butters. The same type of procedure can be applied to fish oils, for removing the saturated acids. The liquid portion remaining after the removal of the saturated triglycerides is a proportionally better drying oil than the original fish oil, and has a higher iodine number.

c. Acidolysis. Acidolysis involves a change quite similar to alcoholysis. In the former case, when the ester is mixed with an excess of an acid, A, a new ester will originate, containing the original alcohol group combined with the added acid, A. In the second type of reaction, the same general change occurs, but it is concerned with the alcohol group.

Low molecular weight acids tend to replace the higher weight acids in the esters. Thus, when coconut oil is treated with acetic acid, mixtures of myristodiacetin and laurodiacetin result. However, the higher fatty acids may, in some cases, replace the lower ones as is evidenced by the fact that the fatty acids of cottonseed or palm oils can replace those of coconut oil when the former are reacted with the latter at 260–300°C., even without a catalyst.⁴²⁷

(4) Rancidity

Like other organic materials, fats are subject to deterioration. The chief form of degradation in the case of fats is referred to as rancidity. The reactions involved in producing rancidity may be hydrolytic or oxidative in character, and may originate from enzymic activity or from spontaneous atmospheric oxidation. The wide variety of changes which are grouped in the category as constituting "off" flavors or odors is indicated by the large number of terms which are used to describe various types of rancidity. Some of these are rancid, pungent, tallowy, soapy, oily, ester-like (in ketonic rancidity), metallic, musty, fishy, bitter, cardboard, and burnt.⁴²⁸

a. Types of Rancidity. (a) *Oxidative Rancidity.* This is the commonest variety and the type which is usually referred to by this term. The oxidation takes place at the unsaturated linkage. Oxygen is required for this reaction, and the rate of development is increased by light, moisture, and heat. Greenbank and Holm⁴²⁹ report that light of wave length, 3600 Å., has the greatest effect on the oxidation of cottonseed oil. The oxidative rancidity of corn and cottonseed oils is believed to be due to the photo-

⁴²⁷ G. Barsky (to Wecoline Products), *U. S. Patent* No. 2,182,332 (Dec. 5, 1939).

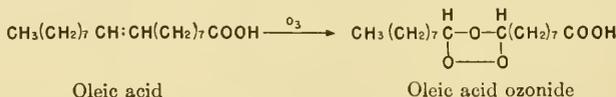
⁴²⁸ H. S. Olcott and H. A. Mattill, *Chem. Revs.*, 29, 257–268 (1941).

⁴²⁹ G. R. Greenbank and G. E. Holm, *Ind. Eng. Chem.*, 25, 167–168 (1933).

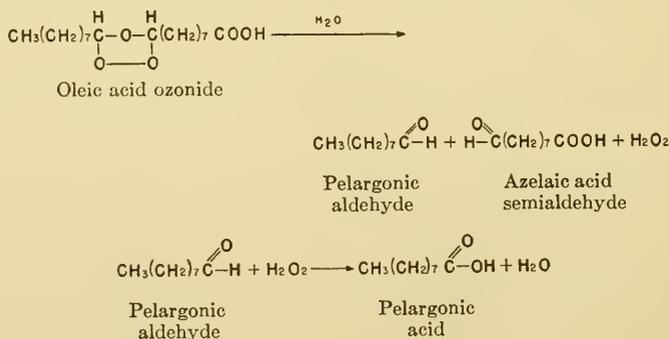
chemical action of light on a compound which is present in the oil or which is produced by compounds which give rise to peroxides.⁴³⁰ Less rancidity develops when the oil is stored in the dark or in the light at wave lengths of 4900 to 5800 A., than when it is kept in ordinary light, even though the peroxide values may be approximately the same in both cases.

Certain metals and their oxides greatly hasten the onset of rancidity by what is believed to be a catalytic action; for example, lard stored in contact with strips of copper or lead was found to become rancid in 5 or 6 days (as determined by the Kreis test). On the other hand, when the lard was stored in the absence of the metals, a positive organoleptic test for rancidity was not given until the 27th day.³³³ It is suggested that the action of metals may be catalytic, in that they form intermediate compounds with the fats, or because they have a directive effect on the fat molecule which causes the unsaturated portions to be directed toward the surfaces, where they become more readily oxidized.³³³

There seems to be little doubt that the ethylenic linkages are the primary site of attack in the development of oxidative rancidity. Oleic acid is known to form an ozonide on treatment with ozone, which might well represent an intermediate. This reaction is illustrated below. When



ozonides are treated with water, they readily split at the oxygen linkage. In the case of oleic acid ozonide, the end products are hydrogen peroxide, azelaic acid semialdehyde, and pelargonic aldehyde. The latter compound is further oxidized to pelargonic acid.

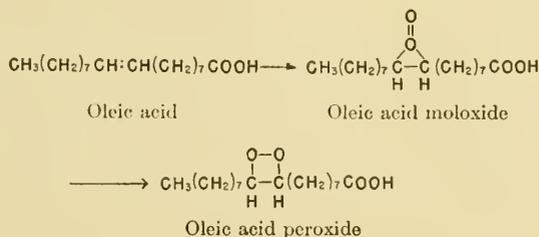


Kerr and Sorber⁴³¹ have shown that, in the development of rancidity, after the early formation of free acid, a drop in iodine number occurs, an

⁴³⁰ M. R. Coe and J. A. LeClerc, *Ind. Eng. Chem.*, **26**, 245-248 (1934).

⁴³¹ R. H. Kerr and D. G. Sorber, *Ind. Eng. Chem.*, **15**, 383-385 (1923).

increase in unsaponifiable fraction takes place, and finally a stable peroxide results. According to Engler and Weissberg (cited by Bull³³³), an intermediate moloxide is first formed, which is then changed into the stable peroxide. These reactions for oleic acid are illustrated below.



It is generally believed that this type of peroxide can act as an oxygen carrier. It can bring about the oxidation of some of the fat components such as the unsaturated acids and the fat-soluble vitamins. Linoleic acid is more readily attacked than oleic acid,^{432,433} while linolenic acid has a still greater susceptibility to oxidation.⁴³⁴ Fats composed of fatty acids having conjugate double bonds are especially reactive. Thus, pseudoelaeostearic acid containing the conjugate bonds is more susceptible to oxidation than is isomeric linolenic acid, in which the double bonds are not conjugated.⁴³⁵ It is believed that small amounts of conjugated acids are present in edible oils, as judged by their absorption spectra.⁴³⁶ Such conjugate acids would be especially susceptible to the formation of peroxides which may initiate reaction chains. Morrell and his group,⁴³⁷⁻⁴⁴¹ as well as Franke and Jerchel,⁴⁴² have carried out extensive research in which the reactivities of various peroxides have been differentiated.

(b) *Hydrolytic Rancidity.* This is also known to occur. Hydrolysis, however, is not a necessary concomitant of rancidity. Kerr and Sorber⁴⁴¹ believe that, in oxidative rancidity, the failure to demonstrate glycerol or free acids indicates the absence of hydrolysis. In the case of dairy products, butyric and other low molecular weight fatty acids are set free by hydrolysis, which usually results from the action of lipase. The odors of

⁴³² K. Täufel and A. Seuss, *Fettchem. Umschau*, 41, 107-113, 131-137 (1934); *Chem. Abst.*, 29, 369 (1935).

⁴³³ G. E. Holm, G. R. Greenbank, and E. F. Deysher, *Ind. Eng. Chem.*, 19, 156-158 (1927).

⁴³⁴ R. Kuhn and K. Meyer, *Z. physiol. Chem.*, 185, 193-216 (1929).

⁴³⁵ J. P. Kass and G. O. Burr, *J. Am. Chem. Soc.*, 61, 3292-3294 (1939).

⁴³⁶ E. S. Miller, W. R. Brown, and G. O. Burr, *Oil & Soap*, 15, 62-65 (1938).

⁴³⁷ R. S. Morrell and S. Marks, *J. Soc. Chem. Ind.*, 50, 27-36T (1931).

⁴³⁸ R. S. Morrell and W. R. Davis, *J. Soc. Chem. Ind.*, 55, 237-246T (1936).

⁴³⁹ R. S. Morrell and W. R. Davis, *J. Soc. Chem. Ind.*, 55, 261-265T (1936).

⁴⁴⁰ R. S. Morrell and W. R. Davis, *J. Soc. Chem. Ind.*, 55, 265-267T (1936).

⁴⁴¹ R. S. Morrell and E. O. Phillips, *J. Soc. Chem. Ind.*, 58, 159-162T (1939).

⁴⁴² W. Franke and D. Jerchel, *Ann.*, 533, 46-71 (1937).

such acids contribute largely to the smell of rancid butter. The higher fatty acids, such as palmitic and stearic, have little odor. Thus, the "off" flavor of fats cannot originate from such acids.

(c) *Ketonic Rancidity*. This type is most frequently encountered as a result of the action of such fungi as *Aspergillus niger* and the blue-green mold, *Penicillium glaucum*, on fats such as coconut oil. Water and some source of nitrogenous matter seem to be necessary for the ketone formation to occur. The "tallowy" odor present in some cases of rancidity may be the result of aldehydes and ketones formed when oxidized unsaturated fatty acids are split. The products formed from oleic acid give the most intense tallowy odor, while the oxidation of linoleic acid causes a much lower amount of "off" odors. Only a slight odor results from the oxidation of linolenic acid.

The products of rancidity include free fatty acids, aldehydes, ketones, and peroxides, as well as some alcohols.⁴⁴³ Methyl amyl, methyl nonyl, and methyl heptyl ketones have been demonstrated,⁴⁴⁴ while it is suggested that methyl alkyl ketone is also found in rancid fats.⁴⁴⁵

b. Organoleptic Tests. The most widely accepted test used as a criterion of rancidity is the Kreis test.⁴⁴⁶ Triebold⁴⁴⁷ has shown that the intensity of the color developed in this procedure as measured spectrophotometrically and as determined by the oxygen absorption method is proportional to the degree of rancidity.

The presence of epihydrin aldehyde, $\begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ \text{CH}_2-\text{CH}-\text{CHO} \end{array}$, is necessary for a positive Kreis test. The products of ketonic rancidity do not give a positive response. Kerr⁴⁴⁸ has reported further investigations on the use of the Kreis test.

Almost any test for aldehydes, as well as for peroxides, can likewise serve as a test for rancidity. Thus, the reduction of methylene blue⁴⁴⁹ and the Schiff test⁴⁵⁰ have been proposed for this purpose. Issoglio⁴⁵¹ used as a test the amount of potassium permanganate that was required to oxidize the water-soluble aldehydes extracted from the fat by shaking with distilled water.

⁴⁴³ M. J. Cummings and H. A. Mattill, *J. Nutrition*, **3**, 421-432 (1930-1931).

⁴⁴⁴ W. N. Stokoe, *Biochem. J.*, **22**, 80-93 (1928).

⁴⁴⁵ H. E. Fierz-David, *Z. angew. Chem.*, **38**, 6-8 (1925).

⁴⁴⁶ M. Winkel, *Z. Untersuch. Nahr. Genussm.*, **9**, 90-96 (1905). M. Winkel and H. Kreis, *ibid.*, **9**, 607 (1905).

⁴⁴⁷ H. O. Triebold, *Cereal Chem.*, **8**, 518-532 (1931).

⁴⁴⁸ R. H. Kerr, *Ind. Eng. Chem.*, **10**, 471-475 (1918).

⁴⁴⁹ H. D. Royce, *Soap*, **7**, No. 9, 25-27, 38 (1931); *Chem. Abst.*, **26**, 1145 (1932).

⁴⁵⁰ H. S. Bailey and H. C. Ebert, *Cotton Oil Press*, **7**, No. 8, 35 (1923).

⁴⁵¹ G. Issoglio, *Ann. chim. applicata*, **6**, 1-18 (1916); *Atti accad. sci., Torino*, **51**, 582-605 (1916).

Tracy *et al.*⁴⁵² have suggested that the rancidity can be predicted from the redox potential. When this value exceeds 0.300 volt in milk, rancidity is apt to develop. Milk was found to be less stable in the winter; this was interpreted as indicating that the lower bacterial growth allowed more oxygen to be available for oxidation. We now know that such a phenomenon can probably best be explained by a lower content of antioxidants in the milk when the cows are no longer given green feed. Oxygen absorption has also been used to estimate rancidity, but it is of more use in the determination of incipient rancidity or stability of fats. Recent reviews on the subject of rancidity include the comprehensive report of Lea,⁴⁵³ and those of Beadle,⁴⁵⁴ Mattill,⁴⁵⁵ Olcott,⁴⁵⁶ Vibrans,⁴⁵⁷ Olcott and Mattill,⁴²⁸ Lundberg,⁴⁵⁸ Riemenschneider and Ault,⁴⁵⁹ and Quackenbush.⁴⁶⁰

c. Tests for Incipient Rancidity. Although oxidative rancidity is largely dependent upon the presence of unsaturated fats, there is a wide variation in the speed at which rancidity develops in fats possessing an equal degree of unsaturation. For example, sweet almond and sesame oils will remain fresh for much longer periods than fats with a similar or lower iodine number.³⁵⁰ Wheat germ oil is extremely stable,⁴⁶¹ in spite of its high degree of unsaturation. We owe to Mattill,⁴⁶² the idea that such stability is to be attributed to antioxidants naturally occurring in the fats. As proof of this concept, it was shown that the addition of wheat germ oil to diets containing lard prevented the early development of rancidity.⁴⁶³ It thus becomes apparent that it is considerably more important to determine the stability of a given fat, *i.e.*, its ability to avoid becoming rancid, than to ascertain to what extent such a reaction has already occurred.

One method for ascertaining the resistance of a fat to oxidation is based upon the amount of peroxides it contains. This value can readily be estimated by the determination of the iodine liberated from an acidified solution of potassium iodide by the so-called "peroxide oxygen" of the fats. Many different procedures have been suggested, all of which are based on

⁴⁵² P. H. Tracy, R. J. Ramsey, and H. A. Ruehe, *Univ. Ill. Agri. Exp. Sta.*, 27, Bull. No. 389, 579-595 (1933).

⁴⁵³ C. H. Lea, *Rancidity in Edible Fats*, Food Investigation Special Report No. 46, H. M. Stationery Office, London, 1938; Chemical Pub. Co., New York, 1939.

⁴⁵⁴ B. W. Beadle, *Oil & Soap*, 23, 33-35 (1946).

⁴⁵⁵ H. A. Mattill, *Oil & Soap*, 18, 73-76 (1941).

⁴⁵⁶ H. S. Olcott, *Oil & Soap*, 18, 77-80 (1941).

⁴⁵⁷ F. C. Vibrans, *Oil & Soap*, 18, 109-112 (1941).

⁴⁵⁸ W. O. Lundberg, *A Survey of Present Knowledge, Researches and Practices in the United States Concerning the Stabilization of Fats*, Publication No. 20, Hormel Institute, University of Minnesota, 1947.

⁴⁵⁹ R. W. Riemenschneider and W. C. Ault, *Food Ind.*, 16, 892-894, 936-939 (1944).

⁴⁶⁰ F. W. Quackenbush, *Oil & Soap*, 22, 336-337 (1945).

⁴⁶¹ L. W. Elder, Jr., *Oil & Soap*, 18, 38-42 (1941).

⁴⁶² H. A. Mattill, *J. Am. Med. Assn.*, 89, 1505-1508 (1927).

⁴⁶³ L. T. Anderegg and V. E. Nelson, *Ind. Eng. Chem.*, 18, 620-622 (1926).

the above reaction, but in which different solvents are employed, or variations in the timing, temperature, or the use of inert gas are indicated.

The procedures of Taffel and Revis⁴⁶⁴ and of Lea⁴⁶⁵ were the earlier standard methods. The Lea technic gives reproducible results, but it involves the heating of the chloroform-acetic acid solvent to boiling. Wheeler⁴⁶⁶ proposed a simplified method with the same solvent in the cold, which was accurately timed at one minute. Lowen *et al.*⁴⁶⁷ found that the peroxide value varied as much as 50% when determined by the Wheeler method, as the sample size was increased from 2 to 10 ml. Stansby⁴⁶⁸ reported that the Lea as well as the Wheeler technics gave decreasing peroxide values when an increasingly large sample was used. One of the methods which he proposed which involved the use of sulfuric instead of acetic or hydrochloric acids was largely free from the above criticism.

The recent procedure of Kokatnur and Jelling⁴⁶⁹ has numerous advantages over the earlier methods in which chloroform or carbon tetrachloride is employed as a solvent. Since the above method employs isopropanol as a solvent, it provides a satisfactory solution for fat, and at the same time also allows ready miscibility with water, thus avoiding a two-phase system when the liberated iodine is titrated with thiosulfate. The Lingenfelter modification⁴⁷⁰ consists in the use of a microtitration apparatus and the use of 0.1 *N* thiosulfate as a standard. Volz and Gortner⁴⁷¹ have recently demonstrated that the recovery of benzoyl peroxide was equally satisfactory in the Lingenfelter method (99.1–101.1%), the Stansby method (98.6–99.1%), and the Lea procedure (100.6–102.2%) when 2.5 to 25.0 mg. of the peroxide was used. Equally effective recoveries (98.9–100.3) were shown when 1 g. of fat was added to the benzoyl peroxide and the latter was determined by the Lingenfelter method. Moreover, the Lingenfelter modification of the Kokatnur and Jelling procedure gave most consistent results when samples of varying size were used, as indicated in Table 40.

Lips *et al.*⁴⁷² compared the several procedures and favored a ferric thiocyanate method. Lea⁴⁷³ has recently suggested a simplification of his earlier technic, while Paschke and Wheeler⁴⁷⁴ have modified the earlier Wheeler method by reducing the sample weight to 1 g. and increasing the

⁴⁶⁴ A. Taffel and C. Revis, *J. Soc. Chem. Ind.*, 50, 87–91T (1931).

⁴⁶⁵ C. H. Lea, *Proc. Roy. Soc. London*, B108, 175–189 (1931).

⁴⁶⁶ D. H. Wheeler, *Oil & Soap*, 9, 89–97 (1932).

⁴⁶⁷ L. Lowen, L. Anderson, and R. W. Harrison, *Ind. Eng. Chem.*, 29, 151–156 (1937).

⁴⁶⁸ M. E. Stansby, *Ind. Eng. Chem., Anal. Ed.*, 13, 627–631 (1941).

⁴⁶⁹ V. R. Kokatnur and M. Jelling, *J. Am. Chem. Soc.*, 63, 1432–1433 (1941).

⁴⁷⁰ J. F. Lingenfelter, *Thesis*, Cornell Univ., 1945. Cited by F. E. Volz and W. A. Gortner, *J. Am. Oil Chemists' Soc.*, 24, 418 (1947).

⁴⁷¹ F. E. Volz and W. A. Gortner, *J. Am. Oil Chemists' Soc.*, 24, 417–420 (1947).

⁴⁷² A. Lips, R. A. Chapman, and W. D. McFarlane, *Oil & Soap*, 20, 240–243 (1943).

⁴⁷³ C. H. Lea, *J. Soc. Chem. Ind.*, 65, 286–290 (1946).

⁴⁷⁴ R. F. Paschke and D. H. Wheeler, *Oil & Soap*, 21, 52–57 (1944).

TABLE 40

PEROXIDE VALUES OF THREE FATS AS DETERMINED BY THREE DIFFERENT METHODS^a

Type of fat	Grams of fat	Method		
		Lingenfelter	Stansby	Lea (1931)
Low peroxide	0.2	3.6	—	—
	0.5	3.3	4.0	—
	1.0	2.8	3.1	4.8
Medium peroxide	0.2	21.0	—	—
	0.5	19.6	17.8	—
	1.0	18.0	17.0	22.0
High peroxide	0.2	108.7	104.8	142.3
	0.5	107.3	100.3	112.7
	1.0	106.5	97.9	110.5

^a F. E. Volz and W. A. Gortner, *J. Am. Oil Chemists' Soc.*, 24, 417-420 (1947).

reaction time to one hour, and by the addition of carbon dioxide to minimize the further oxidation of the fat.

The most widely used method is the so-called Swift stability test.⁴⁷⁵ This involves the determination of the period of "induction" under certain

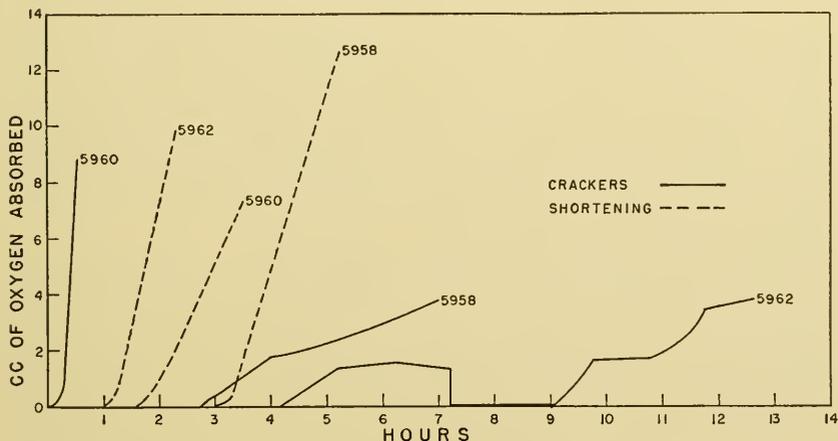


Fig. 16. The variation in the length of time (in hours) and the induction period in the absorption of oxygen for six commercial samples of lard.⁴⁷⁶

standardized conditions of temperature and oxygen addition. The induction period lasts for varying periods of time with different oils. During this interval, practically no oxygen is absorbed and no increase in the blank reading for the peroxide value obtains; as soon as the induction period is completed, oxygen absorption takes place very rapidly and a sudden marked

⁴⁷⁵ A. E. King, H. L. Roschen, and W. H. Irwin, *Oil & Soap*, 10, 105-109 (1933).

⁴⁷⁶ H. O. Triebold and C. H. Bailey, *Cereal Chem.*, 9, 50-64 (1932).

increase in peroxide occurs. The wide variation in induction period between six different samples of lard is graphically represented in Figure 16.

Lea⁴⁷⁷ demonstrated that the induction period is markedly shorter when the fat has previously been exposed to light. The various steps employed in the commercial preparation of corn oil were shown to decrease its induction period to a considerable extent.⁴⁷⁸ The purified fatty acids and glycerol prepared from olive oil were found by Hilditch and Sleightholme⁴⁷⁹ to be much more susceptible to rancidity than was the crude olive oil. These experimental facts all point to the common conclusion that some natural antirancidity principles are present in fat which may be lost on purification.

d. Inhibitors and Antioxidants. Moureu^{480,481} first employed the term *antioxygens* to define those substances which prevent the oxidation of unsaturated triglycerides until they themselves have been destroyed. It has been postulated that the effectiveness of the antioxidants or inhibitors, as they are now called, lies in their ability to break the chain reaction involved in oxidative rancidity. Christiansen⁴⁸² originally proposed that a chain mechanism is involved not only in rancidity but in the drying of oils. Mattill, Olcott, and co-workers⁴⁸³⁻⁴⁸⁸ believe that a "mloxide" is first formed. This latter compound rearranges to the more stable peroxide. Either the mloxide or peroxide spontaneously breaks down, or it reacts with water to facilitate the degradation whereby aldehydes are formed. These aldehydes autoxidize to form peracids which are changed into acids. Such a series of reactions, once started, continues to completion unless it is interrupted at some stage. This chain reaction hypothesis explains why a trace of oxidized fat brings about oxidative rancidity in a large quantity of neutral fat.

Antioxidants presumably act to disrupt this cycle of changes in the following way.⁴⁸⁰ The oxidant, A, unites with oxygen to form a peroxide, AO_2 . The next stage involves the simultaneous oxidation of the antioxidant, B, by the peroxide, and the transformation of the oxidized oxidant to a lower oxide, AO, according to the scheme, $AO_2 + B \rightarrow AO + BO$. It is assumed that these two oxides are mutually antagonistic; hence, they

⁴⁷⁷ C. H. Lea, *J. Soc. Chem. Ind.*, 52, 146-149T (1933).

⁴⁷⁸ H. A. Mattill and B. Crawford, *Ind. Eng. Chem.*, 22, 341-344 (1930).

⁴⁷⁹ T. P. Hilditch and J. J. Sleightholme, *J. Soc. Chem. Ind.*, 51, 39-44T (1932).

⁴⁸⁰ C. Moureu and C. Dufraisse, *Chem. Revs.*, 3, 113-162 (1926).

⁴⁸¹ C. Moureu, C. Dufraisse, and P. Lotte, *Ind. Eng. Chem.*, 22, 549-551 (1930).

⁴⁸² J. A. Christiansen, *Trans. Faraday Soc.*, 24, 714-715 (1928).

⁴⁸³ H. A. Mattill, *J. Biol. Chem.*, 90, 141-151 (1931).

⁴⁸⁴ H. S. Olcott, *J. Am. Chem. Soc.*, 56, 2492-2493 (1934).

⁴⁸⁵ R. B. French, H. S. Olcott, and H. A. Mattill, *Ind. Eng. Chem.*, 27, 724-728 (1935).

⁴⁸⁶ H. S. Olcott and H. A. Mattill, *Oil & Soap*, 13, 98-100 (1936).

⁴⁸⁷ L. A. Hamilton and H. S. Olcott, *Oil & Soap*, 13, 127-129 (1936).

⁴⁸⁸ H. S. Olcott and H. A. Mattill, *J. Am. Chem. Soc.*, 58, 1627-1630 (1936).

react with each other to regenerate the three original molecules in the following manner: $AO + BO \rightarrow A + B + O_2$. The chain reaction is thus broken and the development of rancidity is temporarily forestalled. The antioxidants are gradually destroyed or are transformed to inert products. During the induction period, the antioxidants break up the chain reactions almost as soon as they are started. When they become exhausted, as is true at the end of the induction period, the oxidative reactions proceed at a normal but greatly accelerated pace. Since the inhibitol is destroyed, Stephens,⁴⁸⁹ and also Hamilton and Olcott,⁴⁵⁷ believe that the antioxidant activity cannot be the result of a true anticatalyst as suggested by Moureu and Dufraisse.⁴⁸⁰

(a) *Phenols and Quinones.* Phenols and quinones have been shown to be especially effective antioxidants. Moureu and Dufraisse⁴⁸⁰ reported that one molecule of hydroquinone can protect 40,000 molecules of acrolein from autoxidation. Olcott and Mattill^{488,490} have demonstrated that *o*- and *p*-diphenols are excellent antioxidants, while *m*-diphenols are inactive in preventing oxidation of fats. Such a saturated hydroxybenzene derivative as inositol was shown to be inactive, as were aromatic compounds in which the hydroxyl group was attached to the side chain. On the other hand, only one hydroxyl group is necessary in the naphthalene series to provide an effective antioxidant.

α -Naphthol was found to be a powerful reagent for preventing autoxidation in fats, while β -naphthol was much less effective. Quinone and β -naphthoquinone were found to be extremely effective antioxidants, but α -naphthoquinone was essentially without activity. The phenolic antioxidants were found to be active only when they were in the free state; when they were combined in the form of esters, their activity was completely abolished. A preliminary classification of inhibitols has been made by Olcott and Mattill.⁴⁹⁰

(b) *Tocopherols.* The natural antioxidants were at first concentrated by the procedures useful for the preparation of vitamin E.⁴⁹¹⁻⁴⁹³ It was found that effective antioxidants were present in the sterol-free unsaponifiable extracts prepared from cottonseed, palm, and wheat germ oils. The substance or substances responsible for the stabilizing action were christened *inhibitols*,⁴⁸⁸ since their activity, like that of the phenolic antioxidants, was related to the presence of free hydroxyl groups.

However, although it had been impossible to separate the inhibitols from vitamin E, there was for some time considerable doubt that they were identical, since the relationship between the antisterility and the antioxi-

⁴⁸⁹ H. N. Stephens, *J. Am. Chem. Soc.*, 58, 219-224 (1936).

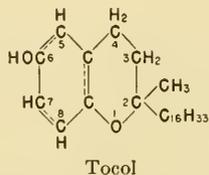
⁴⁹⁰ H. S. Olcott and H. A. Mattill, *J. Am. Chem. Soc.*, 58, 2204-2208 (1936).

⁴⁹¹ E. M. Bradway and H. A. Mattill, *J. Am. Chem. Soc.*, 56, 2405-2408 (1934).

⁴⁹² H. S. Olcott and H. A. Mattill, *J. Biol. Chem.*, 93, 65-70 (1931).

⁴⁹³ H. S. Olcott and H. A. Mattill, *J. Biol. Chem.*, 104, 423-435 (1934).

dant activity proved to be quite variable. However, the isolation⁴⁹⁴ of the several forms of vitamin E, which are now referred to as tocopherols, has largely explained the discrepancies, since it was found that α -tocopherol is most effective as an antisterility agent while β - and γ -tocopherols excel as antioxidants.⁴⁹⁵ The recent demonstration of the presence of another tocopherol, δ -tocopherol, in soybean oil,⁴⁹⁶ which has a higher antioxidant value than γ -tocopherol but an extremely low biological activity, gives further credence to the belief that the tocopherols are actually important natural antioxidants, in spite of the irregularities of biological and antioxidant assays in different oils.



The formula for the tocopherols can readily be compared by considering them as derivatives of tocol:

α -Tocopherol	5,7,8-Trimethyltol
β -Tocopherol	5,8-Dimethyltol ⁴⁹⁷
γ -Tocopherol	7,8-Dimethyltol ⁴⁹⁷
δ -Tocopherol	8-Methyltol ⁴⁹⁶

The tocopherols resemble the phenolic inhibitors in furnishing an aromatic ring and one free hydroxyl group (position 6). However, it has been shown from a study of the parent chromans⁴⁹⁸ that the oxygen in the heterocyclic ring (position 1) is required for antioxygenic activity, just as the second hydroxyl is essential in the diphenols, catechol, and hydroquinone. Moreover, all ethers and esters (except the allophanates) are inactive, as is also the case with the phenolic inhibitors. δ -Tocopherol with one methyl group is the most effective of the series, followed by the β - and γ -compounds, while α -tocopherol is the least active. The relative effectiveness of the tocopherols as antioxidants has been shown by Hove and Hove⁴⁹⁹ to be related to temperature. Whereas the relative activities of α -, β -, and γ -tocopherols were found to be approximately equal in protecting carotene at 35° and 60°C., γ -tocopherol was shown to be the best at 98°C., followed by β -tocopherol, while the α -compound had the lowest activity. A similar

⁴⁹⁴ H. M. Evans, O. H. Emerson, and G. A. Emerson, *J. Biol. Chem.*, **113**, 319-332 (1936).

⁴⁹⁵ H. S. Olcott and O. H. Emerson, *J. Am. Chem. Soc.*, **59**, 1008-1009 (1937).

⁴⁹⁶ M. Stern, C. D. Robeson, L. Weisler, and J. G. Baxter, *J. Am. Chem. Soc.*, **69**, 869-874 (1947).

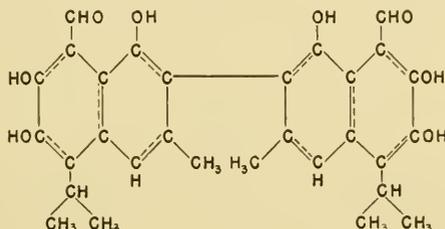
⁴⁹⁷ O. H. Emerson and L. I. Smith, *J. Am. Chem. Soc.*, **62**, 1869-1872 (1940).

⁴⁹⁸ C. Golumbic, *J. Am. Chem. Soc.*, **63**, 1163-1164 (1941).

⁴⁹⁹ E. L. Hove and Z. Hove, *J. Biol. Chem.*, **156**, 623-632 (1944).

decrease in the stabilizing activity of hydroquinone occurs with the successive addition of methyl groups.

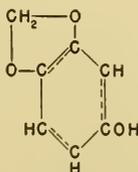
(c) *Gossypol and Sesamol*. Other antioxidants are present in vegetable oils. These include gossypol in cottonseed oil and sesamol in sesame oil. Mattill⁴⁵³ and also Royce^{500,501} demonstrated that gossypol, which may occur in an amount as high as 1% in expeller oil, is an excellent antioxidant. In fact, Royce ascribes the stability of crude cottonseed oil, as compared with the refined product, to this pigment. It has likewise proved effective in the protection of lard from developing rancidity.⁴³³ The formula for gossypol given here⁵⁰² indicates the multiphenolic nature of the molecule to which its stabilizing effect must be ascribed.



Gossypol

Gossypol has been shown to be capable of protecting carotene from destruction *in vitro* by preformed fat peroxides.⁵⁰³ The protective action has likewise been observed *in vivo*, where it was found that 1-milligram doses either of gossypol or of dianilinogossypol were effective in preventing the destruction of carotene fed with lard or with methyl linoleate.⁵⁰⁴ These compounds had an activity only slightly inferior to that of synthetic α -tocopherol. Cottonseed meal was found to be just as effective as wheat germ in stabilizing carotene in ethyl oleate *in vitro*.

Sesame oil has the property of protecting fats from developing rancidity when mixed with them. The effective inhibitor has been shown to be sesamol,⁴²⁸ which is closely related to sesamolign; this is believed to be a glu-



Sesamol

⁵⁰⁰ H. D. Royce, *Oil & Soap*, 10, 123-125 (1933).

⁵⁰¹ H. D. Royce and F. A. Lindsey, Jr., *Ind. Eng. Chem.*, 25, 1047-1050 (1933).

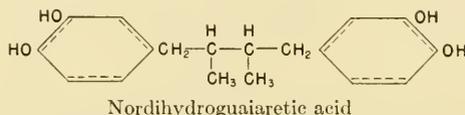
⁵⁰² R. Adams, R. C. Morris, T. A. Geissman, D. J. Butterbaugh, and E. C. Kirkpatrick, *J. Am. Chem. Soc.*, 60, 2193-2204 (1938).

⁵⁰³ E. L. Hove and Z. Hove, *J. Biol. Chem.*, 156, 611-621 (1944).

⁵⁰⁴ E. L. Hove, *J. Biol. Chem.*, 156, 633-642 (1944).

coside of sesamol.⁵⁰⁵ It is suggested that the possible slow transformation of sesamol to sesamol creates an especially effective environment to prevent the development of rancidity. On the other hand, another closely related compound, sesamin, is devoid of antioxidant action.⁴²⁸ A potent antioxidant, presumably sesamol, is concentrated by molecular distillation.⁵⁰⁶

(d) *Nordihydroguaiaretic Acid (NDGA)*. Nordihydroguaiaretic acid has proved to be an excellent antioxidant. Its protective action on fats was first described by Lundberg and associates,⁵⁰⁷ and its use for this purpose is the basis for a public service patent.⁵⁰⁸ This product is prepared by the extraction of the desert plant, *Larrea divaricata*, which is commonly known as the creosote bush. The plant is indigenous to the southwest desert areas of the United States and of Mexico. It can readily be pre-



pared by a method described by Gisvold⁵⁰⁹ and is available commercially.⁵¹⁰ When added to lard, this compound imparts no undesirable flavor, odor, or color to the product⁴⁵⁵; lard so treated has been reported to remain sweet for over a year, as determined by organoleptic tests.⁵⁰⁷ Cream to which 0.00125 or 0.005% of NDGA was added did not develop an oxidized flavor when frozen over an 11-month period.⁵¹¹

Nordihydroguaiaretic acid is a white crystalline solid, melting at 184–185°C., which is slightly soluble in water and dilute acids, moderately soluble in hot benzene and xylene, and very soluble in diethyl ether, alcohol, and glacial acetic acid.⁵⁰⁷ It dissolves sparingly in fats⁵¹² to the extent of 0.5%. NDGA is 2,3-bis-(3,4-dihydroxybenzyl)-butane (4,4-(2,3-dimethyltetramethylene)-dipyrocatechol. Schroeter and co-workers⁵¹³ first synthesized the compound in 1918. Haworth *et al.*⁵¹⁴ synthesized the dimethyl ether, while Lieberman, Mueller, and Stiller⁵¹⁵ have recently re-

⁵⁰⁵ J. Böeseken, W. D. Cohen, and C. J. Kip, *Rec. trav. chim.*, *55*, 816–820 (1936).

⁵⁰⁶ D. P. Grettie, *U. S. Patent* No. 2,052,289 (Aug. 25, 1936).

⁵⁰⁷ W. O. Lundberg, H. O. Halvorson, and G. O. Burr, *Oil & Soap*, *21*, 33–35 (1944).

⁵⁰⁸ W. M. Lauer (to U. S. Secretary of Agriculture), *U. S. Patent* No. 2,373,192 (Apr. 10, 1945).

⁵⁰⁹ O. Gisvold (to Regents, University of Minnesota), *U. S. Patent* No. 2,382,475 (Aug. 14, 1945).

⁵¹⁰ Manufactured by Wm. J. Stange Co., Chicago 12, Ill.

⁵¹¹ J. W. Stull, E. O. Herried, and P. H. Tracy, *J. Dairy Sci.*, *31*, 1024–1028 (1948).

⁵¹² W. O. Lundberg and H. O. Halvorson, *Proc. Inst. Food Tech.*, *1945*, 115–125; *Chem. Abst.*, *40*, 6847 (1946).

⁵¹³ G. Schroeter, L. Lichtenstadt, and D. Irineu, *Ber.*, *51*, 1587–1613 (1918).

⁵¹⁴ R. D. Haworth, C. R. Mavin, and G. Sheldrick, *J. Chem. Soc.*, *1934*, 1423–1429.

⁵¹⁵ S. V. Lieberman, G. P. Mueller, and E. T. Stiller, *J. Am. Chem. Soc.*, *69*, 1540–1541 (1947).

ported its synthesis from two molecules of 1-piperonyl-1-bromoethane. This latest work confirms the structure assigned to it by Haworth *et al.*⁵¹⁴ The addition of NDGA to fats up to a maximum concentration of 0.01% was permitted by the War Food Administration under the Meat Inspection Division (U. S. Department of Agriculture), Memorandum No. 25, issued December, 1943.

(e) *Gum Guaiac*. The active principle in gum guaiac is presumably guaiaretic acid. The gum is a secretion of the tropical tree, *Guajacum officinale* (lignumvitae). Newton and Grettie⁵¹⁶ have described its use for the protection of lard, and the federal government has approved the addition of this product to lard up to a maximum of 0.1%. Grettie⁵¹⁷ has demonstrated its effectiveness as an antioxidant. Higgins and Black⁵¹⁸ have reported that the addition of gum guaiac in amounts of 0.1% to prime steam lard delayed rancidity from 6 to 24 hours, as determined by the active oxygen method, from 7 to 23 days, as determined by the oven test at 60°C., and from 5 to 35 days, according to the oven test on crackers at 60°C. Gum guaiac behaves like the phenolic antioxidants, but it is less effective than most of them. It resembles them in being more effective on animal than on vegetable fats.⁴⁵⁸ The complete innocuousness of gum guaiac, when fed in amounts far in excess of any possible level of consumption in fats, has been demonstrated by Johnson, Carlson, Kleitman, and Bergstrom.^{518a}

(f) *Propyl Gallate and Gallic Acid*. Boehm and Williams^{519,520} first described the use of propyl gallate as a potent phenolic antioxidant. It has a lower toxicity than pyrogallol. It dissolves sparingly in fats, although Lundberg⁴⁵⁸ states that 1.6 g. will dissolve in 100 g. of soybean oil. It has a lower solubility in lard,⁵¹² but it is extremely soluble in ethanol.⁴⁵⁸ Propyl gallate is approved by the Meat Inspection Division of the U. S. Department of Agriculture⁵²¹ as a stabilizer for fats in amounts not exceeding 0.01%. Higgins and Black⁵¹⁸ have reported that propyl gallate is effective for preserving lard but not for crackers.

Lea⁵²² has demonstrated that lower aliphatic esters of gallic acid other than the propyl ester are likewise effective antioxidants. The use of some

⁵¹⁶ R. C. Newton and D. P. Grettie (to Swift & Co.), *U. S. Patent* No. 1,903,126 (March 28, 1933); *French Patent* No. 741,417 (Aug. 20, 1932); *Chem. Abst.*, 27, 2833, 3354 (1932).

⁵¹⁷ D. P. Grettie, *Oil & Soap*, 10, 126-127 (1933).

⁵¹⁸ J. W. Higgins and H. C. Black, *Oil & Soap*, 21, 277-279 (1944).

^{518a} V. Johnson, A. J. Carlson, N. Kleitman, and P. Bergstrom, *Food Research*, 3, 555-574 (1938).

⁵¹⁹ E. Boehm and R. Williams, *Quart. J. Pharm. Pharmacol.*, 16, 232-243 (1943).

⁵²⁰ E. Boehm and R. Williams, *Pharm. J.*, 151, 53, 163-164 (1943).

⁵²¹ U. S. Dept. Agr., Meat Inspection Div., *Memo. No.* 104, Cited by W. O. Lundberg, Publication No. 20, Hormel Institute, University of Minnesota, 1947, p. 25.

⁵²² C. H. Lea, *J. Soc. Chem. Ind.*, 63, 107-112 (1944).

lower alkyl esters of gallic acid including propyl gallate is covered by a patent.⁵²³ Norris and Riemenschneider⁵²⁴ have recently reported the synthesis of the hexyl, octyl, dodecyl, tetradecyl, hexadecyl, and octadecyl esters of gallic acid.

Gallic acid itself has been shown by Olcott and Mattill⁴⁹⁰ and by Golumbic and Mattill⁵²⁵ to have effective antioxidant properties. Thus, the induction period of lard as determined by the oxygen absorption at 75°C. was prolonged from 12 hours to 213 hours by the addition of 0.06% of the acid. Gallic acid has also been shown to exert a synergistic action on other antioxidants, and for this reason it should find an application in the protection of vegetable as well as of animal fats. As yet there is no proof of its innocuousness, and therefore its use has not been approved. It is quite insoluble in fats, although it dissolves to a sufficient extent to exert its antioxidant effect.⁵¹² The stabilizing effects of gallic acid on fats have been demonstrated by Filer *et al.*⁵²⁶ and by Mattil *et al.*⁵²⁷

(g) *Tannins and Tannic Acid.* Spannum, McGuine, and Crapple⁵²⁸ have found that tannins are excellent antioxidants. When added to lard they have protective factors (ratios of keeping time with antioxidant to keeping times of controls) varying from 2.5 to 6.2. The antioxidant activity is presumably related to the gallic acid present in tannins. Several patents cover the use of tannins and tannic acid in fats.^{529,530}

(h) *Ascorbic Acid and Its Esters.* Golumbic and Mattill⁵³¹ have found that ascorbic acid by itself is a relatively ineffective antioxidant in animal fats, although it does have a synergistic action in connection with tocopherol, hydroquinone, and similar products. The poor solubility of ascorbic acid in fat has been overcome by the use of fat-soluble esters such as ascorbyl palmitate. Although these esters are relatively inactive in animal fats, they are especially satisfactory in vegetable fats, and they have a synergistic effect on the phospholipids and tocopherols.⁴⁵³ The use of these esters in fats has been patented.⁵³²

The retarding effects of some of the common antioxidants on the rate of development of rancidity are summarized in Tables 41 and 42.

⁵²³ T. Sabalitschka and E. Böhm (to Heyden Chem. Corp.) *U. S. Patent* No. 2,255,191 (Sept. 9, 1941).

⁵²⁴ S. G. Norris and R. W. Riemenschneider, *J. Am. Chem. Soc.*, **68**, 500-501 (1946).

⁵²⁵ C. Golumbic and H. A. Mattill, *Oil & Soap*, **19**, 144-145 (1942).

⁵²⁶ L. J. Filer, Jr., K. F. Mattil, and H. E. Longenecker, *Oil & Soap*, **21**, 289-292 (1944).

⁵²⁷ K. F. Mattil, L. J. Filer, Jr., and H. E. Longenecker, *Oil & Soap*, **21**, 160-161 (1944).

⁵²⁸ H. T. Spannum, T. H. McGuine, and G. A. Crapple, *Oil & Soap*, **23**, 110-113 (1946).

⁵²⁹ S. Musher (to Musher Foundation), *U. S. Patents* Nos. 2,233,142 (Feb. 15, 1941) and 2,282,811 (May 12, 1942).

⁵³⁰ B. J. Verbeek (to Wilson and Co.), *U. S. Patent* No. 2,354,719 (Aug. 1, 1944).

⁵³¹ C. Golumbic and H. A. Mattill, *J. Am. Chem. Soc.*, **63**, 1279-1280 (1941).

⁵³² P. A. Wells and R. W. Riemenschneider (to Secretary of Agriculture), *U. S. Patent* No. 2,368,435 (Jan. 30, 1945).

TABLE 41
ANTIOXIDANT ACTIVITY OF SEVERAL COMPOUNDS WHEN ADDED ALONE AT SEVERAL LEVELS TO FATS

Antioxidants added	Interval before development of rancidity						Investigator
	Active oxygen method	Oven test at 60°C, days	Oven test with crackers at 60°C, days	Keeping time hrs.	Protection index		
Prime steam lard.....	hrs. 6	7	5	—	—	—	a
0.01% gum guaiac.....	10	9	12	—	—	—	—
0.10% gum guaiac.....	24	23	35	—	—	—	b
Special lard.....	—	—	—	11	—	—	—
0.01% hydroquinone.....	—	—	—	84	8	—	—
0.05% hydroquinone.....	—	—	—	190	17	—	—
0.01% α-tocopherol.....	—	—	—	33	3	—	—
0.05% α-tocopherol.....	—	—	—	97	9	—	—
0.01% NDGA.....	—	—	—	210	19	—	—
0.05% NDGA.....	—	—	—	279	25	—	—
Steam-rendered lard.....	—	—	—	3	—	—	—
0.02% NDGA.....	—	—	—	96	32	—	—
0.10% NDGA.....	—	—	—	168	56	—	—
Prime steam lard.....	6	7	8	—	—	—	d
0.01% propyl gallate.....	33	30	11	—	—	—	—
0.05% propyl gallate.....	135	124	13	—	—	—	—
0.10% propyl gallate.....	145	135	14	—	—	—	—
Refined-bleached cottonseed oil.....	12	10	—	—	—	—	—
0.05% propyl gallate.....	45	35	—	—	—	—	—
Refined-bleached soybean oil.....	18	14	—	—	—	—	—
0.05% propyl gallate.....	54	45	—	—	—	—	c
Lard.....	12	—	—	—	—	—	—
0.06% gallic acid.....	213	—	—	—	—	—	—
Lard #1.....	6 0	—	—	—	—	—	d
0.1% U.S.P. tannic acid.....	37.3	—	—	—	—	6.2	—
Oleo oil.....	7 0	—	—	—	—	—	—
0.1% U.S.P. tannic acid.....	77.8	—	—	—	—	11.1	—

^a J. W. Higgins and H. C. Black, *Oil & Soap*, 21, 277-279 (1944).
^b W. O. Lundberg, H. O. Halvorson, and G. O. Burr, *Oil & Soap*, 21, 33-35 (1944).
^c C. Golumbic and H. A. Mattill, *Oil & Soap*, 19, 144-145 (1942).
^d H. T. Spannuth, T. H. McGuine, and G. A. Crapple, *Oil & Soap*, 23, 110-113 (1946).

TABLE 42
INDUCTION PERIODS OF LARDS CONTAINING 0.01% OF ANTIOXIDANT AS DETERMINED
BY ACTIVE OXYGEN TEST AT 98° C.^a

Antioxidant	Description	Induction period, hours
Control	—	6
α -Tocopherol	Merck	21
40% Tocopherol	Distillation Products Industries	17
Gum guaiac (whole)	S. B. Penick and Co.	10
Guaiaretic acid	M.p., 79.5–80.5°C.	8
α -Guaiaconic acid	M.p., 112–115°C.	12
β -Guaiaconic acid	M.p., 132.5–133.5°C.	8
Hydroquinone	Merck	80
NDGA	Pure crystals; m.p., 186.5–187.5°C.	107
NDGA	Wm. J. Stange Co.; m.p., 184–187°C.	95
Propyl gallate	Purified; m.p., 147°C.	70–77
Propyl gallate	Heyden Chem. Corp.; m.p., 144–148°C.	54–75
Gallic acid	Merck reagent	105

^a W. O. Lundberg, *Publication No. 29, Hormel Institute, University of Minnesota* 1947.

(i) *Butylhydroxyanisole*. Kraybill and associates⁵³³ have recently reported that butylated hydroxyanisole (BHA) is an especially satisfactory antioxidant for animal fats. It is readily soluble in fats and practically insoluble in water. The commercial product consists chiefly of two isomers, namely 3-*tert*-butyl-4-hydroxyanisole (2-*tert*-butyl-4-methoxyphenol) and 2-*tert*-butyl-4-hydroxyanisole. The stability of lard was increased from 4 to 18 hours by the addition of 0.005% of the antioxidant. It was shown to have a synergistic action with acids, hydroquinone, lecithin, methionine, and thiodipropionic acid. When combined with hydroquinone or propyl gallate and an acid synergist, it was found to be very effective for protecting crackers and pastry from spoilage where lard was used in the preparation. The product is harmless when fed at several hundred times the level permitted by the Meat Inspection Division of the Bureau of Animal Industry, U. S. Department of Agriculture (Memorandum No. 118, December, 1948).

(j) *Other Antioxidants*. Compounds related to vitamin K have also been shown to exert antioxidant activity.⁴⁹⁸ This is likewise true for crude lecithin.^{534–537} The lecithin preparations are more active toward

⁵³³ H. R. Kraybill, L. R. Dugan, Jr., B. W. Beadle, F. C. Vibrans, V. Swartz, and H. Rezabek, *J. Am. Oil Chemists' Soc.*, **26**, 449–453 (1949).

⁵³⁴ H. Bollmann, *U. S. Patent* No. 1,575,529 (March 2, 1926).

⁵³⁵ G. I. Evans, *Ind. Eng. Chem.*, **27**, 329–331 (1935).

⁵³⁶ E. W. Kochenderfer and H. G. Smith, *Proc. Iowa Acad. Sci.*, **39**, 169–170 (1932).

⁵³⁷ H. N. Holmes, R. E. Corbet, and R. A. Ragatz, *Ind. Eng. Chem.*, **28**, 133–135 (1936).

vegetable oils than toward animal fats,⁴⁸⁶ although the latter fats are also protected if tocopherols or other inhibitols are added to them. The activity is entirely in the cephalin fraction, and may be related to the ionizable phosphoric acid in the phosphatidyl serine.⁴⁸⁶

Green and Hilditch⁵³⁸ and Hilditch and Paul⁵³⁹ have reported antioxygenic activity in cereal and oil-seed flours, which can be only partially extracted with fat solvents. It is stated that the enzyme catalase, which speeds up the decomposition of hydrogen peroxide, may be a good stabilizer.⁵⁴⁰ However, the effectiveness of liver as an antioxidant continues after the disappearance of the catalase.⁵⁴¹ The activity of cottonseed meal cannot be ascribed to this enzyme.⁴²⁸ Finally, highly purified catalase preparations were found to be ineffective in preventing rancidity in lard.⁴²⁸

In spite of the very potent stabilizing effect of tocopherols and other inhibitols on animal fats, they possess but little activity in the vegetable fats from which they are derived.⁴⁸⁹ On the other hand, a number of acids such as phosphoric, citric, and ascorbic acids have a considerable anti-oxygenic action on vegetable oils,⁴⁹⁰ while they are largely inactive in animal fats. However, since these acids are active only when inhibitols are present, it is assumed that they exert a synergistic effect on the latter compounds. The synergistic effects of the citric acid, phospholipids, and D-isoascorbyl palmitate on the activity of such inhibitols as NDGA and α -tocopherol are outlined in Table 43.

Isopropyl citrate esters (predominantly mono-isopropyl citric ester), in a vehicle consisting of a 1:1 mixture of mono- and diglycerides^{541a} and stearyl citrate esters (predominantly distearyl citrate),^{541b} have recently been shown to be especially effective in preventing the so-called "flavor reversion" which may occur in refined limpid soybean oil or in hydrogenated soybean oil. The isopropyl citrate esters in the mono- and diglyceride vehicle are fat-dispersible, while the stearyl citrate esters are readily fat-soluble. It is proposed that the isopropyl citrate esters plus vehicle be used in margarine at a level not to exceed 0.02%, and the stearyl citrate esters at a level of not more than 0.15%.^{541c} No toxic effects could be demonstrated when these products were administered to rats, rabbits, or

⁵³⁸ T. G. Green and T. P. Hilditch, *J. Soc. Chem. Ind.*, 56, 23-26T (1937).

⁵³⁹ T. P. Hilditch and S. Paul, *J. Soc. Chem. Ind.*, 58, 21-24 (1939).

⁵⁴⁰ M. R. Coe, *Oil & Soap*, 15, 230-236 (1938).

⁵⁴¹ K. Täufel and R. Müller, *Biochem. Z.*, 304, 275-284 (1940).

^{541a} R. H. Neal, C. M. Gooding, and H. W. Vahlteich (to The Best Foods, Inc.), *U. S. Patent* No. 2,485,631 (Oct. 25, 1949).

^{541b} C. M. Gooding, H. W. Vahlteich, and R. H. Neal (to The Best Foods, Inc.), *U. S. Patent* No. 2,485,633 (Oct. 25, 1949).

^{541c} "Oleomargarine. Definitions and Standards of Identity. Notice of Hearing to Amend Definition and Standard of Identity," *Fed. Register*, 16, 1640-1641 (Feb. 16, 1951).

TABLE 43
 SYNERGISTIC EFFECT OF CITRIC ACID, D-ISOASCORBYL PALMITATE, AND PHOSPHOLIPIDS
 ON ANTIOXIDANT ACTIVITY OF NDGA AND α -TOCOPHEROL

Substrate and antioxidants added	Keeping quality (active oxygen method), hours
Lard alone ^a	2
+0.005% NDGA.....	12
+0.005% NDGA + 0.005% D-isoascorbyl palmitate.....	43
+0.005% NDGA + 0.005% citric acid.....	48
+0.005% NDGA (deodorized).....	11
+0.005% NDGA (deodorized) + 0.005% D-isoascorbyl palmitate....	41
Prime steam lard alone ^b	4
+0.06% D-isoascorbyl monoester.....	4
+0.003% phospholipids.....	7.5
+0.03% phospholipids + 0.06% D-isoascorbyl monoester.....	26
+0.12% D-isoascorbyl monoester.....	6.5
+0.12% D-isoascorbyl monoester + 0.03% phospholipids.....	44
+0.001% α -tocopherol.....	4.5
+0.001% α -tocopherol + 0.06% D-isoascorbyl monoester.....	7
+0.001% α -tocopherol + 0.03% phospholipids.....	10
+0.001% α -tocopherol + 0.03% phospholipids + 0.06% D-isoascorbyl monoester.....	40
+0.001% α -tocopherol + 0.03% phospholipids + 0.12% D-isoascorbyl monoester.....	60

^a R. W. Riemenschneider, S. F. Herb, E. M. Hammaker and F. E. Ludly, *Oil & Soap*, 21, 307-309 (1944).

^b R. W. Riemenschneider, J. Turer, P. A. Wells, and W. C. Ault, *Oil & Soap*, 21, 47-50 (1944).

dogs, in amounts as much as 500 times the proposed level of consumption by man.^{541d}

e. Synergists for Antioxidants. The use of synergistic agents to bolster the effectiveness of the antioxidants has been a fairly recent development. Thus, substances in this category will increase the activity of the natural antioxidants in vegetable fats, and they will likewise decrease the amount of inhibitor required to achieve the desired stability in animal fats.

The mechanism of this synergism seems to have been fairly definitely proved with ascorbic acid. Its effect on the stabilization of milk fat was shown by feeding it to cows^{542,543} or by its direct addition to milk.^{544,545} It is also used to prevent rancidity in mayonnaise.⁵⁴⁶ When tocopherol and

^{541d} H. J. Deuel, Jr., S. M. Greenberg, C. E. Calbert, R. Baker and H. R. Fisher, *Food Research*, 16, 258-280 (1951).

⁵⁴² W. C. Brown, L. M. Thurston, and R. B. Dustman, *J. Dairy Sci.*, 20, 133-145 (1937).

⁵⁴³ W. C. Brown, A. H. Vanlandingham, and C. E. Weakley, Jr., *J. Dairy Sci.*, 22, 345-351 (1939).

⁵⁴⁴ F. Kieferle and A. Seuss, *Milchw. Forsch.*, 20, 23-38 (1939).

⁵⁴⁵ G. M. Trout and E. C. Gjessing, *J. Dairy Sci.*, 22, 271-281 (1939).

⁵⁴⁶ P. J. Gray and I. Stone, *Food Industries*, 11, 626-628 (1939).

ascorbic acid were both present in autoxidizing fat, the induction period was greatly prolonged over that noted when only tocopherol was present.⁵³¹ On the other hand, animal fats containing no tocopherol became rancid while much of the ascorbic acid still remained.⁴²³ In the presence of tocopherol, however, the ascorbic acid became oxidized. Thus, while ascorbic acid was found to be an excellent stabilizer for tocopherol,⁵⁴⁷ it is of little value alone in preventing rancidity, since it is not oxidized by fat peroxides and does not prevent their formation. However, because ascorbic acid is able to protect tocopherol, the onset of rancidity is greatly delayed by the so-called synergistic action of this substance.

Clausen, Lundberg, and Burr⁵⁴⁸ have classified the various synergists, in Table 44, according to the degree of protective action which they exert on fats. In addition to ascorbic acid, various amino acids and protein derivatives are included as synergistic compounds.

In addition to the effects listed in Table 44, the authors report definite

TABLE 44
COMPARATIVE SYNERGISM OF SOME AMINO ACIDS AND OTHER SUBSTANCES WITH
ANTIOXIDANT ACTION OF SOME PHENOLIC ANTIOXIDANTS IN LARD^a

Phenolic antioxidant	Synergists having protective indices ^b as follows		
	4.0-3.1	3.0-2.1	2.0-1.6
α -Tocopherol	None	Ascorbic acid, methionine	Threonine, leucine, milk protein hydrolysate #2, norvaline, ascorbyl palmitate, phenylalanine, milk protein hydrolysate #1, cysteine
Hydroquinone	Methionine	Ascorbic acid, milk protein hydrolysate #1, tryptophane	Leucine, milk protein hydrolysate #2, ascorbyl palmitate, proline, phenylalanine, cysteine, alanine, glutamic acid, valine, peptic digest of pancreas protein, asparagin, arginine, barbituric acid, arspenamine, ninhydrin, propanidone, histidine, norleucine, glycerophosphoric acid, trypsin, HCl hydrolysate of casein
Nordihydroxyaraietic acid	None	Methionine	Phenylalanine, leucine, tryptophane, alanine, norleucine, milk protein hydrolysate #2, norvaline, valine, ascorbic acid

^a Adapted from D. F. Clausen, W. O. Lundberg, and G. O. Burr, *J. Am. Oil Chemists' Soc.*, *24*, 403-404 (1947).

^b Protective index is ratio of keeping time in hours of substrate plus 0.01% of phenolic antioxidant and plus 0.01% of synergist to that of substrate with 0.01% of phenolic antioxidant alone.

effects (protective index 1.3-1.1) in the case of other amino acids and related products as well.

f. Prooxidants. Prooxidants may be defined as substances which increase the susceptibility to oxidation.⁵⁴⁹ In this group of substances, the

⁵⁴⁷ O. Isler, *Helv. Chim. Acta*, *21*, 1756-1759 (1938).

⁵⁴⁸ D. F. Clausen, W. O. Lundberg, and G. O. Burr, *J. Am. Oil Chemists' Soc.*, *24*, 403-404 (1947).

⁵⁴⁹ L. A. Hamilton and H. S. Olcott, *Ind. Eng. Chem.*, *29*, 217-223 (1937).

carotenoids and chlorophyll are the most common natural products which act on fats. Carotene, xanthophyll, and lycopene have all been shown to lower the induction period of lard or mixtures of lard and cod liver oil.^{484, 485, 488, 500} However, under the usual conditions, the carotenoids do not have an opportunity to exert their prooxidant effect, since this reaction is prevented by the inhibitols also present. Thus, the xanthophyll in butter or chicken fat has only slight prooxidant activity.⁵⁵⁰ In fact, Newton⁵⁵¹ was formerly of the opinion that carotene is an antioxidant, since the fatty extracts of alfalfa, paprika, and palm oil act as stabilizers for lard. However, it is apparent that this effect cannot be attributed to the carotene, since it is enhanced when the carotene is bleached.⁴⁸⁸ That some specificity in activity is exhibited is shown by the fact that carotene and xanthophyll are prooxidants for oleic and linoleic acids, but are inactive toward linseed and olive oils.⁵⁵²

Chlorophyll is especially effective in augmenting the oxidation of oils when they are exposed to light.⁵⁴⁰ The induction period of the oil is markedly lowered in the presence of chlorophyll, and rancidity develops with a very low peroxide value.⁵⁴⁰ The prooxidant activity of chlorophyll is increased by purification.⁵⁵⁰ The amount of this pigment is appreciable in crude oils, but it is present only in traces in refined oils.⁵⁵³ However, the process of refining usually removes a considerable amount of inhibitols, so that the net result of purification is a product which is less stable, in spite of the fact that it also contains less prooxidant. However, chlorophyll does not lower the induction period of lard when there is only ordinary indoor illumination.⁴²⁸

Although the concentration of metals in oils is extremely minute, it may be sufficient to promote a more rapid oxidation of the fat. Copper and iron salts are the most potent prooxidants.⁴⁵³ The antioxidant effect which has been demonstrated for potassium cyanide⁵³⁵ occurs only when cobalt oleate is present; cyanide is therefore not a true antioxidant but only an antagonist to the prooxidant or an *anti-prooxidant*. In the absence of the metallic catalysts, cyanide is entirely ineffective in preventing oxidation.⁴²⁸

(5) *Drying and Hardening Properties*

The unsaturated linkages of fat are responsible not only for the development of oxidative rancidity but also for the formation of hard insoluble films. This reaction, which is of great importance in the paint and varnish industry, involves the formation of polymers following the absorption of

⁵⁵⁰ K. Täufel and R. Müller, *Biochem. Z.*, *304*, 137-146 (1940).

⁵⁵¹ R. C. Newton, *Oil & Soap*, *9*, 247-252 (1932).

⁵⁵² W. Franke, *Z. physiol. Chem.*, *212*, 234-255 (1932).

⁵⁵³ W. G. Bickford, S. Anderson, and K. S. Markley, *Oil & Soap*, *17*, 138-143, 252-256 (1940).

oxygen. Not all oils are capable of this change; only those which possess a large proportion of unsaturated acids can undergo this reaction. The ability of a fat to act as a drying oil is therefore roughly proportional to the iodine number, *i.e.*, the higher the iodine value the more effectively an oil can serve in such a capacity. However, the latter constant is not an absolute index, and one is forced to resort also to the following practical test. A so-called "drying oil," when spread in a thin layer on a glass plate, will dry to an acetone-insoluble film in 2 to 6 days. The "semidrying" oil will become somewhat sticky after a week, while a "nondrying" oil will still be fluid after exposure to air for 18 to 20 days. The boundaries between these classes of fats are naturally somewhat arbitrary, but the following grouping is fairly satisfactory:

Drying oils	Semidrying oils	Nondrying oils
Tung	Soybean	Olive
Linseed	Corn	Peanut
Poppyseed	Cottonseed	Castor
Sunflower seed	Rapeseed	Coconut
Hempseed	Mustard	
Walnut		
Fish		

Hilditch⁵⁵⁴ has used a formula based upon total polyethenoid acids and the linolenic acid content to ascertain the so-called "quick-drying" index. The formula employed is as follows:

$$\text{Quick-drying index} = P \times Len \times 10^{-2}$$

$$P = Lin \text{ (linoleic acid)} + Len \text{ (linolenic acid)}$$

The percentage of P is an index of drying power and this should not fall below 65–70 in a satisfactory drying oil. The "quick-drying" index based on P multiplied by linolenic acid content is considered as a relative but not as a quantitative measure of the property of this fat. An oil with properties comparable to those of linseed oil should have an index "about 25 (or not much less)." These calculations of Hilditch are summarized in Table 45.

The mechanism by which this transformation takes place is not thoroughly understood. The free fatty acids of linseed and tung oils give completely negative results in film formation, as do their esters with monatomic alcohols.^{555,556} Likewise, the mono- and even the diglycerides of the linseed oil fatty acids will not undergo the drying reaction, although the diglycerides may acquire this property on heating. Also the mono- and diglycol esters of these fatty acids are completely inactive until subjected to heat treatment.⁵⁵⁶

⁵⁵⁴ T. P. Hilditch, *J. Oil Colour Chem. Assoc.*, 31, 1–24 (1948).

⁵⁵⁵ T. F. Bradley, *Ind. Eng. Chem.*, 29, 440–445 (1937).

⁵⁵⁶ T. F. Bradley, *Ind. Eng. Chem.*, 29, 579–584 (1937).

TABLE 45
 INDICES OF DRYING POWER AND COMPONENTS OF DRYING AND SEMIDRYING OILS^a

Oil	Iodine value	Per cent component fatty acids				Polyethenoid acids <i>P</i>	"Quick- drying," index ^b
		Satd.	Oleic	Linoleic	Linolenic		
Perilla (<i>Perilla ocimoides</i>).....	205	7	20 (?)	5 (?)	68 (?)	73 (?)	50
Conophor (<i>Tetracarpidium conophorum</i>).....	200	11	12	12	65	77	50
Linseed (<i>Linum usitatissimum</i>).....	180	10	20	20	50	70	35
Lumbang (candlenut) (<i>Aleurites moluccana</i>)..	164	13	10	49	28	77	22
Rubberseed (sol. fraction) (<i>Hevea brasiliensis</i>)..	149	12	22	42	24	66	16
Rubberseed.....	138	21	20	38	21	59	12
Soybean (sol. fraction).....	152	11	18	57	14	71	10
Soybean (<i>Glycine soja</i>).....	133	19	22	50	9	59	5
Nigerseed (<i>Guizotia abyssinica</i>).....	136	15	15	66	4	70	3
Sunflowerseed (<i>Helianthus annuus</i>).....	136	10	22	68	0	68	0
Safflowerseed (<i>Carthamus tinctorius</i>).....	133	7	56	67	0	67	0
Sesame seed (<i>Sesamum indicum</i>).....	113	18	37	45	0	45	0
Corn (<i>Zea mays</i>).....	113	14	43	43	0	43	0
Cottonseed (<i>Gossypium spp.</i>).....	105	26	29	45	0	45	0

^a Adapted from T. P. Hilditch, *J. Oil Colour Chem. Assoc.*, 31, 1-24 (1948).

^b $P \times$ linolenic acid $\times 10^{-2}$.

Polymerization is now believed to be the essential process which brings about the formation of stable films. This requires the presence of either oxygen or heat. In order for condensation or polymerization to occur, a compound must possess at least two functional groups. It is believed that the phenomena of condensation and polymerization represent the same reaction.⁵⁵⁶

Where a molecule has two such functional groups as the hydroxyl and carboxyl, *linear polymers* of great length may occur as the result of a combination in which a functional group of the second molecule reacts with the third, *etc.*⁵⁵⁷ Such a linkage would be akin to a lactone bond, which may be an exceedingly stable linkage.

Although polymers may arise where only two functional groups are present per molecule, much more stable products are formed where polyfunctional groups occur. Rubber, Bakelite, polyvinyl compounds, and synthetic resins are all substances produced by condensation of such molecules. Because the number of reactive groups is too small, the esters of monatomic alcohols, or even of the ethylene glycol, are ineffective. Triglycerides are able to condense because of the large number of functional groups which render possible the formation of stable three-dimensional interlocked polymers of very high molecular weights. Carothers⁵⁵⁷ points out the relationship between the molecular size of the polymer and the percentage of reacting groups (see Table 46).

TABLE 46
RELATIONSHIP BETWEEN DISAPPEARANCE OF FUNCTIONAL GROUPS AND RESULTING
MOLECULAR SIZE OF POLYMER^a

Compound	Fraction of functional groups used (p)	Degree of polymerization
Dimer	0.50	2
Trimer	0.67	3
Tetramer	0.75	4
Pentamer	0.80	5
Decamer	0.90	10
	0.95	20
	0.99	100
	0.999	1000

^a W. H. Carothers, *Chem. Revs.*, 8, 353-426 (1931); *Trans. Faraday Soc.*, 32, 39-53 (1936).

After the functional groups have acted to the extent of 95% ($p = 0.95$), a very slight increase in the functional groups markedly increases the size of the polymer. When the value of p changes from 0.90 to 0.95, the

⁵⁵⁷ W. H. Carothers, *Chem. Revs.*, 8, 353-426 (1931); *Trans. Faraday Soc.*, 32, 39-53 (1936).

groups in the polymer are increased from 10 to 20; on the other hand, when p is raised from 0.99 to 0.999, the number of groups in the molecular complex is augmented from 100 to 1000.

The formation of polymers with the triglycerides is believed by Bradley^{555,556} and Carothers⁵⁵⁷ to be the result of actual valence cross ties between the molecules, although the existence of covalence forces is not entirely precluded. The triglyceride configuration appears to be particularly appropriate, because of space relationships, for the formation of intermolecular combinations of exceeding complexity. For this reason the unsaturated triglycerides occupy such an important position in the paint industry, in the production of insoluble dry films. In addition to the papers of Bradley^{555,556} a very excellent detailed discussion of the phenomenon of polymerization and condensation is included in a series of reports before the Faraday Society.^{557,558}

⁵⁵⁸ Various Authors, *Trans. Faraday Soc.*, 32, 1-412 (1935).

CHAPTER IV

WAXES, HIGHER ALCOHOLS INCLUDING STEROLS, TRITERPENES, GLYCERYL ETHERS, COLORED FATS, AND HYDROCARBONS

1. Waxes

The term, *wax*, originates from the Anglo-Saxon word, *wear*, which was first applied to the natural material in the honeycomb of the bee. The material in the plant kingdom having similar properties was first called *wear* or *wachs*, and later was referred to as *wax*.¹ At the present time, true waxes are considered to be a group of simple lipids, usually solid at ordinary temperatures, in which a simple fatty acid is combined in ester linkage with a monatomic alcohol of high molecular weight. However, in the broader sense of the term, the waxes include not only the simple esters but also certain hydrocarbons, acids, and alcohols which have physical properties similar to those of the naturally occurring waxes. Certain synthetic compounds which are used as wax substitutes are frequently included in this category.

Waxes are widely distributed in nature in both the vegetable and the animal kingdoms. Because of their great resistance to decomposition and their insolubility in water and most solvents, they are frequently found as protective coverings, not only in the case of plants but also of animals. Thus they may serve as a protective covering for the leaves, stems, and fruit of plants. Carnauba wax occurs as a leaf secretion in the carnauba palm (*Copernicia cerifera*). This wax protects the plant from excessive water loss, since it is a species which is indigenous to the arid regions of Brazil. The so-called candelilla wax apparently has a somewhat similar function in the candelilla plant (*Pedilanthus pavonis*), also called wax slipper flower, which grows in Mexico; this latter wax is obtained not only from the leaves but also from the stems of this shrub. Bayberries, obtained from the bayberry plant (*Myrica cerifera*) which grows on the sand dunes along the Atlantic coast of the United States, also yields a wax. The fruit of trees raised in moist areas of the country may likewise be coated

¹ A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947.

with waxes. Thus, the waxes of apple and pear cuticles apparently play a protective role in the case of these fruits.

On the other hand, lanolin, which consists partly of a mixture of esters of cholesterol with palmitic, stearic, and oleic acids, acts in much the same way as do the cuticle waxes, and provides a resistant coating for wool fibers and for the skin, not only of sheep but of practically all fur-bearing animals. In the case of beeswax, this substance acts as a structural element. Finally, in some cases, wax may supplant the triglyceride in the plant. McKinney and Jamieson² have called attention to the absence of glycerides in the ether-soluble portion of the seed of the *Simmondsia chinensis (californica)* (goat-nut, jojoba); 48.3% of the lipid consists of the alcohols docosenol and eicosenol, which alcohols are combined chiefly with docosenoic and eicosenoic acids. This is a typical wax, and its presence in such a large amount represents a unique situation in the plant. The subject of waxes is treated comprehensively from both the chemical and the technological standpoint in the recent monograph of Warth.¹

(1) *Composition of True Waxes*

Much information about the composition of waxes can be gleaned from an inspection of the hydrolysis products. The alcohols so liberated can readily be separated from the soaps, since they continue to be insoluble in water after saponification, while the soaps can be washed out in water. However, because the rupture of the ester linkage takes place with so much difficulty in the case of the waxes, the saponification is necessarily prolonged and difficult. If it is not complete, not only will the ether-soluble residue contain the alcohols but it will be contaminated with the remaining unhydrolyzed wax. The chemistry of the aliphatic alcohols will first be considered, followed by an examination of the sterols.

a. Aliphatic Alcohols. (a) *Distribution.* Lower alcohols are readily prepared in pure form, and their properties are well known. Alcohols lower than octanol (caprylyl) alcohol are ordinarily not found as components of waxes. The higher members of the series, such as ceryl alcohol, $\text{CH}_3(\text{CH}_2)_{24}\text{CH}_2\text{OH}$, and melissyl alcohol, $\text{CH}_3(\text{CH}_2)_{26}\text{CH}_2\text{OH}$, are not known with certainty to exist, as is frequently reported; the preparations of these alcohols may actually be mixtures of several even-numbered homologues rather than a single homogeneous product. Chibnall *et al.*³ have proposed an important revision in nomenclature to cover such discrepancies. For example, they propose that such mixtures of alcohols should be referred to as $\text{C}_{26} + \text{C}_{28} + \text{C}_{30}$ alcohols rather than by specific names. Table 1 gives the formulas and melting points of some of the common saturated monatomic aliphatic alcohols.

² R. S. McKinney and G. S. Jamieson, *Oil & Soap*, 13, 289-292 (1936).

³ A. C. Chibnall, S. H. Piper, A. Pollard, E. F. Williams, and P. N. Sahai, *Biochem. J.*, 28, 2189-2208 (1934).

TABLE I
 NAMES AND PROPERTIES OF SATURATED MONATOMIC ALIPHATIC ALCOHOLS OBTAINED AS
 HYDROLYTIC PRODUCTS OF WAXES OR PREPARED SYNTHETICALLY^a

Common name	Systematic name	No. of C atoms	F.p., °C.	B.p., °C. ^b	$n_D^{t,c}$	$d_4^{t,c}$
Butyl	<i>n</i> -Butanol	4	—	117.4	1.39931	0.80978
Amyl	<i>n</i> -Pentanol	5	-79 ^d	138	1.40994	0.8144
Caproyl	<i>n</i> -Hexanol	6	-51.6 ^d	157.2	1.41790	0.8186
Heptyl	<i>n</i> -Heptanol	7	-34.1 ^d	176.3	1.42410	0.8219
Caprylyl	<i>n</i> -Octanol	8	-16.3 ^d	194.5	1.42920	0.8246
Pelargonyl	<i>n</i> -Nonanol	9	-5	215	1.43347	0.8274
Capryl	<i>n</i> -Decanol	10	6.88	231 ^e	1.43682	0.8297
Undecyl	<i>n</i> -Undecanol	11	15.85	243 ^e	1.4392 ²³	0.8334 ²³
Lauryl	<i>n</i> -Dodecanol	12	23.95	255	—	0.8309 ²⁴
Tridecyl	<i>n</i> -Tridecanol	13	30.03	155.5 ₁₅	—	0.8277 ^{31 e}
Myristyl	<i>n</i> -Tetradecanol	14	38.26	171.5 ₂₀	—	0.8236 ³⁸
Pentadecyl	<i>n</i> -Pentadecanol	15	43.9	—	—	0.8215 ^{44 e}
Palmityl					—	
(cetyl)	<i>n</i> -Hexadecanol	16	49.62	190 ₁₅	—	0.8105 ⁶⁰
Margaryl	<i>n</i> -Heptadecanol	17	53.9	—	—	0.8150 ^{55 e}
Stearyl	<i>n</i> -Octadecanol	18	57.98	210 ₁₅	—	0.8124 ⁵⁹
Nonadecyl	<i>n</i> -Nonadecanol	19	61.65	166.5 _{0.32}	—	0.8090 ^{63 e}
Arachidyl	<i>n</i> -Eicosanol	20	65.5 ^d	220 ₃	—	0.8060 ^{67 e}
Heneicosyl	<i>n</i> -Heneicosanol	21	68.5 ^d	178 _{0.4}	—	—
Behenyl	<i>n</i> -Docosanol	22	70.6	180 _{0.22}	—	0.8000 ^{73 e}
Tricosyl	<i>n</i> -Tricosanol	23	74 ^d	192 _{0.7}	—	—
Lignoceryl	<i>n</i> -Tetracosanol	24	74.8	210 _{0.40}	—	0.7950 ^{77 e}
Pentacosyl	<i>n</i> -Pentacosanol	25	79 ^d	215 _{0.36}	—	—
Ceryl	<i>n</i> -Hexacosanol	26	78.8	—	—	0.7890 ^{81 e}
Octacosyl	<i>n</i> -Octacosanol	28	82.25	—	—	0.7830 ^{85 e}
Nonacosyl	<i>n</i> -Nonacosanol	29	84.1	—	—	—
<i>n</i> -Myricyl	<i>n</i> -Triacosanol	30	86.3	—	—	0.7770 ^{88 e}
<i>n</i> -Lacceryl	<i>n</i> -Dotriacontanol	32	88.9	—	—	—
Tetratriacontyl	<i>n</i> -Tetratriacontanol	34	91.6	—	—	—
			(94.0) ^e			
Hexatriacontyl	<i>n</i> -Hexatriacontanol	36	92.6	—	—	—
			(96.0) ^e			
Takakibyl	<i>n</i> -Tetratetracontanol	44	99.0 ^e	—	—	—

^a Data adapted from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, and A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947.

^b Subscript figures indicate the pressure in millimeters of mercury at which the boiling point was determined.

^c Value of *t* is 20°C. except where superscript appears.

^d Denotes melting point.

^e Figures reported by Warth.

The alcohols found naturally consist chiefly of those having an even number of carbons. The most frequently occurring members are primary alcohols, although several secondary alcohols have been reported, as well as a keto alcohol from cochineal wax.

n-Octanol, *n*-decanol, and *n*-dodecanol have been found in traces in the blubber oil of the sperm whale (*Physeter macrocephalus*).⁴ Gill and Tucker⁵ noted the presence of both C₁₂ and C₁₄ alcohols in the head oil of the porpoise (*Phocaena communis*), although Lovern⁶ failed to confirm this observation. Dodecanol is the lowest alcohol to be reported as a component of a vegetable wax; this has been found in the cuticle wax of the cascara sagrada (*Rhamnus purshiana*).⁷ *n*-Tetradecanol occurs to the extent of about 8% in the wax esters of the head oil of the sperm whale^{8,9} and porpoise.⁶

Cetyl alcohol, or *n*-hexadecanol, was observed as early as 1817 by Chevreul¹⁰ in sperm head oil,⁸ where it comprises about 45% of the total alcohols.⁹ This compound has been reported, also, as a component of Arctic sperm blubber oil,^{11,12} as well as of porpoise head oil.⁶ Cetyl alcohol is present to the extent of 25% in the total alcohols of sperm blubber oil.⁹ The wax found in the skulls of whales and dolphins is frequently referred to as "spermaceti."

Octadecanol is likewise a component of the so-called "spermaceti." It is known to be present in small amounts in sperm head^{8,9} and blubber oils,^{9,12} as well as in porpoise and dolphin blubber oils.⁶ In most cases it does not exceed 5% of the total alcohols present. *n*-Eicosanol has been found as a component of dermoid cysts.¹³ It may also occur in some of the preceding sources in amounts too small to be identified. This C₂₀ alcohol has been reported in the seed oil of the *Simmondsia chinensis* (*californica*) or goat-nut (jojoba) in the amount of 14.6%, and the next higher homologue (*n*-docosanol) to the extent of 33.7%.² Carnaubyl alcohol, C₂₄H₄₉OH, occurs in wool fat,⁷ alkanet root wax (*Anchusa tinctoria*),¹⁴ and in the felted beech coccus (*Cryptococcus fagi*).¹⁵

Although there is some doubt as to whether ceryl alcohol (hexacosanol), as frequently separated, is a single substance or rather a mixture of several homologues, there seems to be some proof that it is distributed widely.

⁴ S. Ueno and R. Koyama, *Bull. Chem. Soc. Japan*, 11, 394-403 (1936).

⁵ A. H. Gill and C. M. Tucker, *Oil & Fat Industries*, 7, 101-102 (1930).

⁶ J. A. Lovern, *Biochem. J.*, 28, 394-402 (1934).

⁷ R. A. Gortner, *Outlines of Biochemistry*, 3rd ed., Wiley, New York, 1949, p. 790.

⁸ E. André and M. T. Francois, *Compt. rend.*, 183, 663-665 (1926).

⁹ T. P. Hilditch and J. A. Lovern, *J. Soc. Chem. Ind.*, 48, 365-368T (1929).

¹⁰ M. E. Chevreul, *Ann. chim. phys.* [2], 7, 155-181 (1817).

¹¹ M. Tsujimoto, *Chem. Umschau Fette, Öle, Wachse Harze*, 32, 127-128 (1925); *Chem. Abst.*, 19, 2882 (1925).

¹² Y. Toyama, *J. Soc. Chem. Ind. Japan*, 30, 527-532 (1927); *Chem. Abst.*, 21, 4079, (1927).

¹³ F. Ameseder, *Z. physiol. Chem.*, 52, 121-128 (1907).

¹⁴ M. V. Betrabet and G. C. Chakravorti, *J. Indian Inst. Sci.*, A16, 41-51, 52-53 (1933).

¹⁵ B. K. Blount, *J. Chem. Soc.*, 1935, 391-393.

This alcohol has been found as a component of bent grass (*Agrostis*) lipids,¹⁶ in brussels sprouts (*Brassica oleracea gemmifera*),¹⁷ in the wax from the blades of barnyard grass (*Echinochloa*) (*Panicum crusgalli*),¹⁸ in spinach (*Spinacia oleracea*),¹⁹ in the cortex of the juneberry or serviceberry (*Amelanchier ovalis* Moench),²⁰ in the resinous coating of the South African "bushman's candle" (*Sarcocaulum rigidum* Schinz),²¹ and in apple cuticle wax.²² Ceryl alcohol has been known also to be a component of such well-known natural products as carnauba wax, beeswax, and wool fat.⁷

Pollard, Chibnall, and Piper²³ have demonstrated the presence of *n*-octacosanol in the wax extracted from the wheat leaves; it has also been isolated from apple cuticle wax.²² It is not clear whether or not the saturated C₂₈ alcohol C₂₈H₅₇OH, isolated by Ueno and Yamasaki²⁴ from the non-saponifiable fraction of oil from the Koryan corn, or beard grass (also called blue-stem) (*Andropogon sorghum* or *Sorghum vulgare*), and called "koryanyl alcohol," is identical with *n*-octacosanol.

Lucerne leaf wax or alfalfa (*Medicago sativa*) was shown by Chibnall *et al.*²⁵ to be a source of *n*-triacontanol, CH₃(CH₂)₂₈CH₂OH, and this appears to be its principal alcohol. This C₃₀ alcohol has been reported as a component of rice polishings, and is frequently referred to as "melissyl" or "myricyl" alcohol.²⁶ Other sources of *n*-triacontanol are apple cuticle wax,²² sugar cane wax (*Saccharum officinarum*), (where it comprises 80% of the non-saponifiable fraction),²⁷ beeswax and carnauba wax (*Copernicia cerifera*),²⁸ and from the growing tips of the slash pine (*Pinus caribaea*).²⁹

Several higher alcohols are present in beeswax (to C₃₄) and lac wax (to C₃₆). Jermstad³⁰ has reported an alcohol called "sorbol" from the wax-like material in the berries of *Sorbus aucuparia* (mountain ash or rowan tree) to which he assigns the formula C₃₄H₆₉OH. Takakibyl alcohol is the

¹⁶ C. E. Bills and G. E. Steel, *Proc. Soc. Exptl. Biol. Med.*, **31**, 134-135 (1933).

¹⁷ P. N. Sahai and A. C. Chibnall, *Biochem. J.*, **26**, 403-412 (1932).

¹⁸ A. Pollard, A. C. Chibnall, and S. H. Piper, *Biochem. J.*, **25**, 2111-2122 (1931).

¹⁹ D. L. Collison and I. Smedley-MacLean, *Biochem. J.*, **25**, 606-613 (1931).

²⁰ J. Rabate, *Bull. soc. chim. biol.*, **12**, 758-764 (1930).

²¹ P. Karrer and K. Schwarz, *Vierteljahrsschr. Naturforsch. Ges. Zürich*, **77**, 78-82 (1932).

²² A. C. Chibnall, S. H. Piper, A. Pollard, J. A. B. Smith, and E. F. Williams, *Biochem. J.*, **25**, 2095-2110 (1931).

²³ A. Pollard, A. C. Chibnall, and S. H. Piper, *Biochem. J.*, **27**, 1889-1893 (1933).

²⁴ S. Ueno and R. Yamasaki, *J. Soc. Chem. Ind. Japan*, **38**, suppl., 113-116 (1935).

²⁵ A. C. Chibnall, E. F. Williams, A. L. Latner, and S. H. Piper, *Biochem. J.*, **27**, 1885-1888 (1933).

²⁶ U. Tange, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **14**, No. 275-277 (1930).

²⁷ N. L. Vidyarthi and M. Narasingarao, *J. Indian Chem. Soc.*, **16**, 135-143 (1939).

²⁸ T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, Wiley, New York, 1947.

²⁹ J. A. Hall and O. Gisvold, *J. Biol. Chem.*, **113**, 487-496 (1936).

³⁰ A. Jermstad, *Pharm. Acta Helv.*, **8**, 69-70 (1933); *Chem. Abst.*, **27**, 3973 (1933).

name given to another alcohol isolated from *Sorghum vulgare* (blue-stem),²⁴ which has the formula $C_{44}H_{89}OH$.

Saturated secondary alcohols are occasionally found in waxes. Reeves and Anderson³¹ have reported such unusual products as *d*-eicosanol-2 (C_{20}) and *d*-octadecanol-2 (C_{18}) in a wax prepared from the tubercle bacilli. Hydroxy fatty acids were present also, as well as the disaccharide trehalose, but the tests for glycerol were negative. Two other alcohols which are unique not only because they are secondary alcohols but also because they consist of an odd number of carbon atoms are 10-nonacosanol (C_{29})³² and 15-nonacosanol,¹⁷ which have been found in apple cuticle wax and brussels sprouts, respectively. Hall and Gisvold²⁹ have reported the presence of 10-nonacosanol in the growing tips of the slash-pine (*Pinus caribaea* Morelet).

Several diatomic alcohols have been reported in the saturated series. These include "pemphigus" alcohol, $C_{34}H_{68}(OH)_2$ (m.p., 100–105°C.), which has been prepared from the wax of the wool louse (*Pemphigus xylostei*), which feeds on the stems of the European honeysuckle (*Lonicera xylosteum*).³³ Another dihydric alcohol has been isolated from the nema-

TABLE 2
NAMES AND PROPERTIES OF UNSATURATED MONATOMIC ALCOHOLS OBTAINED AS
HYDROLYTIC PRODUCTS OF WAXES OR OBTAINED SYNTHETICALLY^a

Common name	Systematic name	M.p., °C.	B.p., °C./b	$(n_D^t)^c$	$(d_4^t)^c$
—	10-Undecenol	— ^d	122 ₃	—	—
—	11-Dodecenol	—	13S ₁₁	—	0.840 ¹⁵
—	12-Tridecenol	—	149.5 ₉	—	0.845 ¹⁵
Physeteryl	5-Tetradecenol ^e	—	—	1.4573 ¹⁵	0.8507 ¹⁵
—	14-Pentadecenol	32.5	171 ₁₀	—	—
Zoomaryl (palmitoleyl)	9-Hexadecenol ^e	196.5 ^f ₁₆	—	1.4605 ¹⁵	0.8537 ¹⁵
Oleyl	<i>cis</i> -9-Octadecenol	2 ^d	209 ₁₅	1.4607 ²⁰	0.8489 ²⁰
Elaidyl	<i>trans</i> -9-Octadecenol	36.5	216 ₁₈	1.4552 ¹⁰	0.8338 ¹⁰
—	11-Eicosenol	—	—	—	—
Erucyl	<i>cis</i> -13-Docosenol	35	241 ₁₀	—	—
Linoleyl	<i>cis-cis</i> -9,12-Octadecadienol	−3	—	1.4782 ⁶⁷	0.8612 ²⁰

^a Adapted from A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 733.

^b Subscript figures represent atmospheric pressure in millimeters of mercury at which the boiling point was determined.

^c Superscript figures indicate the value of *t*.

^d Freezing or solidification point.

^e Data from Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 10, 573–579 (1935).

^f Boiling point of acetate ester.

³¹ R. E. Reeves and R. J. Anderson, *J. Am. Chem. Soc.*, 59, 858–861 (1937).

³² K. S. Markley, S. B. Hendricks, and C. E. Sando, *J. Biol. Chem.*, 98, 103–107 (1932).

³³ F. N. Schulz and M. Becker, *Biochem. Z.*, 235, 233–239 (1931).

tode (*Ascaris megaloccephala*) and from the round worm (*Ascaris lumbricoides*); this alcohol probably also contains some carbonyl groups, as it has four oxygens ($C_{32}H_{68}O_4$).³⁴

Another interesting ketonic alcohol which has been prepared from cochineal wax (*Coccus cacti*) is 15-keto-*n*-tetratriacontanol, $CH_3(CH_2)_{18}CO(CH_2)_{13}CH_2OH$, which melts at 100.5–100.7.³⁵ Becker³⁶ reports the preparation of *cocceryl* alcohol from the cochineal wax coccerin, which has two less carbons than the ketonic alcohol, and which is diatomic. The formula is given as $C_{32}H_{64}(OH)_2$, and it has a melting point of 102°C. One wonders whether these two products may be identical. Phthiocerol is a dihydroxy monomethoxy alcohol, $C_{35}H_{72}O_3$, which has been found to be a component of the wax of tubercle bacilli. Although it was first separated from the human tubercle bacilli,^{37,38} it has since been shown to be a component of the bovine type.³⁹ This alcohol is optically active, $[\alpha]_D = -4.8^\circ$, and it melts at 73–74°C.

A list of some of the unsaturated alcohols which have been found as components of waxes is given in Table 2.

Octadecyl alcohol (or oleyl alcohol), $CH_3(CH_2)_7CH:CH(CH_2)_7CH_2OH$, is the most important member of the group of unsaturated alcohols. It is found largely in fish oils. Tsujimoto⁴⁰ and Toyama^{12,41} first discovered it in certain shark oils; they later found it in sperm oils,¹¹ including the oil from the Arctic sperm whale. The blubber oil is composed of 66 to 70% of oleyl alcohol, while the head oil contains 27 to 30% of this component.⁴² Oleyl alcohol comprises 25% of spermaceti.⁴³ About 30% of the head oil of the porpoise also consists of octadecyl alcohol.⁶

In addition to oleyl alcohol, most of the other unsaturated alcohols are of marine origin. Thus, Toyama and Tsuchiya,⁴⁴ citing Tsujimoto,⁴⁰ have reported 5-tetradecenol (physeteryl) and 9-hexadecenol (zoomaryl alcohol) in sperm head oil. Moreover, Toyama and Akiyama have demonstrated that hexadecenol in sperm blubber oil is identical with zoomaryl alcohol

³⁴ F. N. Schulz and M. Becker, *Biochem. Z.*, **265**, 253–259 (1933).

³⁵ A. C. Chibnall, A. L. Latner, E. F. Williams, and A. Ayre, *Biochem. J.*, **28**, 313–325 (1934).

³⁶ M. Becker, *Biochem. Z.*, **239**, 235–242 (1931).

³⁷ F. H. Stodola and R. J. Anderson, *J. Biol. Chem.*, **114**, 467–472 (1936).

³⁸ C. W. Wiegand and R. J. Anderson, *J. Biol. Chem.*, **126**, 515–526 (1938).

³⁹ J. Cason and R. J. Anderson, *J. Biol. Chem.*, **126**, 527–541 (1938).

⁴⁰ M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, **24**, 41–45 (1920); *Chem. Abst.*, **15**, 2006 (1921).

⁴¹ Y. Toyama, *Chem. Umschau Fette, Öle, Wachse Harze*, **29**, 237–240, 245–247 (1922); *Chem. Abst.*, **17**, 892–893 (1923).

⁴² T. P. Hilditch and J. A. Lovern, *J. Soc. Chem. Ind.*, **48**, 359–364T (1929).

⁴³ M. T. Francois, *J. pharm. chim.*, **12**, 189–191 (1930).

⁴⁴ Y. Toyama and T. Tsuchiya, *Bull. Chem. Soc. Japan*, **10**, 563–569, 570–573, 573–579 (1935).

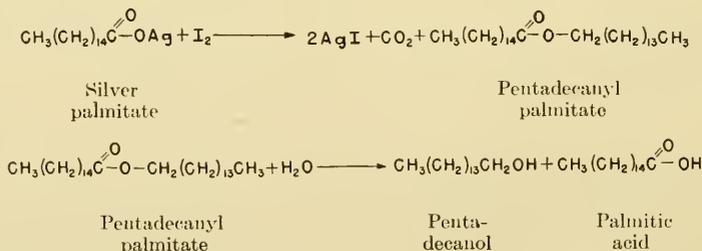
previously found in sperm head oil.⁴⁵ Hilditch²⁸ reported the possible occurrence of linoleyl alcohol in the oil from the sperm whale, while Ueno and Koyama⁴ found an unsaturated decenol in sperm blubber. An unsaturated C₁₀ alcohol of unknown structure has likewise been isolated from the liver oil of the Japanese crab, tarabakani (*Paralithoides camtschatica* Tilesius).⁴⁶ The elementary composition would suggest the formula C₁₀H₁₈O₂.

Only two of the alcohols have been found exclusively in plant sources. Green, Hilditch, and Stainsby⁴⁷ have demonstrated the presence of 11-eicosenol, CH₃(CH₂)₇CH:CH(CH₂)₉CH₂OH and 13-docosenol, CH₃(CH₂)₇CH:CH(CH₂)₁₁CH₂OH, in the seeds of *Simmondsia chinensis* (*californica*), or goat-nut, which is known as jojoba oil. Hexacosenol has also been reported in jojoba oil.¹

Oleyl alcohol can be changed from the *cis* configuration to the *trans* form (elaidyl alcohol) by the action of the nitrogen oxides in a manner similar to that in which oleic acid is transformed into elaidic acid. The latter alcohol is solid at room temperature (m.p., 35–35.5°C.). Although the elaidyl alcohol cannot be resolved from the mixture after its formation from oleyl alcohol, it has been synthesized by the reduction of ethyl elaidate.^{48,49}

(b) *Methods of Synthesis.* The newer methods of synthesis of the higher aliphatic alcohols are simple and readily adaptable to commercial use; hence, the alcohols are no longer a laboratory curiosity but a product as readily available as are the fatty acids.

The first method employed for the synthesis of alcohol was that of Simonini,⁵⁰ which involves treating the silver salts of the fatty acids with iodine. The alcohol which results has one less carbon than the original acid. The application of this procedure for the preparation of pentadecanol from palmitic acid is illustrated below.⁵¹



⁴⁵ Y. Toyama and G. Akiyama, *Bull. Chem. Soc. Japan*, 10, 579–584 (1935); 11, 29–34 (1936).

⁴⁶ M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, 32, 362–364B (1929); *Chem. Abst.*, 24, 4650 (1930).

⁴⁷ T. G. Green, T. P. Hilditch, and W. J. Stainsby, *J. Chem. Soc.*, 1936, 1750–1755

⁴⁸ Y. Toyama, *Chem. Umschau Fette, Ole, Wachse Harze*, 31, 13–17 (1924); *Chem. Abstr.*, 18, 1270 (1924).

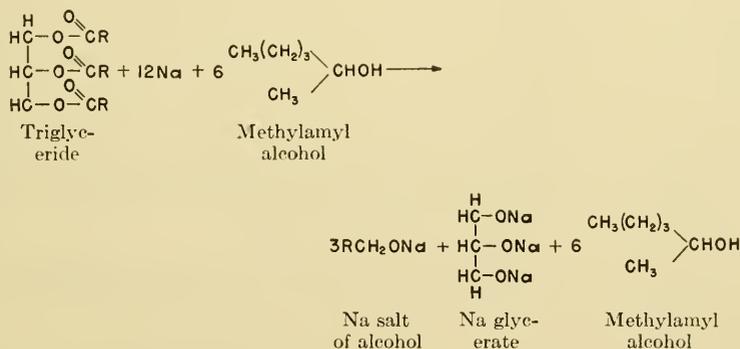
⁴⁹ E. André and M. T. Francois, *Compt. rend.*, 185, 279–281, 387–388 (1927).

⁵⁰ A. Simonini, *Monatsh.*, 13, 320–325 (1892); 14, 81–92 (1893).

⁵¹ L. Panics, *Monatsh.*, 15, 9–16 (1894).

A second procedure for the preparation of alcohols is by reduction of the corresponding aldehydes. Bouis and Carlet⁵² were the first to report the synthesis of an alcohol by the reduction of the corresponding aldehyde with zinc-acetic acid, followed by hydrolysis of the resulting acetate ester. A similar procedure was used by Krafft⁵³ for making the C₁₀-C₁₈ aliphatic alcohols with an even number of carbons. The use of the aldehyde as a starting material has not proved commercially practical because of the unsatisfactory methods used for the large-scale production of the aldehydes.

The procedure which has recently proved most practical commercially has been the reduction of esters with metallic sodium in the presence of a reducing alcohol. This reaction is based upon the classical work of Bouveault and Blanc,^{54,55} which was the subject of several patents.⁵⁶⁻⁵⁸ Improved methods for the production of alcohol by this technique^{59,60} have been applied by Procter and Gamble for the large-scale production of the higher alcohols from coconut oil as described by Kastens and Peddicord.⁶¹ Methylamyl alcohol is used as the reducing alcohol, and ordinary carbon steel vessels are employed for the reaction:



Garber⁶² has discussed the sodium reduction process and listed a large number of research reports on this subject during 1945-1947. According

⁵² J. Bouis and H. Carlet, *Ann.*, 124, 352-355 (1862).

⁵³ F. Krafft, *Ber.*, 16, 1714-1726 (1883).

⁵⁴ L. Bouveault and G. Blanc, *Compt. rend.*, 136, 1676-1678 (1903); 137, 60-62, 328-329 (1903).

⁵⁵ L. Bouveault and G. Blanc, *Bull. soc. chim.* [3], 31, 666-672, 672-675, 1203-1206 (1904).

⁵⁶ L. Bouveault and G. Blanc, *French Patent* No. 338,895 (1903). Cited by M. I. Kastens and H. Peddicord, *Ind. Eng. Chem.*, 41, 438-446 (1949).

⁵⁷ L. Bouveault and G. Blanc, *German Patent* No. 164,294 (July 5, 1903).

⁵⁸ L. Bouveault and G. Blanc, *U. S. Patent* No. 868,252 (Oct. 15, 1907).

⁵⁹ C. O. Henke and R. G. Benner, *U. S. Patent* No. 2,070,597 (Feb. 16, 1947).

⁶⁰ N. D. Scott and V. L. Hansley, *U. S. Patent* No. 2,019,022 (Oct. 29, 1935).

⁶¹ M. I. Kastens and H. Peddicord, *Ind. Eng. Chem.*, 41, 438-446 (1949).

⁶² H. J. Garber, *Ind. Eng. Chem.*, 40, 1684-1694 (1948).

to a recent French report,⁶³ oleyl alcohol in 95% purity can be produced by a modification of the above procedure. Sodium amalgam has been recommended as the reduction agent.⁶⁴

Another highly successful commercial method for the synthesis of the higher alcohols is the use of high-pressure catalytic processes as developed both in Europe⁶⁵⁻⁶⁷ and in the United States.⁶⁸⁻⁷¹ The catalysts employed include copper chromite,⁷⁰ nickel or copper carbonate,⁶⁵ or copper or chromium oxides,⁷¹ while the pressures range between 100 and 220 atmospheres at temperatures of 200-300°C. The catalysts ordinarily employed for hydrogenation of the ethylenic bonds in the hydrogenation of fats, namely nickel or cobalt, are unsatisfactory for the production of alcohols, since hydrocarbons are the final products. High-pressure hydrogenation is presumably the result of the independent discoveries made almost simultaneously by a large number of different workers.^{66,71-76} The first commercial application of this process⁷⁷ was by the Deutsche-Hydrierwerke, A.-G., at Berlin-Charlottenburg in 1927. In the United States,^{78,79} the method has been used by the E. I. du Pont de Nemours & Company, Inc., since 1933.

Prins⁸⁰ obtained better yields of alcohols by a modification of the Bouveault-Blanc procedure. This involves the solution of the ester in ether and placing the ethereal solution over aqueous sodium acetate. Sodium strips and acetic acid are dropped in slowly while the ether layer is main-

⁶³ Anonymous, *Bull. mens. inst. technique études, recherches corps gras*, No. 9, 3-9 (1947). Cited by M. L. Kastens and H. Peddicord, *Ind. Eng. Chem.*, *41*, 446 (1949).

⁶⁴ S. Shikata and Y. Inoue, *U. S. Patent* No. 2,263,195 (Nov. 18, 1941).

⁶⁵ A. F. Kertress, *J. Soc. Dyers Colourists*, *48*, 7-9 (1932).

⁶⁶ W. Normann, *Z. angew. Chem.*, *44*, 714-717 (1931).

⁶⁷ W. Schrauth, O. Schenck, and K. Stickdorn, *Ber.*, *B64*, 1314-1318 (1931).

⁶⁸ R. Adams and C. S. Marvel, *Univ. Ill. Bull.*, *20* [8], 54 (1922). Cited by M. L. Kastens and H. Peddicord, *Ind. Eng. Chem.*, *41*, 438 (1949).

⁶⁹ H. Adkins and R. Cramer, *J. Am. Chem. Soc.*, *52*, 4349-4358 (1930).

⁷⁰ H. Adkins and R. Conner, *J. Am. Chem. Soc.*, *53*, 1091-1095 (1931).

⁷¹ H. Adkins and K. Folkers, *J. Am. Chem. Soc.*, *53*, 1095-1097 (1931). K. Folkers and H. Adkins, *ibid.*, *54*, 1145-1154 (1932). H. Adkins, B. Wojcik, and L. W. Covert, *ibid.*, *55*, 1669-1676 (1933).

⁷² H. Adkins, *Reactions of Hydrogen*, Univ. Wisconsin Press, Madison, 1937, p. 97.

⁷³ R. Oda, *J. Soc. Chem. Ind. Japan*, *35*, suppl., 349-352 (1932); *Chem. Abst.*, *26*, 5544 (1932).

⁷⁴ O. Schmidt, *Ber.*, *B64*, 2051-2053 (1931).

⁷⁵ W. Schrauth, *Ber.*, *B65*, 93-95 (1935).

⁷⁶ S. Ueno and S. Ueda, *J. Soc. Chem. Ind. Japan*, *33*, suppl., 482-486 (1935); *Chem. Abst.*, *29*, 8371 (1935).

⁷⁷ M. Briscoe, *Chem. Trade J.*, *90*, 76-78 (1932).

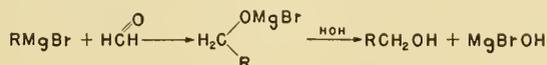
⁷⁸ W. A. Lazier, *U. S. Patent* Nos. 1,746,783 (Feb. 11, 1930); 1,839,974 (Jan. 5, 1932); 1,964,000 (June 26, 1934); 1,984,884 (Dec. 18, 1934); 2,079,414 (May 4, 1937); 2,109,844 (Mar. 1, 1938).

⁷⁹ P. L. Salzberg, *U. S. Patent* Nos. 2,089,433 (Aug. 10, 1937); 2,129,507 (Sept. 6, 1938).

⁸⁰ H. J. Prins, *Rec. trav. chim.*, *42*, 1050-1052 (1923).

tained at a slightly acid pH. Much lower temperatures may be used than those of Bouveault-Blanc. A yield of 90% of 1-octanol from ethyl caprylate has been reported, as well as a satisfactory production of chaulmoogryl alcohol from the parent acid.⁸¹

Another practical method, of especially wide application, for preparing primary, secondary, and tertiary alcohols, is the well-known Grignard reaction. Primary alcohols are readily synthesized by this method by treating the alkyl magnesium halide with formaldehyde and hydrolyzing the intermediate product:



One obtains an alcohol with one additional carbon besides that contained in the alkyl group.

In addition to the synthesis of oleyl alcohol by the use of sodium and a reducing alcohol,⁶³ a number of these methods may be employed for preparing unsaturated alcohols from their parent unsaturated acids. One may use catalytic reduction with copper-cadmium catalysts⁸² or a mixed catalyst containing the chromites of zinc, copper, and cadmium.⁸³ 1-Docosanol is the chief product when erucic acid is reduced with zinc chromite, although small amounts of 1-docosanol and 1-docosene are obtained.⁸⁴ Komori⁸⁵ reported an 80% yield of unsaturated alcohols when rice fatty acids were reduced in the presence of iron chromite at 320°C. and 120 atmospheres. Erucyl, oleyl, and linoleyl alcohols have been produced in high yield from the ethyl esters of the corresponding acids in the presence of a zinc-chromium catalyst.⁸⁶ When soybean oil is hydrogenated at 450°C. using ammonium zinc chromate, or at 550°C. in the presence of a mixture of chromium trioxide and zinc oxide, highly unsaturated alcohols are also produced.⁸⁷

(c) *Properties of Alcohols.* The aliphatic straight-chain alcohols melt and boil at lower temperatures than do the corresponding acids. The lower members up to the C₁₂ alcohol are liquid at ordinary temperature.

⁸¹ M. M. Dewar, *U. S. Pub. Health Service, Pub. Health Bull.*, No. 168, 33-35 (1927).

⁸² H. T. Böhme, *Fettchemie G.m.b.H.*, *French Patent* No. 819,255 (1937); *Brit. Patent* No. 479,642 (Feb. 16, 1937).

⁸³ W. A. Lazier, *U. S. Patent* No. 2,094,127 (Sept. 28, 1937).

⁸⁴ S. Komori, *J. Soc. Chem. Ind. Japan*, 43, suppl., 122-125 (1940); *Chem. Abst.*, 34, 5411 (1940).

⁸⁵ S. Komori, *J. Soc. Chem. Ind. Japan*, 43, suppl., 428-430 (1940); *Chem. Abst.*, 35, 4345 (1941).

⁸⁶ S. Komori, *J. Soc. Chem. Ind. Japan*, 42, suppl., 46-47 (1939); *Chem. Abst.*, 33, 7273 (1939).

⁸⁷ Y. Sinozaki and S. Sumi, *J. Agr. Chem. Soc. Japan*, 14, 1113-1116, 1117-1122 (1938). Y. Sinozaki, S. Sumi, and S. Adati, *ibid.*, 14, 1123-1128; 1129-1134 (1938); *Chem. Abst.*, 33, 8044-8045 (1939).

The short-chain alcohols have sharp, pungent odors; those with an intermediate chain length have a pleasant fruity aroma, while the higher members (above C_{14}), which are solid, are odorless. Data on the melting (or freezing) points, boiling points, specific gravity, and refractive indices are included in Tables 1 and 2.

The alcohols apparently crystallize in double-chain-length structures with the two hydroxyl groups in approximation to each other. The higher alcohols show dimorphism, which can readily be observed, since the α -form is transparent, while the β -modification is opaque. Lauryl alcohol and the lower homologues crystallize as the α -form on cooling, but change to the opaque and higher melting β -modification on standing. In the case of this C_{12} alcohol, the freezing point of the α -crystals is $21.6^{\circ}\text{C}.$, while the β -form melts at $23.8^{\circ}\text{C}.$ Transition from the α - to the β -form is monotropic. In the case of myristyl (C_{14}) alcohol and its higher homologues, the α -type of crystals (the higher melting form) results on solidification; on cooling this changes enantiotropically to the β -variety. However, on heating, the β -polymorph reverts to the α -form, and their melting points are those of the α -modification.

There is some evidence that, in lower members of the series (below C_{12}), the odd- and even-chain-length alcohols vary in structure. This deduction is based on the fact that the melting points of the alcohols below lauryl show alternation, while those of the higher homologues follow a straight line. Malkin⁸⁸ concluded that the odd-carbon members of the series have vertical chains while, in the stable modifications of the even-carbon homologues, the chains are tilted at an angle of $55^{\circ}40'$. Such tilted-chain structures are believed by Malkin to change into the vertical-type structure at a temperature slightly below the fusion point. On the other hand, Phillips and Mumford⁸⁹ disagree with Malkin's statement that the vertical structure is characteristic of the odd-chain alcohols, since they also exhibit a polymorphic change from the α -modification (supposedly the vertical structure) to the β -type (believed to have the tilted arrangement). Another possible explanation of the phenomenon is that the transition of the α - to the β -form involves the transformation from a vertical rotating structure (α) to a vertical stationary chain (β).⁹⁰ Such an alteration would not result in a change in chain length or a difference in the angle of tilt. Further evidence in support of the vertical-chain structure of the odd-carbon alcohols was adduced by Bernal^{91,92} and Malkin.⁹³ The latter worker studied the short spacings of the C_{15} , C_{17} , and C_{19} alcohols. The fact that

⁸⁸ T. Malkin, *J. Am. Chem. Soc.*, *52*, 3739-3740 (1930).

⁸⁹ J. W. C. Phillips and S. A. Mumford, *J. Chem. Soc.*, *1934*, 1657-1665.

⁹⁰ D. A. Wilson and E. Ott, *J. Chem. Phys.*, *2*, 231-238, 239-244 (1934).

⁹¹ J. D. Bernal, *Nature*, *129*, 870 (1932).

⁹² J. D. Bernal, *Z. Krist.*, *83*, 153-155 (1932).

⁹³ T. Malkin, *J. Chem. Soc.*, *1935*, 726.

two spacings (3.7 and 4.2 Å.) were noted in the β -form and only one in the α -modification (4.2 Å.), indicates that the change, $\alpha \rightarrow \beta$, of the odd-chain alcohols does not necessarily involve a transition from a vertical to a tilted chain.

Francis *et al.*⁹⁴ have reported the following long spacings (in Å.) for the aliphatic alcohols: C₁₈ (β) 41.35; C₂₂ (β) 50.0; C₂₄ (β) 54.25; C₂₆ (α) 70.7, (β) 58.0; C₂₈ (α) 75.95, (β) 62.3; C₃₀ (β) 66.4; C₃₂ (β) 71.0; C₃₄ (α) 90.8, (β) 74.65.

The solubilities of the C₁₀ to C₁₈ alcohols have been studied in a number of organic solvents by Hoerr, Harwood, and Ralston.⁹⁵ The solubility properties are comparable with those of the acids. The alcohols dissolve well

TABLE 3

SOLUBILITY OF ALIPHATIC C₁₀ TO C₁₈ EVEN-CHAIN ALCOHOLS IN ETHYL ETHER, ACETONE, AND 95% ETHANOL AT VARYING TEMPERATURES^a

Solvent	Temp., °C.	Solubility, g./100 g. solvent				
		C ₁₀	C ₁₂	C ₁₄	C ₁₆	C ₁₈
Ethyl ether	-40	8.0	1.4	0.1	—	—
95% Ethanol		7.1	0.6	—	—	—
Ethyl ether	-20	38.9	5.3	1.2	0.1	—
Acetone		13.6	1.6	<0.1	—	—
95% Ethanol		43.6	4.2	0.4	—	—
Ethyl ether	0	520	44.2	9.3	3.0	0.5
Acetone		335	12.9	2.4	0.1	—
95% Ethanol		1150	52	6.4	1.8	0.2
Acetone	10	∞	75	8.7	1.3	0.1
Ethyl ether	20	∞	960	100	26.1	7.7
Acetone		∞	1150	38.6	6.7	1.1
95% Ethanol		∞	2120	105	15.9	5.0
Ethyl ether	30	∞	∞	380	76	26.4
Acetone		∞	∞	340	30.9	7.0
95% Ethanol		∞	∞	630	89	22.2
Ethyl ether	34.5	∞	∞	1180	123	46
Acetone	40	∞	∞	∞	290	41.4
95% Ethanol		∞	∞	∞	430	120

^a Adapted from C. W. Hoerr, H. J. Harwood, and A. W. Ralston, *J. Org. Chem.*, *9*, 267-280 (1944).

in such a non-polar substance as diethyl ether and in such slightly polar substances as trichloromethane, ethyl acetate, and butyl acetate. They form eutectic mixtures with benzene, cyclohexane, and tetrachloromethane. The alcohols are quite soluble in such moderately polar solvents as acetone

⁹⁴ F. Francis, S. H. Piper, and T. Malkin, *Proc. Roy. Soc. London*, *A128*, 214-252 (1930). F. Francis, F. J. E. Collins, and S. H. Piper, *ibid.*, *A158*, 691-718 (1937).

⁹⁵ C. W. Hoerr, H. J. Harwood, and A. W. Ralston, *J. Org. Chem.*, *9*, 267-280 (1944).

and 2-butanone. On the other hand, the alcohols are largely insoluble in the highly polar solvents, nitroethane and acetonitrile, with the result that over a wide range they form two immiscible solutions. The solubilities of the C₁₀ to C₁₈ alcohols in diethyl ether, acetone, and 95% ethyl alcohol at widely varying temperatures are listed in Table 3.

As in the case of the corresponding aliphatic acids, the solubility of the alcohols is progressively reduced as the chain length is increased. As would be expected, solubility in all cases increases with a rise in temperature, so that at 20° and higher the 1-decanol is infinitely soluble in the three solvents reported in Table 3. At 30°C., infinite solubility also obtains for 1-dodecanol, and at 40°C. the same is true for 1-tetradecanol. As in the case of the fatty acids, the relative effectiveness of different solvents for a given alcohol varies with the temperature. Furthermore, the proportions of the alcohols which dissolve in the several solvents at a fixed temperature vary with the particular alcohol. Thus, in order to state the best solvent under a given condition, one must consider both the alcohol to be dissolved and the temperature of the solution.

(d) *Chemical Reactions of Alcohols.* The reaction which is considered most characteristic of alcohols is esterification. Such an interaction occurs readily not only with organic acids but also with inorganic acids. The subject of esterification has been considered in Chapter II.

On oxidation, primary alcohols are changed first to aldehydes and then to the fatty acid derivatives. When the alcohols are subjected to hydrogenation at 350°C. under 200 atmospheres pressure with nickel as a catalyst, they become reduced to the corresponding saturated hydrocarbon.

Two different products may originate on thermal decomposition of alcohols. Thus they may lose hydrogen to yield an aldehyde:



When the reaction conditions are varied, the product on dehydration will be an olefin:

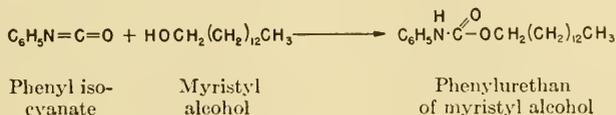


The alcohols are converted to the saturated hydrocarbons having one less carbon when they are subjected to hydrogenolysis by passing them over reduced nickel catalysts at 250°C. under a pressure of 100 to 200 atmospheres of hydrogen.⁹⁶ Thus, Böeseken and van Senden⁹⁷ have reported the production of hexane after hydrogenolysis is applied to 1-heptanol.

⁹⁶ B. Wojcik and H. Adkins, *J. Am. Chem. Soc.*, 55, 1293-1294 (1933).

⁹⁷ J. Böeseken and G. H. van Senden, *Rec. trav. chim.*, 32, 23-38 (1913).

There are a number of substances with which the alcohols react to form stable crystalline compounds readily identifiable by their melting points. The most common derivative is the urethan which is formed by a reaction of the alcohol and isocyanate. The formation of the phenylurethan of myristyl alcohol (1-tetradecanol) is illustrated here. In addition to phenyl-



urethan, the α -naphthylurethans,^{98,99} the *p*-nitrophenylurethans,¹⁰⁰ and the 3,5-dinitrophenylurethans¹⁰¹ give satisfactory derivatives for identification. A number of other substituted urethans, including the *o*-, *m*-, and *p*-nitrophenyl derivatives, are crystalline. The higher alcohols form carbamates on heating with carbamic acid.

Esters with characteristic melting points result when the higher alcohols react with 3,5-dinitrobenzoic acid.¹⁰² *p*-Nitrobenzoates have likewise been prepared.¹⁰³

b. Sterols. The sterols comprise one of the most interesting groups of lipids. These products are alcohols which possess a cyclic structure containing the cyclopentenophenanthrene ring. In nature they occur free, as well as combined with fatty acids in an ester linkage. In the latter case the products are considered to be waxes.

Although the sterols are a most important group in themselves, they are merely a class of a larger division to which Callow and Young¹⁰⁴ have given the names "steroids." The term "steride" is sometimes employed in the same connection. These compounds containing a steroid nucleus are widely distributed in nature. They are of especial interest because they possess very specific and potent physiological effects. In this category are included the sex hormones, the adrenocorticohormones, the bile acids, a number of provitamins D, as well as those saponins which comprise the glucosides of the digitalis group, and the so-called saponinins.

Few fields in organic chemistry have been as fruitful during the last several decades as has that of steroid chemistry. During this time not only has the structure of a large group of sterols been elucidated, but also the chemistry of the much larger number of hormones and related steroids has been disclosed. The subject of steroids has been exhaustively dis-

⁹⁸ C. Neuberg and E. Kinsky, *Biochem. Z.*, **20**, 445-449 (1909).

⁹⁹ V. T. Bickel and H. E. French, *J. Am. Chem. Soc.*, **48**, 747-751 (1926).

¹⁰⁰ R. L. Shriner and R. F. B. Cox, *J. Am. Chem. Soc.*, **53**, 1601-1605 (1931).

¹⁰¹ F. Hoeke, *Rec. trav. chim.*, **54**, 505-517 (1935).

¹⁰² G. B. Malone and E. E. Reid, *J. Am. Chem. Soc.*, **51**, 3424-3427 (1929).

¹⁰³ H. Henstock, *J. Chem. Soc.*, **1933**, 216.

¹⁰⁴ R. K. Callow and F. G. Young, *Proc. Royal Soc. London*, **A157**, 194-212 (1936).

cussed from various viewpoints in recent years, and the reader is referred to the specific articles for more details. The chemical aspects have been periodically reviewed, during the period of rapid development, by a number of masters in the field. These include chapters by Windaus¹⁰⁵, Rosenheim and King,¹⁰⁶ Schoenheimer and Evans,¹⁰⁷ Heilbron and Jones,¹⁰⁸ Shoppee,¹⁰⁹ Sobotka and Bloch,¹¹⁰ Koch,¹¹¹ Ruigh,¹¹² Reichstein and Reich,¹¹³ and Sarett and Wallis.¹¹⁴ An excellent treatment of the historical aspects developed largely from a physiological standpoint is contained in the review by Bills,¹¹⁵ which includes a discussion of the relationship to vitamin D. Probably the most complete treatment of the subject is afforded in the comprehensive monograph of L. F. and M. Fieser.¹¹⁶ Another extensive discussion is to be found in the section written by Winterstein and Schön in the monograph of Hefter and Schönfeld¹¹⁷ on lipids.

(a) *History of Sterols.* Cholesterol was the initial compound of this group to be separated and identified. Poulletier¹¹⁸ prepared it from gallstones (1769). De Fourcroy¹¹⁹ classified it with spermaceti and "adipocire" because of its wax-like properties. Its true nature was first recognized by Chevreul,¹²⁰ who demonstrated that it was unsaponifiable and hence could be differentiated from other wax-like materials. This same worker¹²¹ coined the name "cholesterine" for the product now known as cholesterol, from the derivatives, *chole*, bile, and *stereos*, solid. Another milestone in understanding the chemical nature of cholesterol was the discovery by Berthelot¹²² that it is an alcohol. This investigator prepared a number of cholesterol esters and anticipated the finding of these compounds in the body, and suggested their relationship to bile acids. However, the term "sterol" did not come into general use¹¹⁵ until 1911 as a term to include all the animal and plant alcohols of this series.

¹⁰⁵ A. Windaus, *Ann. Rev. Biochem.*, **1**, 109-134 (1932).

¹⁰⁶ O. Rosenheim and H. King, *Ann. Rev. Biochem.*, **3**, 87-110 (1934).

¹⁰⁷ R. Schoenheimer and E. A. Evans, Jr., *Ann. Rev. Biochem.*, **6**, 139-162 (1937).

¹⁰⁸ I. M. Heilbron and E. R. H. Jones, *Ann. Rev. Biochem.*, **9**, 135-172 (1940).

¹⁰⁹ C. W. Shoppee, *Ann. Rev. Biochem.*, **11**, 103-150 (1942).

¹¹⁰ H. Sobotka and E. Bloch, *Ann. Rev. Biochem.*, **12**, 45-80 (1943).

¹¹¹ F. C. Koch, *Ann. Rev. Biochem.*, **13**, 263-294 (1944).

¹¹² W. L. Ruigh, *Ann. Rev. Biochem.*, **14**, 225-262 (1945).

¹¹³ T. Reichstein and H. Reich, *Ann. Rev. Biochem.*, **15**, 155-192 (1946).

¹¹⁴ L. H. Sarett and E. S. Wallis, *Ann. Rev. Biochem.*, **16**, 655-688 (1947).

¹¹⁵ C. E. Bills, *Physiol. Revs.*, **15**, 1-97 (1935).

¹¹⁶ L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, 3rd ed., Reinhold, New York, 1949.

¹¹⁷ A. Winterstein and K. Schön, in G. Hefter and H. Schönfeld, *Chemie und Technologie der Fette und Fettprodukte*, Vol. I, Springer, Vienna, 1936, Chap. IV, pp. 111-144.

¹¹⁸ Poulletier de la Salle, *circa* 1769. Cited by de Fourcroy, *Ann. chim. phys.* [1], **3**, 242-252 (1789), and by C. E. Bills, *Physiol. Revs.*, **15**, 1 (1935).

¹¹⁹ De Fourcroy, *Ann. chim. phys.* [1], **3**, 242-252 (1789).

¹²⁰ M. E. Chevreul, *Ann. chim. phys.* [1], **95**, 5-50 (1815).

¹²¹ M. E. Chevreul, *Ann. chim. phys.* [2], **2**, 339-372 (1816).

¹²² M. Berthelot, *Ann. chim. phys.* [3], **56**, 51-98 (1859).

Although the present empirical formula of $C_{27}H_{46}O$ was proved, in 1888, on the basis of an analysis of cholesterol acetate dibromide by Reinitzer,¹²³ it was not until the period of the early thirties of this century that the structural formulas of cholesterol and other sterols were first established.

In addition to cholesterol, other sterols have also been known for a long time. For example, in 1811 Braconnot¹²⁴ isolated a product from mushrooms which he named *adipocire*. Vauquelin¹²⁵ referred to this same product as *fungine*, while Gobley¹²⁶ gave it the name *agaricine*. It remained for Tanret^{127,128} to coin the term *ergosterine* for it, and to point out its relationship to cholesterol.

(b) *Classification of Sterols*. Thanks to the development of our knowledge on the structural relationships of the various sterols, and in view of the increased information now available on their distribution in nature, a satisfactory classification based upon the latter data has recently been drawn up. This classification is as follows:

1. *Zoosterols* or animal sterols. This group includes cholesterol, coprosterol, allo-cholesterol, and dihydrocholesterol.

2. *Phytosterols* or plant sterols characteristic of the higher plants or *phanerogams*. The common sterols included in this category are sitosterol, stigmasterol, and brassicasterol.

3. *Mycosterols* or sterols from lower plants (especially fungi). The general plant group in which the mycosterols occur includes the *cryptogams*. The best known example in this group is ergosterol. Other representatives are zymosterol, dihydroergosterol, and fucosterol.

(c) *Structure and General Properties of the Sterols*. Although the correct empirical formula for cholesterol was established many years ago by Reinitzer,¹²³ the first approximation to the structural formula was not proposed until 1928. This Wieland formula¹²⁹ was soon shown to require modification. This investigator realized that the position to which the ethyl group was attached was not unequivocally proven, and the task of placing the two "homeless" carbon atoms continued to be the objective of Wieland and collaborators, according to Rosenheim and King.¹⁰⁶ As a result of the new conception of the structure of the hydrocarbon, cholane, proposed by Rosenheim and King,¹³⁰⁻¹³² a new formula for cholesterol was proposed by these same workers¹³⁰⁻¹³² and by Wieland and Dane.¹³³ This formula has now been unanimously accepted. The revised structure was

¹²³ F. Reinitzer, *Monatsh.*, 9, 421-441 (1888).

¹²⁴ H. Braconnot, *Ann. chim. phys.* [1], 79, 265-304 (1811).

¹²⁵ M. Vauquelin, *Ann. chim. phys.* [1], 85, 5-25 (1813).

¹²⁶ M. Gobley, *J. pharm. chim.* [3], 29, 81-91 (1856).

¹²⁷ C. Tanret, *Compt. rend.*, 108, 98-100 (1889).

¹²⁸ C. Tanret, *Ann. chim. phys.* [6], 20, 289-297 (1890).

¹²⁹ H. Wieland, *Z. angew. Chem.*, 42, 421-424 (1929).

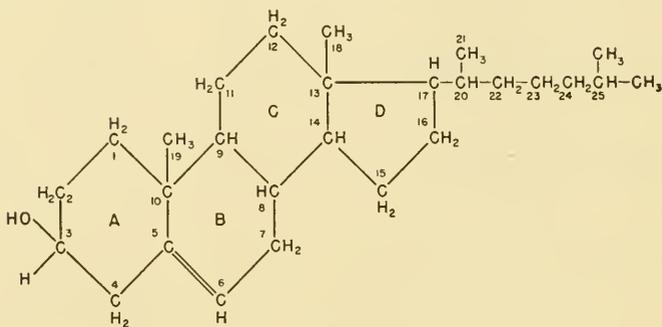
¹³⁰ O. Rosenheim and H. King, *Chemistry & Industry*, 51, 464-466 (1932).

¹³¹ O. Rosenheim and H. King, *Nature*, 130, 315 (1932).

¹³² O. Rosenheim and H. King, *J. Soc. Chem. Ind.*, 52, 299-301 (1933).

¹³³ H. Wieland and E. Dane, *Z. physiol. Chem.*, 210, 268-281 (1932).

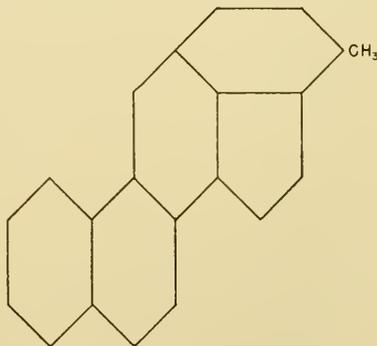
considered by Windaus and Wieland to offer a satisfactory explanation for the difficulties inherent in the original formula.



Cholesterol

The small arabic numbers indicate the designations generally assigned to the different carbon atoms in cholesterol, as well as in other sterols which have a similar structure. A, B, C, and D are commonly used to designate the rings, which may also be referred to as I, II, III, and IV, respectively.

In addition to establishing the basic unit of the steroids as a phenanthrene ring to which a five-carbon ring is attached, the new formula also involved placing the side chain at C₁₇ instead of at C₁₉. According to Rosenheim and King,¹³⁰⁻¹³² the length of the calciferol and ergosterol molecule determined experimentally by Bernal,¹³⁴ by the use of the x-ray diffraction patterns, could not be reconciled with the original Wieland formula. Moreover, certain data on the surface films of sterols obtained by Adam and Rosenheim¹³⁵ were "nearly impossible to interpret" on this earlier basis. The observation of Wieland and Dane¹³⁶ was also cited as strong proof that the side chain on the sterols was attached to C₁₇. When the potential intramolecular ring structure of cholic acid was closed, it was demonstrated that the condensation product formed was methylcholanthrene. All of



Methylcholanthrene

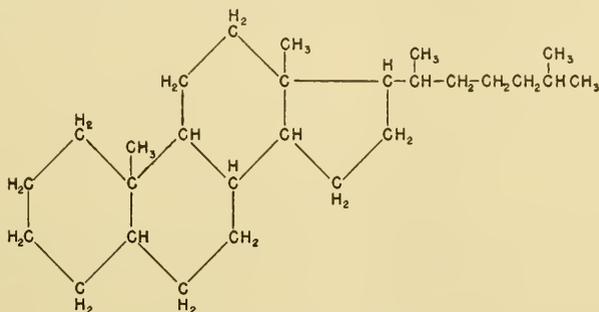
¹³⁴ J. D. Bernal, *Nature*, 129, 277-278 (1932).

¹³⁵ N. K. Adam and O. Rosenheim, *Proc. Roy. Soc. London*, A126, 25-34 (1929).

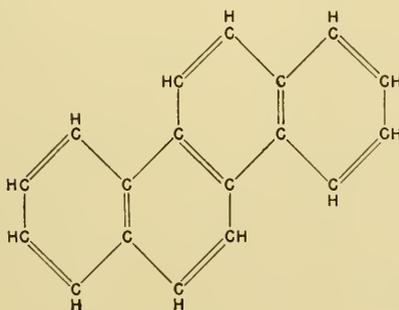
¹³⁶ H. Wieland and E. Dane, *Z. physiol. Chem.*, 219, 240-244 (1933).

these objections to the original Wieland formula were satisfied by the new postulated structure.

Cholestane has long been recognized as the mother substance of the sterols. The new structure proposed for cholestane was based upon its dehydrogenation to chrysene, which was accomplished by Diels and co-workers^{137,138} when cholesterol or cholic acid was reduced with palladium, charcoal, or selenium.



Cholestane ($C_{27}H_{48}$)



Chrysene ($C_{18}H_{12}$)

Apparently chrysene is produced only when cholesterol or cholic acid is subjected to very energetic dehydrogenation, whereby the original ring D is modified. Ruzicka¹³⁹ and others¹⁴⁰⁻¹⁴² showed that the product, $C_{18}H_{16}$, obtained by Diels *et al.*^{143,144} from cholesterol retained the 5-carbon structure for the D ring under certain conditions. The latter compound has frequently been referred to as *Diels' hydrocarbon*. It is also obtained on dehydrogenation of ergosterol, from the sex hormones, from some of the

¹³⁷ O. Diels and W. Gädke, *Ber.*, 58, 1231-1233 (1925).

¹³⁸ O. Diels, *Ber.*, 66, 487-488, 1122-1127 (1933).

¹³⁹ L. Ruzicka, L. Ehmann, M. W. Goldberg, and H. Hösl, *Helv. Chim. Acta*, 16, 833-841 (1933).

¹⁴⁰ E. Bergmann and H. Hillemann, *Ber.*, 66, 1302-1306 (1933).

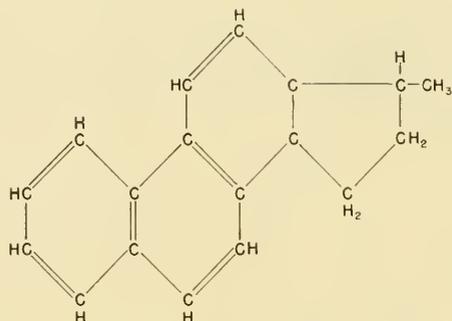
¹⁴¹ J. W. Cook and C. L. Hewett, *J. Soc. Chem. Ind.*, 52, 451-452, 603 (1933).

¹⁴² O. Diels and H. Klare, *Ber.*, 67, 113-122 (1934).

¹⁴³ O. Diels and W. Gädke, *Ber.*, 60, 140-147 (1927).

¹⁴⁴ O. Diels, W. Gädke, and P. KÖrding, *Ann.*, 459, 1-26 (1927).

toad poisons (bufotoxin, bufotalin, and cinobufagin), as well as from cardiac glycosides and sapogenins:



Diels' hydrocarbon ($C_{18}H_{16}$)

Other information useful in establishing the structural relationships of cholesterol can now be interpreted. For example, it had been known that the molecule contained a double bond which could take up halogens. The double bond and the hydroxyl group were on two rings which were adjacent since, on oxidation, a tetracarboxylic acid was obtained with the same number of carbons as cholesterol.^{145,146} From the chemical properties of this acid and of numerous oxidation products, it could be deduced that the two rings, A and B, were condensed with each other, and that the unsaturated linkage was in a β,γ -position to the hydroxyl group.

The structure of the side chain of cholesterol had been well known for a number of years after Windaus and Resau¹⁴⁷ demonstrated, in 1913, that methyl heptanone, $(CH_3)_2CH \cdot CH_2 \cdot CH_2 \cdot CO \cdot CH_3$, originates on energetic oxidation of this sterol. This would indicate that the ketone group (C_{20}) is the site of a combination of the side chain with the cyclopentenophenanthrene ring, while the adjacent methyl group obviously represents a side chain attached at C_{20} . The rest of the aliphatic chain is apparently unaltered by this treatment.

The sterols are quite similar in certain general physical properties. They are white crystalline solids which are insoluble in water, but which readily dissolve in fats and fat solvents. For the most part, they are optically active. They contain 26 to 30 carbons, and all have four carbocyclic rings. The side chain is invariably attached to the fourth ring in position 17. They have a single secondary alcohol group on the first ring (position 3) which may be esterified in the presence of acids or oxidized to a ketone. Bile acids and other steroids, such as the adrenocortical and sex hormones, may have additional hydroxyl groups on the cyclopentenophenanthrene

¹⁴⁵ A. Windaus and I. Stein, *Ber.*, *37*, 3699-3708 (1904).

¹⁴⁶ A. Windaus and M. Deppe, *Ber.*, *66*, 1563-1565 (1933).

¹⁴⁷ A. Windaus and C. Resau, *Ber.*, *46*, 1246-1248 (1913).

nucleus. Cholesterol and a number of closely related compounds have at least one unsaturated linkage in ring B of the cyclic part of the molecule. Ergosterol and other provitamins D have two unsaturated linkages in ring B.

Many sterols are readily precipitable by the saponin, digitonin, with which they form an insoluble precipitate. However, no digitonide results when this test is applied to a sterol ester, as a free hydroxyl group is a necessary requirement for the reaction. Lanolin and other sterol esters can easily be separated from the readily precipitable free sterols by the application of this method.

Sterols show a marked tendency to separate in the form of mixed crystals with closely related compounds. In some cases it is almost impossible to remove the contaminant completely, even by repeated recrystallizations. It has recently been found that unsaturated sterols are usually mixed with a small amount of the corresponding dihydro compound.^{148,149}

(d) *Occurrence and Properties of Individual Zoosterols.* a'. Cholesterol: Cholesterol is by far the oldest recognized and also the most important member of the sterol group. It belongs to the class of zoosterols. This alcohol is widely distributed in the animal kingdom but it is completely absent from the plant world. It is the sterol which is almost exclusively present in warm-blooded animals, while closely related forms may replace it in the lower animals and insects.

(1') *Distribution of Cholesterol:* Cholesterol is present in most animal cells. Highest concentrations occur in nervous tissue, liver, and in fat deposits. The white matter of the brain contains 14% of cholesterol on the dry weight basis, while the grey matter has a content of 6% of this alcohol.¹⁵⁰ Fieser¹¹⁶ indicates that the human brain may contain as much as 17% of cholesterol in the dry substance. A sufficiently high proportion of cholesterol occurs in the spinal cord of cattle, so that this tissue is widely used commercially as a source. Other tissues have the following amounts of cholesterol (in per cent) based on dry weight¹⁵⁰: kidney 1.6, spleen 1.5, skin 1.3, liver 0.93, mammary gland 0.70, whole blood 0.65, heart muscle 0.65, smooth muscle 0.55, diaphragm 0.34, and skeletal muscle 0.25. Bile also contains a considerable amount of cholesterol; this secretion may become supersaturated with this alcohol, with the result that gallstones are formed. The adrenal cortex is known to be a site where cholesterol occurs in relatively high concentration. It is considered to function in this organ as the precursor of the adrenocortical hormones.

Cholesterol is frequently associated with small amounts of related com-

¹⁴⁸ R. J. Anderson, F. P. Nabenhauer, and R. L. Shriner, *J. Biol. Chem.*, 71, 389-399 (1926-1927).

¹⁴⁹ F. W. Heyl and O. F. Swoap, *J. Am. Chem. Soc.*, 52, 3688-3690 (1930).

¹⁵⁰ W. R. Bloor, *Biochemistry of the Fatty Acids*, Reinhold, New York, 1943.

pounds, from which it can be separated only with extreme difficulty. For example, Schönheimer and his collaborators¹⁵¹⁻¹⁵² reported that cholesterol prepared from various organs contains 1 to 2% of dihydrocholesterol, as well as traces of ergosterol or of some other highly unsaturated sterol. Provitamin D₃, or 7-dehydrocholesterol, is present in the material isolated from the spinal cord to the extent of 0.1%, while a larger proportion occurs in the sterol separated from skin.¹⁵³ Only after precipitation of the sparingly soluble cholesterol dibromide¹⁵⁴⁻¹⁵⁶ and regeneration of cholesterol by zinc dust and acetic acid^{154,155,157} or sodium iodide can a product of high purity be obtained.¹⁵⁸

Hilditch²⁸ reports that the liver fat of several species of fishes contains a non-saponifiable fraction which is largely cholesterol. These include the liver fat of the skate (*Raia maculata*) (0.37%), of the angel-fish (*Rhina squatina*) (1.5%), of the thresher shark (*Alopias vulpes*) (1.8%), and of the spotted dogfish (*Scyllium canicula*) (2%).

The relatively high cholesterol value in the mammary gland is probably related to its excretion in milk. Whole milk has been reported to contain 0.013%, while powdered whole milk has a cholesterol content of 0.088%.¹¹⁷ Nataf and collaborators¹⁵⁹ have found cholesterol to be lowest in the milk of Holstein cows (7.0, 8.2, and 11.1 milligram per cent) and considerably higher in Jersey milk (11.1-15.6 milligram per cent) and in the milk from Guernsey cows (11.8-16.0 milligram per cent). The concentration in butter fat is given as 0.185%, while lard is stated to have 0.108% of cholesterol.¹¹⁷ The presence of cholesterol in beef tallow has also been reported.

The high proportion of cholesterol in skin is of especial interest because it presumably is a precursor of 7-dehydrocholesterol, which is provitamin D₃. It is believed that the activation of this provitamin D to vitamin D takes place at or near the surface of the skin when it is exposed to sunlight or to other sources of ultraviolet light. Large amounts of cholesterol are also excreted by the sebaceous glands. Wool grease, which is more commonly called lanolin, is an important source of cholesterol. Separation in a pure form is hampered by the fact that the isocholesterols, which are now known

¹⁵¹ R. Schönheimer, H. v. Behring, R. Hummel, and L. Schindel, *Z. physiol. Chem.*, **192**, 73-76 (1930).

¹⁵² R. Schönheimer, H. v. Behring, and R. Hummel, *Z. physiol. Chem.*, **192**, 86-93, 93-96 (1930).

¹⁵³ H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, pp. 346, 348.

¹⁵⁴ A. Windaus, *Ber.*, **39**, 518-523 (1906).

¹⁵⁵ A. Windaus and A. Hauth, *Ber.*, **39**, 4378-4384 (1906).

¹⁵⁶ R. J. Anderson, *J. Biol. Chem.*, **71**, 407-418 (1927).

¹⁵⁷ L. Ruzicka, H. Brügger, E. Eichenberger, and J. Meyer, *Helv. Chim. Acta*, **17**, 1407-1416 (1934).

¹⁵⁸ R. Schoenheimer, *J. Biol. Chem.*, **110**, 461-462 (1935).

¹⁵⁹ B. Nataf, O. Mickelsen, A. Keys, and W. E. Petersen, *J. Nutrition*, **36**, 495-506 (1948).

to be mixtures of agnol (agnosterol), $C_{30}H_{48}O$, and lanol (lanosterol), $C_{30}H_{50}O$, are also present in a considerable amount. Of the common foods, the most concentrated source of cholesterol is egg-yolk, in which it is present¹¹⁷ to the extent of 1.342%. Egg-white, on the other hand, is devoid of this lipid. Certain types of gallstones are almost pure cholesterol.

Cholesterol occurs in nature both free and in the form of esters of palmitic, stearic, or oleic acids. That present in brain, spinal cord, gallstones, and red blood cells is in the free state, while both the ester and the free alcohol are found in blood serum, as well as in the fat of the epidermis. In lanolin obtained from sheep wool, the cholesterol and related sterols occur exclusively as the esters.

Although cholesterol is a constant constituent in the higher animals, it is also widely distributed in the lower forms. Dorée¹⁶⁰ has demonstrated cholesterol in reptiles, while several investigators have shown it to be present in such fishes as the mackerel,¹⁶⁰ carp (roe¹⁶¹ and milt¹⁶²), and salmon.¹⁶³ Cholesterol occurs, likewise, in mollusks such as the whelk (*Buccinum undatum*),¹⁶⁰ the octopods,^{164,165} arthropods (crab),¹⁶⁰ annulates (earthworm),¹⁶⁰ coelenterates (sea anemone),¹⁶⁰ and in several varieties of snails¹⁶⁶ and shellfish.¹⁶⁷ Although Dorée failed to detect this sterol in the insects, Welsch¹⁶⁵ reported its presence in the Spanish fly (*Cantharis vesicatoria*) and in the cockchafer beetle (*Melolantha vulgaris*). Dorée¹⁶⁰ was also unable to detect cholesterol in starfish (*Asterias vulgaris*), but Mathews¹⁶⁸ indicated the possibility of its presence in this species; it was found in the sea-urchin (*Echinus*), which is another echinoderm. The former worker claimed that the silkworm (*Bombyx mori*) contains only a modified sterol, the so-called bombicesterol,¹⁶⁰ but cholesterol has also been detected.¹⁶⁹ Apparently, cholesterol is absent from the sponges,¹⁶⁰ which are on the borderline between metazoa and protozoa. However, a closely allied sterol, first demonstrated by Henze¹⁷⁰ and called "spongosterine" (spongosterol), is present in this species. The reduction product of chole-

¹⁶⁰ C. Dorée, *Biochem. J.*, **4**, 72-106 (1909).

¹⁶¹ M. Gobley, *J. pharm. chim.* [3], **17**, 401-417 (1850); **18**, 107-119 (1850).

¹⁶² M. Gobley, *J. pharm. chim.* [3], **19**, 406-421 (1851).

¹⁶³ F. Miescher, *Ber.*, **7**, 376-379 (1874).

¹⁶⁴ M. Henze, *Z. physiol. Chem.*, **55**, 433-444 (1908).

¹⁶⁵ A. Welsch, "Ueber das Vorkommen und die Verbreitung der Sterine im Thier- und Pflanzenreich," *Dissertation*, Freiburg im Breisgau, 1909. Cited by C. E. Bills, *Physiol. Revs.*, **15**, 4 (1935).

¹⁶⁶ C. A. Kind, S. G. Slater, and A. Vinci, *J. Org. Chem.*, **13**, 538-541 (1948).

¹⁶⁷ M. Tsujimoto and H. Koyanagi, *J. Soc. Chem. Ind. Japan*, **37**, suppl., 81-85B, 85-86B, 436-439B (1934); **38**, 118-120B (1935); *Chem. Abst.*, **28**, 3257, 7566 (1934); **29**, 3865 (1935).

¹⁶⁸ A. P. Mathews, *J. Biol. Chem.*, **14**, 465-467 (1913).

¹⁶⁹ A. Menozzi and A. Moreschi, *Atti accad. Lincei, Rend.* [5], **19**, 1 sem., 126-129 (1910).

¹⁷⁰ M. Henze, *Z. physiol. Chem.*, **41**, 109-124 (1904); **55**, 427-432 (1908).

terol, cholestanol, has been isolated from certain sponges.¹⁷¹ Chalinasterol has also been found.¹⁷² Panzer¹⁷³ has demonstrated the presence of cholesterol in one protozoon, namely, *Goussia gadi*, which he isolated from the air bladders of the pollack. It is possible that cholesterol may have originated from the host rather than from the parasitic coccidia. Although cholesterol is found in many bivalves, the oyster contains a modified form referred to as ostreasterol.¹⁷⁴ Gastropods (snails) have been found to contain cholesterol regardless of feeding habits.¹⁷⁵

(2') Physical Properties of Cholesterol: Cholesterol occurs in two types of crystals. When it is prepared from solutions of anhydrous solvents, it forms needle-like crystals. On the other hand, when the solvent contains water, for example, 95% alcohol, the cholesterol separates as shiny monoclinic platelets resembling mother of pearl, having one molecule of water of crystallization. The water of crystallization is lost when these platelets are heated at 100°C., or when the product is dried over concentrated sulfuric acid. The crystals readily disintegrate on standing.

Like other lipids, cholesterol is soluble in organic solvents, such as alcohol, ether, chloroform, benzene, and petroleum ether, as well as in fats and oils. It readily dissolves in cold ethyl acetate, which fact enables it to be easily separated from other lipoids. It is soluble with difficulty in cold alcohol, but it readily dissolves in hot 95% (5.5 parts) and 85% (9 parts) alcohol. Colloidal solutions of cholesterol, which remain remarkably stable over long periods, can be prepared by the addition of an acetone solution to water.¹⁷⁶ Cholesterol melts sharply at 148.5°C. It can be distilled under a high vacuum and can also be sublimed.

Because of the presence of several asymmetric carbon atoms, cholesterol exhibits optical rotation. $[\alpha]_D^{15}$ is given as -31.1^{177} and -29.9^{115} in 4% ethereal solution. Apparently, the specific rotation is independent of concentration. After a prolonged exposure to air, oxidation occurs, with a yellowing of the crystals and a resultant lowering of the melting point and a change in solubility.

Another interesting property exhibited by cholesterol deposits in the tissues is that of birefringence. Such double refraction is of considerable assistance in the qualitative identification of this lipid in tissues, as it is not exhibited by other lipids.

Considerable data are available on the turbidity points, melting points, and optical rotation of the cholesteryl esters (see Table 4). Cholesteryl

¹⁷¹ W. Bergmann, D. H. Gould, and E. M. Low, *J. Org. Chem.*, **10**, 570-579 (1945).

¹⁷² W. Bergmann, H. P. Schedl, and E. M. Low, *J. Org. Chem.*, **10**, 587-593 (1945).

¹⁷³ T. Panzer, *Z. physiol. Chem.*, **73**, 109-127 (1911).

¹⁷⁴ W. Bergmann, *J. Biol. Chem.*, **104**, 317-328, 553-557 (1934).

¹⁷⁵ W. Bergmann and E. M. Low, *J. Org. Chem.*, **12**, 67-75 (1947).

¹⁷⁶ L. S. Moyer, *Biochem. Z.*, **273**, 122-131 (1934).

¹⁷⁷ O. Hesse, *Ann.*, **192**, 175-179 (1878).

acetate, $C_{27}H_{45}O \cdot OCCH_3$, is readily prepared by the interaction of cholesterol and acetic anhydride; it crystallizes in plates or needles from an alcohol-ether solution and melts at $115^\circ C$. Cholesterol palmitate, $C_{27}H_{45}O \cdot OC(CH_2)_{14}CH_3$, can be synthesized by heating one mole of cholesterol with two moles of palmitic acid for 3 hours at $200^\circ C$. This ester is present in blood serum, skin, adrenal glands, and wool fat. The stearic acid ester, $C_{27}H_{45}O \cdot OC(CH_2)_{16}CH_3$, is not found in blood serum, but it does occur in the adrenal glands. Cholesteryl stearate can be prepared in a manner analogous to that used for the palmitate. It can be crystallized from an ether-alcohol mixture in the form of white platelets. Cholesteryl oleate, $C_{27}H_{45}O \cdot OC(CH_2)_7CH:CH(CH_2)_7CH_3$, is another common ester. It is present in blood serum, wool fat, and the adrenal glands. The synthesis is identical with that of the palmitate and stearate. The oleate ester separates from solution in the form of long thin needles which are readily soluble in ether, chloroform, and hot acetone, but only difficultly soluble in hot alcohol.

On heating, the cholesteryl esters exhibit the unusual property of changing to a turbid liquid state before melting to a clear liquid at a higher temperature. Reinitzer¹²³ observed this phenomenon in 1888 in the case of cholesteryl benzoate; it has since been shown to be a general property of the natural cholesteryl esters, as well as of the synthetic ones. The turbid liquid state has properties actually intermediate between a solid and a liquid phase. Lehmann¹⁷⁸ referred to this as the "liquid crystalline" state, which is a misnomer, in that there is actually no crystalline phase. The more appropriate name, *mesomorphic* state, was proposed by Friedel¹⁷⁹ and is in current usage.

Two types of the mesomorphic phase are now recognized, *i.e.*, *smectic* and *nematic*. In the first case, the liquid does not exhibit a normal flow, but rather gliding movements in one plane. In the nematic type of mesomorphism, the liquid flows readily, but it has a thread-like structure and a comparatively low viscosity. A third type of mesomorphism is likewise demonstrable in some cholesteryl esters; this is known as the *cholesteric* phase. This is quite similar to the nematic phase, but differs in having a definite layering, and in showing distinct color effects in polarized light. The molecular layers appear to be thicker than in the smectic phase.

The cholesteryl esters from the formate to the myristate can assume the cholesteric form, although the esters from the caprylate to the myristate usually appear first in the smectic modification, and then change to the cholesteric phase on further heating, before melting to the clear liquid state. These changes are reversible and occur in the opposite order on cooling. The cholesteryl esters having fatty acid chains longer than those

¹⁷⁸ O. Lehmann, *Z. physik. Chem.*, 4, 462-472 (1889).

¹⁷⁹ G. Friedel, *Ann. phys.* [9], 18, 273-474 (1922).

TABLE 4
TURBIDITY POINTS, MELTING POINTS, SPECIFIC ROTATIONS, AND SOLUBILITIES OF CHOLESTERYL ESTERS^a

Cholesteryl ester	Formula	Turbidity point, °C.	Melting point, °C.	[α] _D ²⁰	Solubility, g./100 ml.		
					Ethanol (20°C.)	Ethanol (boiling)	Acetone (20°C.)
Free alc. ^b	C ₂₇ H ₄₈ OH	—	149	-39	—	—	—
Formate	C ₂₇ H ₄₈ O·OCH	—	96	-51.4	0.6	11.9	6.2
Acetate	C ₂₇ H ₄₈ O·OCCH ₃	—	115	-47.4	0.5	8.7	2.5
Propionate	C ₂₇ H ₄₈ O·OCCH ₂ CH ₃	112	114	-40.9	0.4	5.1	2.3
Butyrate	C ₂₇ H ₄₈ O·OC(CH ₂) ₂ CH ₃	103	111.5	-36.3	0.4	4.5	2.3
Isobutyrate	C ₂₇ H ₄₈ O·OCCH(CH ₃) ₂	—	127.5	-37.9	0.4	3.1	1.0
Valerate	C ₂₇ H ₄₈ O·OC(CH ₂) ₃ CH ₃	92	94	-34.0	0.4	5.2	1.3
Isovalerate	C ₂₇ H ₄₈ O·OCCH ₂ CH(CH ₃) ₂	112	114.5	-34.0	0.4	4.7	1.0
Caproate	C ₂₇ H ₄₈ O·OC(CH ₂) ₄ CH ₃	85	101	-34.0	0.3	3.7	0.6
Caprylate	C ₂₇ H ₄₈ O·OC(CH ₂) ₆ CH ₃	103	108	-31.3	0.2	2.3	0.6
Caprate	C ₂₇ H ₄₈ O·OC(CH ₂) ₈ CH ₃	82	93	-29.8	0.1	1.7	0.5
Laurate	C ₂₇ H ₄₈ O·OC(CH ₂) ₁₀ CH ₃	—	91	-27.6	0.1	1.3	0.3
Myristate	C ₂₇ H ₄₈ O·OC(CH ₂) ₁₂ CH ₃	80	86	-26.6	0.03	1.0	0.2
Palmitate	C ₂₇ H ₄₈ O·OC(CH ₂) ₁₄ CH ₃	78	90	-25.1	0.03	1.0	0.1
Stearate	C ₂₇ H ₄₈ O·OC(CH ₂) ₁₆ CH ₃	78	82.5	-24.3	0.03	0.7	0.1
Lignocerate	C ₂₇ H ₄₈ O·OC(CH ₂) ₂₂ CH ₃	87	89	-18.7	0.2	0.8	0.3
Undecenoate	C ₂₇ H ₄₈ O·OCC ₁₀ H ₉	79	83.5	-28.3	0.1	4.1	1.1
Oleate (<i>cis</i>)	C ₂₇ H ₄₈ O·OC(CH ₂) ₇ CH=CH(CH ₂) ₇ CH ₃	—	44.5	-23.4	0.3	1.4	2.4

Cholesteryl ester	Formula	Turbidity point, °C.	Melting Point °C.	[α] _D ²⁰	Solubility, g./100 ml.			
					Ethanol (20°C.)	Ethanol (boiling)	Acetone (boiling)	
Elaidate (<i>trans</i>)	C ₂₇ H ₄₈ O·OC(CH ₂) ₇ CH(CH(CH ₂) ₇)CH ₃	56	65.5	-23.7	0.1	1.7	0.4	6.4
Petroselinate (<i>cis</i>)	C ₂₇ H ₄₆ O·OC(CH ₂) ₆ CH(CH(CH ₂) ₁₀)CH ₃	—	—	-21.9	0.6	1.6	1.6	3.5
Erucate	C ₂₇ H ₄₆ O·OC(CH ₂) ₁₁ CH(CH(CH ₂) ₇)CH ₃	44	48	-20.8	0.2	1.6	1.4	6.8
Linoleate	C ₂₇ H ₄₆ O·OC(CH ₂) ₇ CH:CHCH ₂ CH(CH(CH ₂) ₆)CH ₃	41	42	-23.9	0.7	1.6	1.8	3.8
Linolenate	C ₂₇ H ₄₅ O·OC(CH ₂) ₇ CH:CHCH ₂ CH:CHCH ₂ CH ₃	49	74	-24.3	0.8	2.1	1.9	4.1
Mono-oxalate	C ₂₇ H ₄₆ O·OCCOOH	—	69	-32.5	1.0	17.1	6.4	18.5
Dioxalate	C ₂₇ H ₄₅ O·OC·CO·OC ₂₇ H ₄₅	—	220-230	-25.8	0.03	0.3	0.07	0.5
Monosuccinate	C ₂₇ H ₄₅ O·OC(CH ₂) ₂ COOH	—	175	—	—	—	—	—
Disuccinate	C ₂₇ H ₄₆ O·OC(CH ₂) ₂ CO·OC ₂₇ H ₄₆	220	235	-38.6	0.06	0.2	0.04	0.5
Diadipate	C ₂₇ H ₄₅ O·OC(CH ₂) ₈ CO·OC ₂₇ H ₄₅	—	195.5	-34.7	0.04	0.2	0.03	0.4
Diadipate	C ₂₇ H ₄₅ O·OC(CH ₂) ₈ COOH	—	144	-30.6	1.9	7.3	1.3	4.2
Diadipate	C ₂₇ H ₄₅ O·OC(CH ₂) ₈ CO·OC ₂₇ H ₄₅	195	222	-36.1	0.06	0.2	0.03	0.3
Monosuberate	C ₂₇ H ₄₅ O·OC(CH ₂) ₈ COOH	127	130	-29.2	0.4	5.1	0.7	5.2
Disuberate	C ₂₇ H ₄₅ O·OC(CH ₂) ₈ CO·OC ₂₇ H ₄₅	—	179.5	-34.9	0.02	0.1	0.04	0.3

^a I. H. Page and H. Rudy, *Biochem. Z.*, 220, 304-326 (1930). Adapted from A. W. Kralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 556.

^b L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, 3rd ed., Reinhold, New York, 1949.

of myristic acid do not exhibit the cholesteric phase, but only the smectic modification. In the case of cholesteryl myristate, Friedel¹⁷⁹ found that the cholesteric state develops at 72°C., the smectic phase at 78°C., and that the compound melts to a clear liquid at 83°C. Numerous cholesteryl esters have been synthesized, and their properties studied.¹⁸⁰⁻¹⁸⁴

The cholesteryl esters of the fatty acids are quite soluble in boiling ethanol and in boiling acetone. They are only slightly soluble in ethanol at 20°C., and somewhat more so in acetone at this temperature.¹⁸⁴ In the case of the dicarboxylic acids, the monoesters have a considerably greater solubility than do the diesters.

(3') Chemical Properties of Cholesterol. One of the most important reactions of cholesterol is the ability to form esters, not only with organic acids but also with inorganic acids. In addition to the series of cholesteryl esters the physical properties of which are reviewed in Table 4, a number of non-physiological esters are known. These include the benzoate, $C_{27}H_{45}O \cdot OCC_6H_5$, which can be prepared from cholesterol and benzoyl chloride in dry pyridine solution. The plate-like crystals melt at 146.5°C. to a turbid liquid which clears at 178°C. On cooling the melted product exhibits a violet fluorescence. Cholesteryl allophanate, $C_{27}H_{45}O \cdot OCNHCONH_2$, results from the action of hydrogen cyanide on cholesterol in benzene solution. This ester is quite difficultly soluble in most solvents. The melting point is recorded as 235-236°C. by Tange and McCollum,¹⁸⁵ and as 277-278°C. by Fabre.¹⁸⁶ The dicholesterol ester of oxalic acid, $C_{27}H_{45}O \cdot OCCO \cdot OC_{27}H_{45}$,¹⁸⁴ is sufficiently insoluble to be useful commercially in the separation of cholesterol from other sterols.

Other cholesterol compounds are those in which this alcohol combines in glucoside linkage with the sugars. Plattner and Uffer¹⁸⁷ have synthesized glucosides, maltosides, and cellobiosides of cholesterol. When the reaction was carried out in the presence of mercuric acetate or of mercuric acetamide, a 30 to 50% yield was obtained. It is possible that a combination between cholesterol and glucuronic acid similar to that with the sugars may be the water-soluble form in which cholesterol is excreted in the urine. Although this fraction is ordinarily very small, Bloch and Sobotka¹⁸⁸ have found it present at ten times the normal level in the urine of cancer patients. Although these workers did not prove the nature of the cholesterol compound, they were able to recover the bulk of it only after hydrolysis of the

¹⁸⁰ F. M. Jaeger, *Rec. trav. chim.*, **25**, 334-351 (1906).

¹⁸¹ P. Gaubert, *Compt. rend.*, **145**, 722-725 (1907).

¹⁸² P. Gaubert, *Compt. rend.*, **147**, 498-500 (1908).

¹⁸³ A. Prins, *Z. physik. Chem.*, **67**, 689-723 (1909).

¹⁸⁴ I. H. Page and H. Rudy, *Biochem. Z.*, **220**, 304-326 (1930).

¹⁸⁵ U. Tange and E. V. McCollum, *J. Biol. Chem.*, **76**, 445-456 (1928).

¹⁸⁶ R. Fabre, *Compt. rend.*, **183**, 679-681 (1926).

¹⁸⁷ P. A. Plattner and A. Uffer, *Helv. Chim. Acta*, **28**, 1049-1053 (1945).

¹⁸⁸ E. Bloch and H. Sobotka, *J. Biol. Chem.*, **124**, 567-572 (1938).

urine. In the case of the closely related sex hormones, it is known that these steroids may be secreted as glucuronates or ethereal sulfates. Marx¹⁸⁹ has confirmed the fact that cholesterol occurs in the urine in a water-soluble compound, not extractable with fat solvents until after hydrolysis. Bloch and Sobotka¹⁸⁸ have suggested the possibility that urinary cholesterol might be combined with albumin, although this possibility seems doubtful in view of their finding that clear urine contained as much bound cholesterol as did cloudy samples of urine. In view of the recent report of cholesterol complexes of protein in blood, which may contain as much as 30% of cholesterol,¹⁹⁰ further studies in this field would seem to be advisable.

In line with the behavior of aliphatic alcohols, cholesterol can readily be transformed to an ether, dicholesteryl ether, which has the formula, $C_{27}H_{45} \cdot O \cdot C_{27}H_{45}$. This ether was first prepared synthetically by Mauthner and Suida,¹⁹¹ in 1896, from cholesterol treated with copper sulfate as a dehydrating agent. Other more recent procedures for its preparation have been reviewed by Bills and McDonald,¹⁹² Silberman and Silberman-Martyncewa¹⁹³ have proved that this compound is a physiological one, since it can be isolated from the spinal cord of cattle in amounts of 1.5 to 2.0% of the dry weight. Their product melted at 205–209°C.; it was soluble in boiling benzene, toluene, and alcohol, slightly soluble in cold benzene or toluene, and it dissolved to only a slight extent in cold alcohol, warm ether, or acetone.

Because of the double bond at the 5:6 position, cholesterol possesses a number of characteristic reactions. Not only will it add hydrogen and the halogens, but it is acted on by perbenzoic acid and ozone. When it is catalytically hydrogenated, dihydrocholesterol (also called β -cholestanol), $C_{27}H_{47}OH$, originates. Isomeric compounds result when reduction is brought about by sodium and amyl alcohol.

Halogens readily react to saturate the cholesterol molecule. One of the best known of these compounds, cholesterol dibromide ($C_{27}H_{45}Br_2OH$), originates when cholesterol is treated with an excess of bromine in an ethereal acetic acid solution. The dibromide crystallizes in characteristic needles which are useful for the detection of cholesterol. They exist in two modifications which melt at 109–111°C. and 123–124°C., respectively. Cholesterol dibromide can be reconverted to cholesterol on reduction with sodium amalgam.

¹⁸⁹ W. Marx, *Personal communication* (1950).

¹⁹⁰ I. W. Gofman, F. Lindgren, H. Elliott, W. Mantz, J. Hewitt, B. Strisower, V. Herring, and T. P. Lyon, *Science*, *111*, 166–171, 186 (1950).

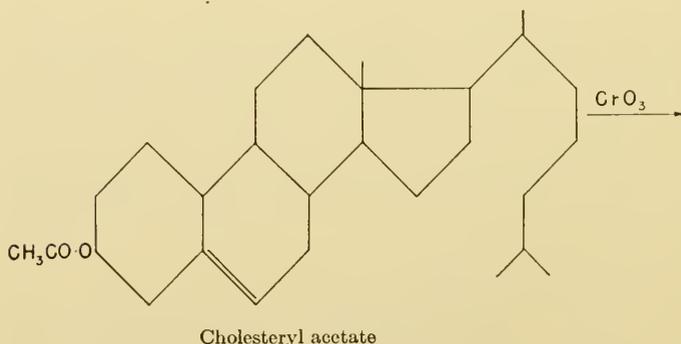
¹⁹¹ J. Mauthner and W. Suida, *Monatsh.*, *17*, 29–49 (1896).

¹⁹² C. E. Bills and F. G. McDonald, *J. Biol. Chem.*, *72*, 1–19 (1927).

¹⁹³ H. Silberman and S. Silberman-Martyncewa, *J. Biol. Chem.*, *159*, 603–604 (1945).

The iodine absorption value, which is a function of the double bond, may be useful in the identification of cholesterol. Hübl reagent gives a theoretical result (65.8), as does the Rosenmund-Kuhnhehn pyridine sulfate-bromine reagent.¹⁵⁰ The Wijs and Hanus solutions, on the other hand, yield erroneous values. According to Rolls,¹⁹⁴ such irregularities in iodine absorption may be the result of variations in temperature or in the solvent employed. Although the Rosenmund-Kuhnhehn reagent gives theoretical results with cholesterol, the values are too high with ergosterol.¹⁹⁵ Bloor¹⁵⁰ has indicated that when the latter reagent is modified by substitution of iodine for bromine, it serves satisfactorily for the determination of ergosterol; the modified reagent no longer gives a theoretical result with cholesterol. However, the original Rosenmund-Kuhnhehn reagent can be employed satisfactorily with both cholesterol and ergosterol if the medium is changed to one containing three parts of methanol and one part of acetic acid.

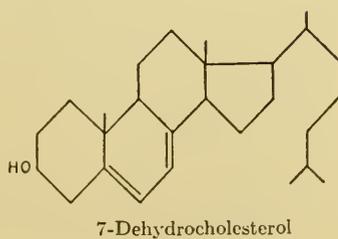
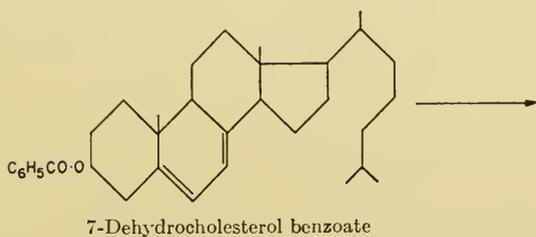
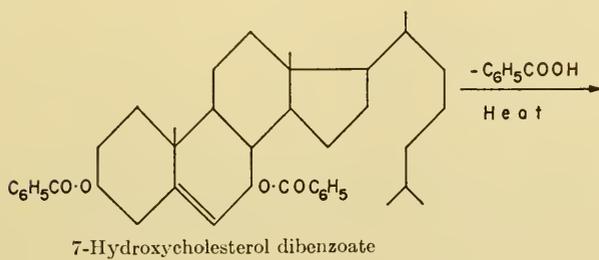
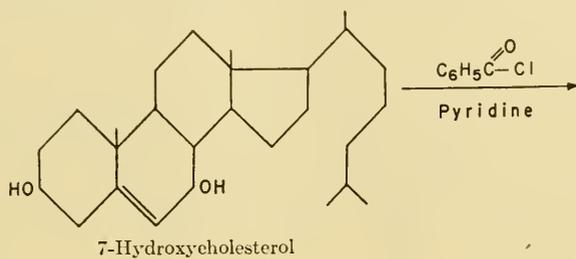
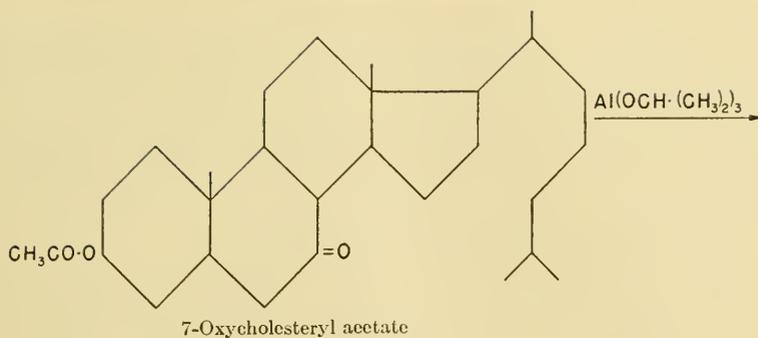
One important transformation which can be effected on the cholesterol molecule is the introduction of a second double bond in ring B with the formation of 7-dehydrocholesterol. This compound, which is found to some extent in nature and presumably may be synthesized in limited amounts by the higher animals, is of especial interest, since it has been shown to be provitamin D₃. The classical synthesis of this compound has been accomplished by Windaus and his co-workers¹⁹⁶ by oxidation of cholesteryl acetate with chromic acid to 7-oxycholesteryl acetate, followed by reduction with aluminum isopropoxide. This yields 7-hydroxycholesterol. The latter compound is benzoylated, and the dibenzoate is then decomposed by heat and by saponification into 7-dehydrocholesterol and benzoic acid. These reactions are illustrated in the following equations:



¹⁹⁴ J. O. Rolls, *J. Am. Chem. Soc.*, *55*, 2083-2094 (1933).

¹⁹⁵ M. Yasuda, *J. Biochem. Japan*, *25*, 417-433 (1937).

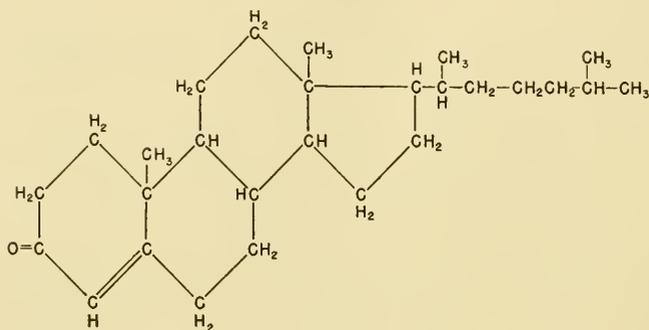
¹⁹⁶ A. Windaus, H. Lettré, and F. Schenek, *Ann.*, *520*, 98-106 (1935).



The second double bond can likewise be introduced into the sterol nucleus at the 7:8 position by treatment of the acetate with *N*-bromosuccinimide.¹⁹⁷ Bromine enters the sterol nucleus at position 7. It may be dislodged from this position by treatment with dimethylaniline, as a result of which 7-dehydrocholesterol originates. Yields as high as 50% may result.¹⁹⁸

Cholesterol forms a series of unstable addition products with the fatty acids (acetic, butyric, palmitic, stearic, and oleic), as well as with oxalic acid. It also yields an addition product with saponin.

On oxidation, cholesterol forms a series of derivatives which vary with the severity of the oxidizing agent. Thus, on mild oxidation with cupric oxide, it is converted to the ketone cholestenone, in which the double bond



Cholestenone

has shifted from the 5:6 to the 4:5 position. On more violent oxidation with chromic acid, potassium permanganate, or hydrogen peroxide, decomposition products result which have been useful in establishing the structural configuration of the sterol nucleus. As mentioned earlier, dehydrogenation with a selenium catalyst yields chrysenes,¹⁴⁴ or the so-called Diels' hydrocarbon.^{143,144} Thermal decomposition products likewise include naphthalene as well as small amounts of styrene.¹⁹⁹

Stereoisomerism is a most important property of cholesterol and related sterols. The metabolism of such stereoisomeric forms is quite unique, and varies with the compound in question. Moreover, the response of the sterol to precipitation reactions is closely related to the stereochemical configuration. The most important point of asymmetry in cholesterol itself is at C₃, while a second important focal point for such cholesterol derivatives as cholestenone or dihydrocholesterol obtains at position 5. The latter carbon is not an asymmetric one in cholesterol, because of the double bond

¹⁹⁷ H. B. Henbest, E. R. H. Jones, A. E. Bide, R. W. Peevers, and P. A. Wilkinson, *Nature*, 158, 169 (1946).

¹⁹⁸ E. F. Week, *Personal communication*.

¹⁹⁹ H. Fischer and A. Triebs, *Ann.*, 446, 241-259 (1925).

between carbons 5 and 6. However, on reduction of cholesterol, asymmetry develops at C₅, and two important classes of naturally occurring steroids can be prepared, depending upon the configuration around this carbon. A third point of asymmetry not occurring in cholesterol, but of considerable importance in the phytosterols, is on C₂₄ (see page 351).

The hydroxyl group on C₃ assumes a position in dihydrocholesterol, stigmasterol, ergosterol, sitosterol, and fucosterol similar to that in cholesterol. Only the compounds which possess this configuration for the hydroxyl group (the so-called B-type) are precipitable by digitonin. When the positions of the hydrogen and hydroxyl groups on C₃ are reversed, one obtains a compound which is no longer precipitable by digitonin. Such a configuration occurs in *epi-compounds*. According to Fieser¹¹⁶ no exceptions to this rule of precipitability with digitonin exist, and Fernholz²⁰⁰ has demonstrated that the same relationship exists for cholesterol derivatives where the side chain on C₁₇ has been shortened or even replaced by a ketonic group, as occurs in the sex hormones. The precipitability of the sterols with digitonin is not affected by the *allo* or *normal* configuration; therefore, the linkage between the A and B rings is apparently not concerned with the reaction.

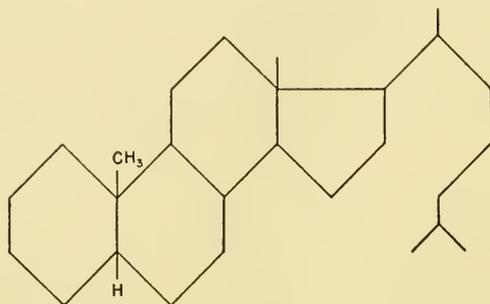
The two classes of stereoisomers in which a difference in configuration obtains on the C₅ atom are called the *normal* and the *allo* series. Coprosterol, a natural saturated sterol formed from cholesterol, which is present in feces, belongs to the *normal* series; dihydrocholesterol, which is prepared by reduction of cholesterol, is a member of the *allo* type of sterols. The differences are not concerned with the arrangement of the atoms and groups around C₃, since the differences are still maintained in the compounds formed when coprosterol and dihydrocholesterol are reduced to their corresponding hydrocarbons, namely, coprostane and cholestane. Windaus²⁰¹ has suggested that the hydrocarbons vary in the nature of the union between rings A and B. In the case of coprostane (also referred to as pseudocholestane) the two rings correspond to a *cis*-decalin or to two "bed" forms. In cholestane, the rings A and B are pictured as two "chair" rings which occur in *trans*-decalin. The results of Ruzicka *et al.*²⁰² have confirmed this view by demonstrating that differences in densities and molecular refractions exist between the two hydrocarbons. In the case of this series of stereoisomers, it is also necessary to develop the concept of the structural formula by fixing the position of the hydrogen on C₅. The methyl group attached to C₁₀ is used as a reference point. The compounds in which the hydrogen and methyl group are in *cis* relationship are con-

²⁰⁰ E. Fernholz, *Z. physiol. Chem.*, 232, 97-100 (1935).

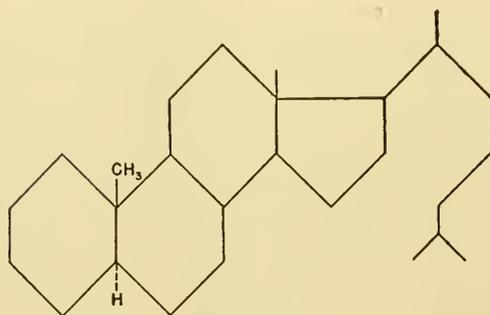
²⁰¹ A. Windaus, *Ann.*, 447, 233-258 (1926).

²⁰² L. Ruzicka, M. Furter, and G. Thomann, *Helv. Chim. Acta*, 16, 327-336 (1933).

sidered to be the *normal* series while, those in which a *trans* relationship exists between these groups are referred to as the *allo* series. The structural relationship between the two series is illustrated in the diagrammatic formulas for coprostane and cholestane. Bonds which are pictured as a solid line project upward while interrupted lines extend in the opposite direction:



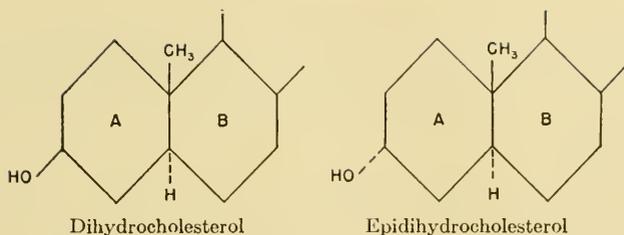
Coprostane
(A/B: *cis*-decalin type)



Cholestane
(A/B: *trans*-decalin type)

In order to establish the position of the hydroxyl group on C₃, it must be related to some other group on the steroid nucleus. Ruzicka²⁰³ has chosen the C₅, which can serve only when the compound is saturated in the 5, 6 position. According to this scheme, cholesterol has a *trans* relationship at these points, while the epi isomer possesses a *cis* relationship. This is illustrated by the structural formulas assigned to dihydrocholesterol and epi-dihydrocholesterol (only the A and B rings are included, since it is in these rings that the differences exist).

²⁰³ L. Ruzicka, cited by H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, p. 359.



According to the Ruzicka scheme, the configuration of the hydroxyl group on C_3 for epicholesterol and epiergosterol is similar to its position in epidihydrocholesterol, even though the reference asymmetric $C(C_5)$ no longer exists as a coordination point.

TABLE 5
COMPARATIVE CONFIGURATION OF HYDROXYL GROUP ON C_3 OF SOME STEROLS AS
COMPARED WITH H ON C_5 OR WITH CH_3 GROUP ON C_{10} ^a

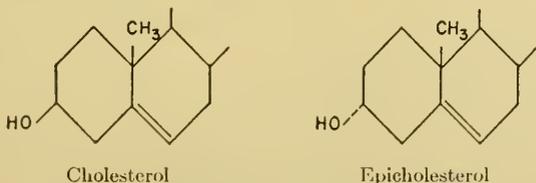
Sterol	Schoenheimer-Evans ^b		Ruzicka <i>et al.</i> ^c	
	3,10	5,10	3,5	5,10
Dihydrocholesterol	<i>cis</i>	<i>trans</i>	<i>trans</i>	<i>trans</i>
Epidihydrocholesterol	<i>trans</i>	<i>trans</i>	<i>cis</i>	<i>trans</i>
Coprosterol	<i>cis</i>	<i>cis</i>	<i>cis</i>	<i>cis</i>
Epicoprosterol	<i>trans</i>	<i>cis</i>	<i>trans</i>	<i>cis</i>
Cholesterol	<i>cis</i>	(<i>trans</i>)	(<i>trans</i>)	(<i>trans</i>)
Epi-allo-cholesterol	<i>trans</i>	(<i>cis</i>)	(<i>trans</i>)	(<i>cis</i>)

^a The designations in parentheses do not exist, since C_5 is not asymmetric in these cases. They are based upon the theory that the hydroxyl on C_3 and the methyl on C_{10} maintain similar relations to C_5 as when C_5 is asymmetric.

^b R. Schoenheimer and E. A. Evans, Jr., *J. Biol. Chem.*, 114, 567-582 (1936).

^c L. Ruzicka, cited by H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945.

A second method for classifying the ordinary and epi forms has been suggested by Schoenheimer and Evans²⁰⁴ which corrects the above difficulty. The basis for their classification of asymmetry on C_3 consists in referring it to the methyl group on C_{10} . Those compounds such as cholesterol, ergosterol, 7-dehydrocholesterol, 7-dihydrocholesterol, and coprosterol all belong to the *cis* group and are the ordinary forms; the epi isomers are those in which the $OH(C_3)$ and $CH_3(C_{10})$ relationships are *trans*. The diagram-



²⁰⁴ R. Schoenheimer and E. A. Evans, Jr., *J. Biol. Chem.*, 114, 567-582 (1936).

matic relation of cholesterol and epicholesterol is pictured here. According to the Ruzicka scheme, cholesterol and epi-*allo*-cholesterol are both *trans*, although the configurations of the hydroxyl group are opposite. The comparative relationship of the terminology by these two methods is shown in Table 5.

The Schoenheimer-Evans designation would appear to be the more satisfactory, since the methyl group on C₁₀ occupies the same position on all known sterols and on bile acids, in contrast to the hydrogen atom on C₅, which may be in one of two positions. Using this system of nomenclature, all sterols precipitable with digitonin are *cis* derivatives, while all epi forms have a *trans* configuration and are not precipitated with digitonin. According to Lettré²⁰⁵ this system of terminology provides a satisfactory means of indicating which saturated sterols will show molecular compound formation because of opposite configurations at C₃ and C₅. One difficulty in the terminology does exist, however, since lumisterol, formed on irradiation of cholesterol, does have the methyl group on C₁₀ on the opposite side of the molecule.²⁰⁶

Although the conversion of the normal to the epi series cannot be demonstrated directly with cholesterol, the interrelationship can be shown in the test tube when the ketones cholestanone or coprostanone are reduced. When cholestanone is reduced in neutral solution,²⁰⁷ or with sodium and amyl alcohol, dihydrocholesterol is formed, while in an acidic medium the epidihydrocholesterol results.²⁰⁸ Windaus and Uibrig²⁰⁹ had previously obtained epidihydrocholesterol by epimerization of dihydrocholesterol with sodium ethylate, but the equilibrium point was reached when only 10% of the dihydrocholesterol was changed. The conditions are reversed with coprostanone, the normal alcohol arising when reduction occurs in acidic solution,²¹⁰ while the epi isomer arises in neutral solution.¹⁵⁷ Windaus and Uibrig²¹¹ and Windaus²¹² working by himself had previously been able to demonstrate the interconversions of the epimers.

The interrelationship of the normal and *allo* compounds where the variation depends upon the configuration on C₅ is more difficult to demonstrate chemically than is that of the ordinary and epi derivatives having differences in configuration on C₃. In the case of *allo*cholesterol, the configuration of A/B rings cannot be altered on hydrogenation, followed by oxidation to cholestanone and then by reduction to the epimeric alcohols dihydrocholesterol and epidihydrocholesterol. Both of these isomers still retain

²⁰⁵ H. Lettré, *Ber.*, 68, 766-767 (1935).

²⁰⁶ K. Dimroth, *Ber.*, 69, 1123-1129 (1936).

²⁰⁷ O. Diels and E. Abderhalden, *Ber.*, 39, 884-890 (1906).

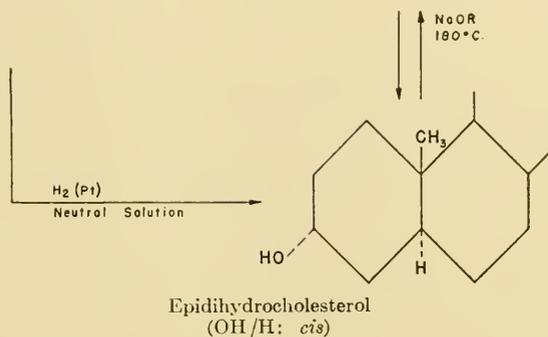
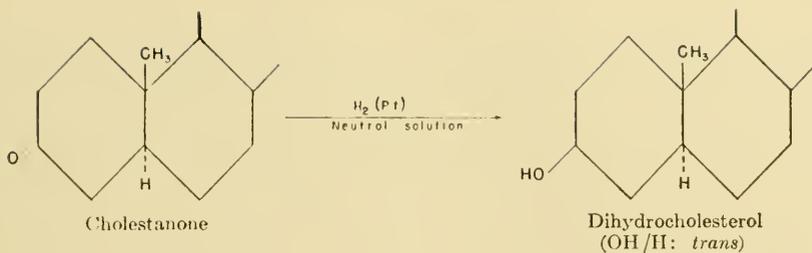
²⁰⁸ G. Vavon and Z. Jakubowicz, *Bull. soc. chim.* [4], 53, 581-588 (1933).

²⁰⁹ A. Windaus and C. Uibrig, *Ber.*, 47, 2384-2388 (1914).

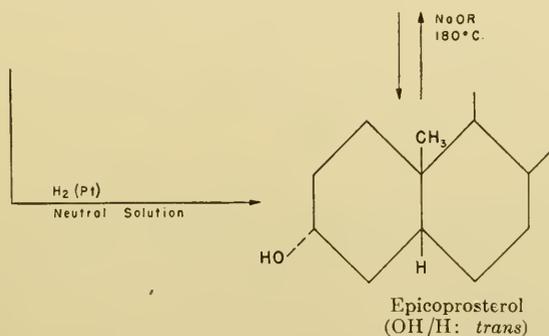
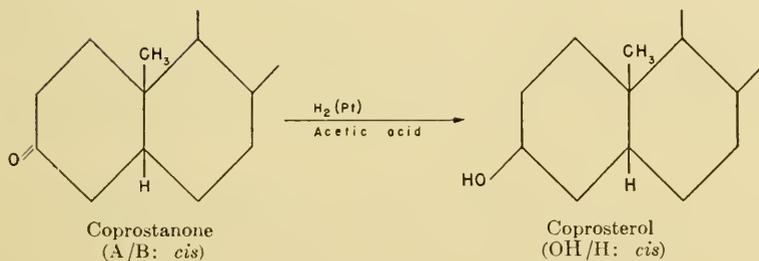
²¹⁰ H. Grasshof, *Z. physiol. Chem.*, 225, 197-198 (1934).

²¹¹ A. Windaus and C. Uibrig, *Ber.*, 48, 857-863 (1915).

²¹² A. Windaus, *Ber.*, 49, 1724-1734 (1916).



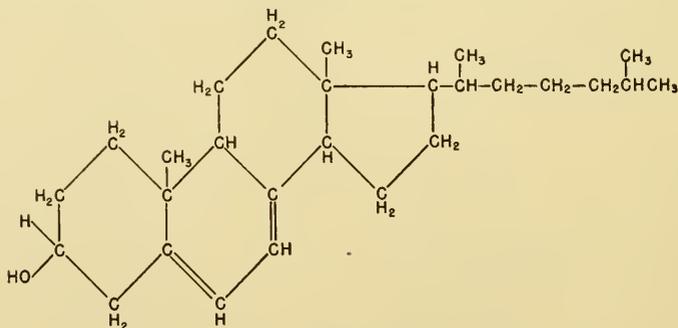
Epimerization of cholestanone



Epimerization of coprostanone

the original allo arrangement. However, Grasshof²¹³ was able to transform the normal to the allo series by using the unsaturated ketone cholestenone as the starting material. This compound is readily reduced to the saturated ketone coprostanone, which, on further reduction, gives rise to the epimers coprosterol and epicoprosterol. The transformation of the allo to the normal series is thus readily demonstrated. It is interesting that the animal also follows this circuitous course in transforming dietary or metabolic cholesterol into fecal coprosterol.²¹⁴ Cholestenone can be readily synthesized from cholesterol by oxidation with copper oxide.^{144,207} It is also readily formed when cholesterol dibromide is oxidized to the ketone with potassium permanganate or chromic acid, followed by debromination to reintroduce the unsaturated linkage.^{154,157} Schoenheimer¹⁵⁸ was able to improve the yield to 89% of the theoretical by using sodium or potassium iodide, which results in debromination and a regeneration of the double bond by the iodine which is liberated (see p. 343).

b'. 7-Dehydrocholesterol: While cholesterol itself is inactive as a precursor of vitamin D, 7-dehydrocholesterol is one of the most active provitamins D. Although it has a limited distribution in nature, it is present to the extent of 17 to 27% of the total sterols of a certain species of whelk (*Buccinum undatum*),^{215,216} while as much as 6% of the sterols of pig skin consist of this provitamin.¹⁵³ The methods of synthesis of 7-dehydrocholesterol from cholesterol are described earlier (see pages 334 and 335). The properties of this alcohol closely simulate those of ergosterol. It melts at 149–150°C.,²¹⁷ and it has a specific rotation of -122.5° in benzene, -82° in ethyl acetate, and -113.6° in chloroform. The formula is given here.



7-Dehydrocholesterol

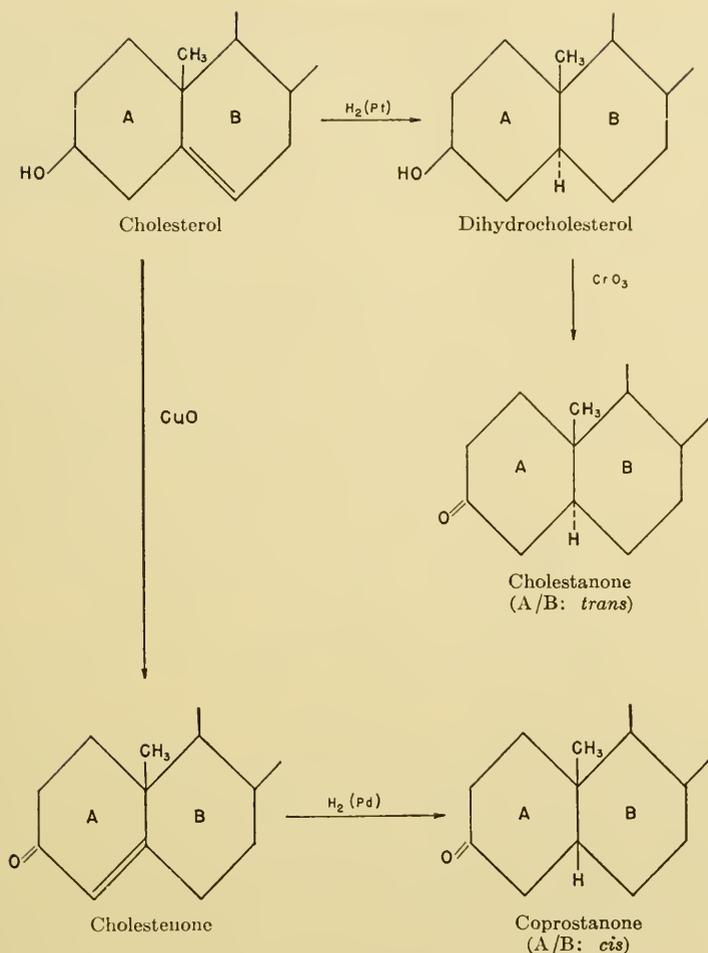
²¹³ H. Grasshof, *Z. physiol. Chem.*, **223**, 249–251 (1934).

²¹⁴ R. Schoenheimer, D. Rittenberg, and M. Graff, *J. Biol. Chem.*, **111**, 183–192 (1935).

²¹⁵ A. Windaus, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, Fachgruppen, III*, **1936**, 185–192.

²¹⁶ F. Bock and F. Wetter, *Z. physiol. Chem.*, **256**, 33–41 (1938).

²¹⁷ A. G. Boer, E. H. Reerink, A. van Wijk, and J. van Niekerk, *Proc. Acad. Sci. Amsterdam*, **39**, 622–632 (1936).



Transformation of allo to normal series

c'. Dihydrocholesterol. This substance closely resembles cholesterol, with which it is frequently associated in nature. As noted earlier, most preparations of cholesterol made from natural sources contain 1 to 2% of this saturated sterol.^{151,152} It was discovered by von Boehm²¹⁸ in intestinal contents and was later isolated from the feces by Windaus and Ubrig.²¹¹ Presumably, it is formed by hydrogenation of cholesterol in the body, and is excreted through the intestinal wall, which explains its presence in feces.²¹⁹ Dihydrocholesterol can readily be prepared in the laboratory by controlled reduction of cholesterol. It also originates, along with equal amounts of

²¹⁸ R. von Boehm, *Biochem. Z.*, 33, 474-479 (1911).

²¹⁹ R. Schönheimer and H. v. Behring, *Z. physiol. Chem.*, 192, 102-111 (1930).

epidihydrocholesterol, by reduction of cholestanone. Windaus²²⁰ found that it occurs in association with coprosterol in the reduction of allocholesterol.

Dihydrocholesterol forms anhydrous hexagonal platelets, which melt sharply at 142°C. As in cholesterol, the crystals contain one molecule of water of crystallization when they are prepared from a saturated solution in 95% ethanol. The sterol is optically active, having a specific rotation of +28.8° at 22°C. in ether.

It is readily distinguished from cholesterol because of the absence of a double bond. Thus, it does not form the characteristic insoluble dibromide, nor will it absorb iodine; however, it will react with one molecule of ozone. The color reactions which are given by cholesterol and which depend upon the unsaturated linkage are not shown by dihydrocholesterol. However, those reactions of cholesterol which are attributable to the alcohol group are, of course, also produced by dihydrocholesterol. The latter forms esters with the fatty acids. Since dihydrocholesterol has the hydroxyl group in the *normal* position, it reacts with digitonin to form a difficultly soluble digitonide whose acetyl derivative melts at 110.5 to 111°C. Epidihydrocholesterol does not form a precipitate with digitonin.

d'. Coprosterol: As early as 1862, Flint²²¹ recognized that the sterol which he had separated from human feces differed from cholesterol. He called it *stercorine*, but it was later renamed *koprosterin* by Bondzynski,²²² who rediscovered it in 1896. In accordance with the current terminology for alcohols, it is now commonly referred to as *coprosterol*. The name originates from a Greek word, *kopro*, which means dung; the second part, from which cholesterol is also derived, *stercos*, means solid. Although coprosterol occurs as the chief sterol component in the feces of practically all animals, Krueger²²³ has proved that the so-called guanosterol obtained from Peruvian guano is identical with cholesterol rather than with coprosterol. This is taken to indicate that marine birds do not excrete coprosterol. Ambergris, which is a product of the sperm whale, has been shown to contain largely coprosterol and epicoprosterol, with small amounts of cholesterol.²²⁴

Bondzynski and Humnicki²²⁵ established the empirical formula of coprosterol as C₂₇H₄₈O. Thus, it has the same composition as dihydrocholesterol, but its properties differ markedly from those of the latter. Windaus²²⁶

²²⁰ A. Windaus, *Ann.*, 453, 101-112 (1927).

²²¹ A. Flint, *Am. J. Med. Sci.*, 44, 305-365 (1862).

²²² S. Bondzynski, *Ber.*, 29, 476-478 (1896).

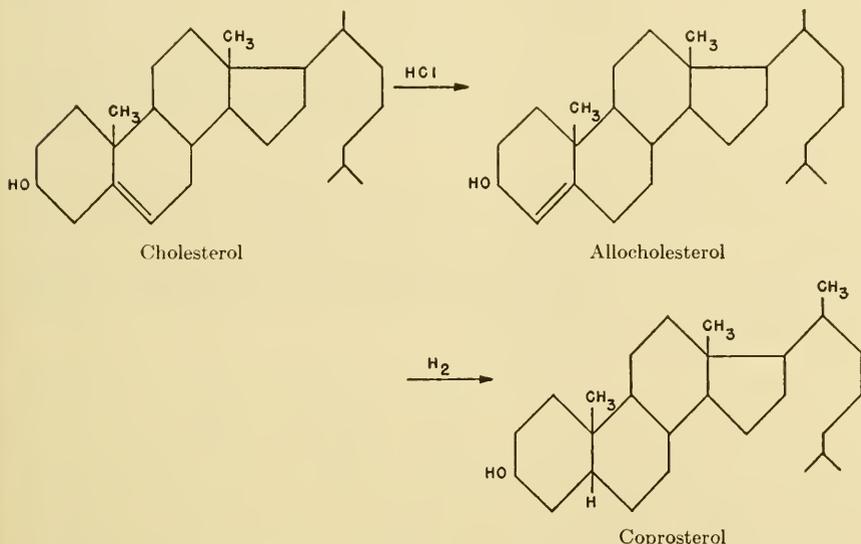
²²³ J. Krueger, *J. Am. Chem. Soc.*, 66, 1795-1797 (1944).

²²⁴ H. Janistyne, *Fette u. Seifen*, 48, 501-504 (1941); *Chem. Abstr.*, 38, 2163 (1944); *Chem. Zentr.*, 112, II, 3003-3004 (1941).

²²⁵ S. Bondzynski and V. Humnicki, *Z. physiol. Chem.*, 22, 396-410 (1896).

²²⁶ A. Windaus and K. Neukirchen, *Ber.*, 52, 1915-1919 (1919).

showed that it is a reduction product of allocholesterol which, in turn, may be derived from cholesterol. The structure of coprosterol was further elucidated by the demonstration of the relationship of its hydrocarbon coprostane, or pseudocholestane, to cholanic acid.²²⁶ This latter compound is known to be related to the bile acids.



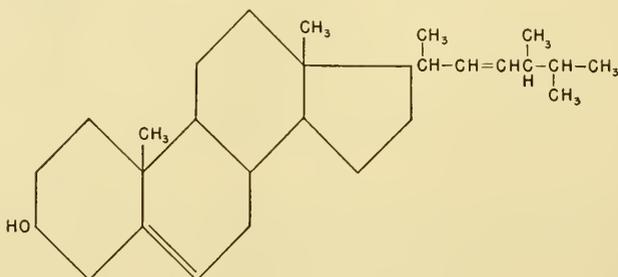
Coprosterol crystallizes from ethanol in fine needles but, in contrast to cholesterol and dihydrocholesterol, they contain no water of crystallization. Like other sterols, it is insoluble in water and alkali but readily dissolves in alcohol, ether, chloroform, and other organic solvents, including fats. It can be separated from cholesterol chromatographically.²²⁷ Coprosterol melts at 102–104°C., and it has a specific rotation of +24° (in 13.7% concentration in chloroform).

Because it is saturated, coprosterol adds no halogens and has an iodine value of zero. In spite of this, ozone reacts with it just as is the case with dihydrocholesterol. Because of the fact that the position of the hydroxyl group on C₃ is the same as in cholesterol, it is precipitated most completely by digitonin. The color given in the Salkowski reaction is yellow, but this changes gradually to a reddish color. The Liebermann-Burchard test is similar to that given by cholesterol, but the intensity is only one-third that of the latter sterol. It forms esters, of which the best known are coprosterol acetate (C₂₇H₄₇O·OCCH₃), which forms needles with a melting point of 88–89°C., and coprosterol propionate (C₂₇H₄₇O·OCCH₂CH₃). When crystallized from methanol, the latter ester forms long needles which melt at 99–100°C.

²²⁷ A. von Christiani, *Z. physiol. Chem.*, 280, 127–128 (1944).

e'. Ostreasterol: Another type of zoosterol was first prepared by Bergmann¹⁷⁴ from the non-saponifiable matter of oysters (*Ostrea virginica*), and of the clam (*Venus mercenaria*). Apparently, this is the only sterol present in the oyster; it is possible that it replaces cholesterol in all species of the *Lamellibranchiata*. It occupies a position midway between the zoosterols and the phytosterols. Ostreasterol melts at 143°C. and, like cholesterol, it has a specific rotation which is on the levo side (-43.6°). Although Bergmann¹⁷⁴ originally believed that ostreasterol belonged to the C_{29} series, this conception now seems incorrect and the formula $C_{28}H_{46}OH$ has been suggested.¹⁷⁵ Bergmann and Low¹⁷⁵ consider that it is probably identical with chalinasterol.

f'. Chalinasterol: Bergmann and collaborators¹⁷² have isolated a sterol from the sponges (*Chalina arbuscula* Verrill and *Tetilla laminaris* Wilson) which melts at 144°C. and has a specific rotation of -42° . When it is hydrogenated, it is converted into campesterol. It is therefore identified as the 24a-epimer of brassicasterol.



Chalinasterol

g'. Isocholesterol: Schulze²²⁸⁻²³⁰ called the cholesterol-like material present in lanolin or wool grease "isocholesterol." This was later shown to consist of at least two substances,²³¹ which are now referred to as agnosterol and lanosterol. Agnosterol has the empirical structure $C_{30}H_{47}OH$ (m.p., $162^\circ C.$, $[\alpha]_D = +70.6^\circ$), while lanosterol has the composition $C_{30}H_{49}OH$ (m.p., $141^\circ C.$, $[\alpha]_D = +58^\circ$). Agnosterol has three unsaturated linkages, whereas lanosterol possesses only two double bonds. Windaus and Tschesche²³¹ obtained 50 grams of isocholesterol from 1 kilogram of lanolin; this isocholesterol contained 4 grams of agnosterol and 45 grams of lanosterol. These were readily separated from each other by fractional crystallization of the acetates from diethyl ether. Agnosteryl acetate is only slightly soluble in this solvent, while lanosteryl acetate is extremely so.

Neither of these compounds is isomeric with cholesterol; in fact, it is now

²²⁸ E. Schulze, *Ber.*, 5, 1075-1078 (1872).

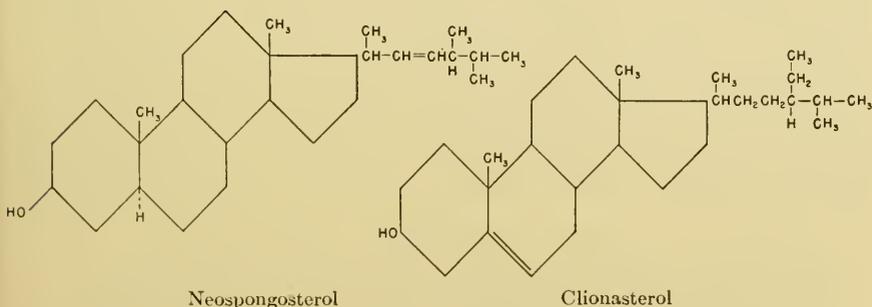
²²⁹ E. Schulze, *Ber.*, 6, 251-254 (1873).

²³⁰ E. Schulze, *J. prakt. Chem.*, N. S. 7, 163-178 (1873).

²³¹ A. Windaus and R. Tschesche, *Z. physiol. Chem.*, 190, 51-61 (1930).

known that they should not be included among the sterols, as they have a different structure. On selenium reduction, they give 1, 2, 8-trimethylphenanthrene instead of Diels' hydrocarbon ($C_{18}H_{16}$); they apparently do not contain the five-membered ring attached to phenanthrene. They are now considered to be members of the triterpenes. These are products composed of 30 carbon atoms whose carbon skeleton is believed to be divided into isopentane units. Lanosterol and agnosterol are the only triterpenes which have been prepared from an animal source²³¹; one of these, *i.e.*, lanosterol, has also been separated from yeast.²³² The method of molecular rotation differences, which has been frequently employed to establish the structure of the steroids, enables one to distinguish the triterpenes readily from the steroids.²³³ Because these do not belong to the sterol group, the term "ischolesterol," as well as the names lanosterol and agnosterol, are misleading and should be dropped. For a further discussion to the triterpenes see Section 2 (page 383).

h'. Other Zoosterols: A number of other sterols, which belong to the category of animal products, have been described. These include spongosterol^{170,171} from the sponge (*Suberites domuncula*), as well as from the similar form *Suberites compacta*¹⁷¹ (the so-called neospongosterol), and clionasterol from the boring sponge (*Cliona celata*),^{160,234,235} sterols from the freshwater sponge (*Spongilla lacustris*),²³⁶ from the sea anemone (*Anemonia sulcata*),²³⁷ rose coral (*Manicina areolata*),²³⁸ the branched coral (*Xiphogorgia sp.*),²³⁸ and the crab (*Limulus polyphemus*), as well as from three types of sea urchins (*Echinidae*).²³⁸ Poriferasterol,^{234,239} also, is found in sponges, in association with clionasterol. On hydrogenation they yield the same saturated alcohol, poriferastanol. From this fact it has been deduced that poriferasterol is 22-dehydroclionasterol:



²³² H. Wieland and W. Benend, *Z. physiol. Chem.*, **274**, 215-222 (1942).

²³³ D. H. R. Barton, *J. Chem. Soc.*, **1945**, 813-819.

²³⁴ F. R. Valentine and W. Bergmann, *J. Org. Chem.*, **6**, 452-461 (1941).

²³⁵ C. A. Kind and W. Bergmann, *J. Org. Chem.*, **7**, 341-345 (1942).

²³⁶ A. Mazur, *J. Am. Chem. Soc.*, **63**, 883-884, 2442-2444 (1941).

²³⁷ M. Deffner, *Z. physiol. Chem.*, **278**, 165-168 (1943).

²³⁸ W. Bergmann, M. L. McLean, and D. Lester, *J. Org. Chem.*, **8**, 271-284 (1943).

²³⁹ A. M. Lyon and W. Bergmann, *J. Org. Chem.*, **7**, 428-431 (1942).

Bombicsterol has been reported as obtainable from the pupae of the silkworm (*Bombyx mori*), while stellerol has been prepared from the testicles of echinoderms and asteroids²⁴⁰ as well as from the eggs of the starfish (*Asterias forbesi*).^{241,242} Bombicsterol is apparently the only animal sterol (except dihydrocholesterol) which has no double bond between C₅ and C₆. It is also distinguished by being the only sterol of the C₂₈ series in the animal group, except for ergosterol in the case of the earthworm, and ostreasterol in the oyster. Another compound of this group, called microcionasterol, has been prepared²⁴³ from the sponge (*Microciona prolifera*), while still another sterol, actiniasterol, which has two double bonds, has been separated from the sea anemone (*Anemonia sulcata*).²⁴⁴

(e) *Occurrence and Properties of Phytosterols.* The presence in plants of sterols which are different from cholesterol has long been recognized. Until recently, all non-animal sterols have been classed as phytosterols. However, with a more complete appraisal of the chemical nature of the compounds in different plant forms, it has now become evident that the higher plants (phanerogams) contain sterols which are quite different from those found in lower plant forms (cryptogams). The term phytosterol has been retained for the sterols from the higher plants, while those from the cryptogams are classed as mycosterols.

Hesse,¹⁷⁷ in 1878, first prepared a plant sterol from Calabar beans and from peas, although Beneke^{245,246} had previously separated a sterol from peas which he called cholesterine. Hesse recognized that this sterol differed from cholesterol, although he believed that it was isomeric with it. Because of its wide distribution in the plant kingdom, it was given the name, "phytosterin." Actually, the product which Hesse prepared was a mixture of sterols which Windaus and Hauth¹⁵⁵ separated into sitosterol and stigmasterol. It has only recently been shown that the empirical formula for sitosterol^{247,248} is C₂₉H₄₉OH, and that for stigmasterol²⁴⁹ is C₂₇H₄₇OH. Neither of these can, therefore, be isomeric with cholesterol.

a'. *Sitosterols:* Sitosterol is the most widely distributed of the phytosterols. It occurs in an especially high concentration in corn, wheat germ, and soybean oils. Ueno and Yamasaki²⁴ found Koryan corn oil (*Sorghum vulgare*) an excellent source of sitosterol, which can be readily purified, as no

²⁴⁰ A. Kossel and S. Edlbacher, *Z. physiol. Chem.*, *94*, 264-283 (1915).

²⁴¹ I. H. Page, *J. Biol. Chem.*, *57*, 471-476 (1923).

²⁴² W. Bergmann and H. A. Stansbury, Jr., *J. Org. Chem.*, *9*, 281-289 (1944).

²⁴³ W. Bergmann and T. B. Johnson, *Z. physiol. Chem.*, *222*, 220-226 (1934).

²⁴⁴ E. Klenk and W. Diebold, *Z. physiol. Chem.*, *236*, 141-144 (1935).

²⁴⁵ F. W. Beneke, *Studien über das Vorkommen, die Verbreitung und die Function von Gallenbestandtheilen in den thierischen und pflanzlichen Organismen*, Rieckert, Giessen, 1862.

²⁴⁶ G. M. R. Beneke, *Ann.*, *122*, 249-255 (1862).

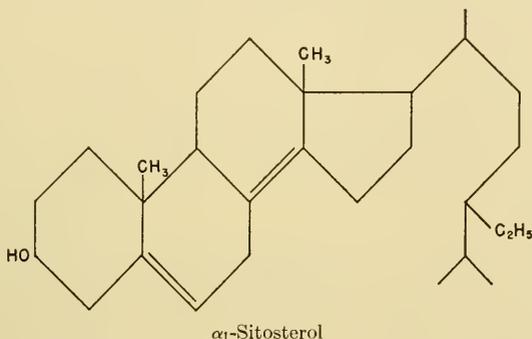
²⁴⁷ H. Sandqvist and E. Bengtsson, *Ber.*, *64*, 2167-2171 (1931).

²⁴⁸ A. Windaus, F. v. Werder, and B. Gschaider, *Ber.*, *65*, 1006-1009 (1932).

²⁴⁹ H. Sandqvist and J. Gorton, *Ber.*, *63*, 1935-1938 (1930).

other phytosterols are found in this oil. Burián²⁵⁰ discovered it in wheat germ and rye germ oil. However, the original sitosterol was shown by Anderson and co-workers^{148, 251, 252} to consist of at least three isomeric forms, which they designated as α , β , and γ . Some dihydro-sitosterol has also been shown to be present in wheat germ oil. More recent studies have indicated that the α -form, which is the most soluble fraction, consists of at least three isomers,^{253, 254} which are designated α_1 , α_2 , and α_3 . The constants of some of these sitosterols are given in Table 6.

The tetracyclic nucleus has been shown to be the same in sitosterols and in cholesterol; therefore, the variation must be in the side chain.²⁵⁵ However, the α -sitosterols apparently have a second double bond on C₈ which may be in ring B or C. Bernstein and Wallis²⁵⁶ have suggested the following structure for α_1 -sitosterol. Presumably, α_2 and α_3 are essentially the same, with some shifts in the position of the double bond on C₈:



A number of workers have proved that β -sitosterol is actually 22-dihydro-stigmasterol.²⁵⁷⁻²⁵⁹ It is now believed that γ -sitosterol is a stereoisomer of β -sitosterol and that they differ only in the configuration on the C₂₄ atom.

Fieser and Fieser¹¹⁶ suggest that an important center of asymmetry of the sterols exists on C₂₄ for those products with side chains in that position. They point out that in addition to the relationship of β - and γ -sitosterol, a corresponding similarity exists between campesterol and ergostanol (22,23-

²⁵⁰ R. Burián, *Monatsh.*, 18, 551-574 (1897). Reprinted in *Sitzber. Akad. Wiss. Wien, Math. naturw. Klasse, Abl. IIb*, 106, 549-572 (1897).

²⁵¹ R. J. Anderson and R. L. Shriner, *J. Am. Chem. Soc.*, 48, 2976-2986 (1926).

²⁵² R. J. Anderson, R. L. Shriner, and G. O. Burr, *J. Am. Chem. Soc.*, 48, 2987-2996 (1926).

²⁵³ E. S. Wallis and E. Fernholz, *J. Am. Chem. Soc.*, 58, 2446-2449 (1936).

²⁵⁴ S. Bernstein and E. S. Wallis, *J. Am. Chem. Soc.*, 61, 1903-1904 (1939).

²⁵⁵ L. Ruzicka and E. Eichenberger, *Helv. Chim. Acta*, 18, 430-434 (1935).

²⁵⁶ S. Bernstein and E. S. Wallis, *J. Am. Chem. Soc.*, 61, 2308-2313 (1939).

²⁵⁷ B. E. Bengtsson, *Z. physiol. Chem.*, 237, 46-51 (1935).

²⁵⁸ R. E. Marker and E. L. Whittle, *J. Am. Chem. Soc.*, 59, 2704-2708 (1937).

²⁵⁹ S. Bernstein and E. S. Wallis, *J. Org. Chem.*, 2, 341-344 (1937).

TABLE 6
PHYSICAL PROPERTIES OF SITOSTEROL AND SITOSTEROL DERIVATIVES^a

Isomer	Sterol		Acetate		Benzoate		3,5-Dinitrobenzoate		R _e
	M.p., °C.	[α] _D	M.p., °C.	[α] _D	M.p., °C.	[α] _D	M.p., °C.	[α] _D	
α ₁	164-166	- 1.7	137	+ 29	168-172	+ 42	222	+ 37	b
	163	- 1.8	—	—	167-168	+ 40.8	225	+ 37	c
	156	+ 3.5	124-126	+ 17	164-166	+ 27	206	+ 26	d
α ₂	142	+ 1.7	—	—	167.5-168	+ 14.9	202.5-203	+ 15.4	c
	142-143	+ 5.2	152-153	+ 6.1	173-175	+ 12.0	210-211.5	+ 12.2	b
β	136-137	- 36.6	125-126	- 41.0	146-147	- 13.8	202-203	- 10.4	e
	135-135.5	- 34.2	126-127	- 34.7	145-146	- 14.2	207-209	- 21.7	f
	136-137	- 31.5	122-123	- 36.6	146-147	- 12.2	—	—	g
γ	146	- 42.0	143-143.5	- 45.0	—	—	—	—	c
	147	- 42.8	143-143.5	- 45.8	152	- 19.6	—	—	g
δ	146-147	- 23.9	113.5-114.5	- 24.4	157-158	- 16.0	—	—	g
	143-144	- 38.7	127-128	- 44.7	—	—	215-217	—	f

^a I. M. Heilbron and E. R. H. Jones, *Ann. Rev. Biochem.*, **9**, 135-172 (1940).

^b S. Bernstein and E. S. Wallis, *J. Am. Chem. Soc.*, **61**, 1903-1904 (1939).

^c S. W. Gloyer and H. A. Schuetz, *J. Am. Chem. Soc.*, **61**, 1901-1903 (1939).

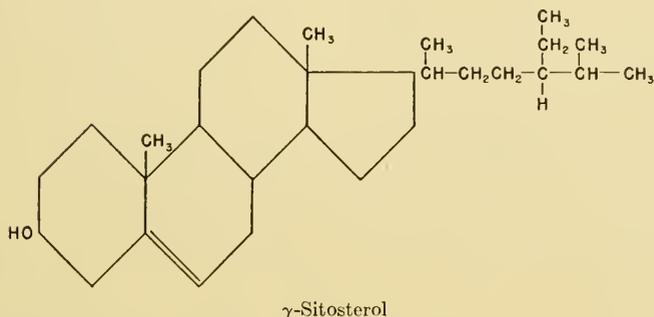
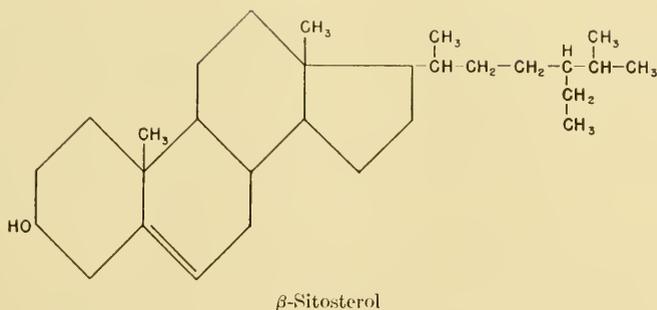
^d E. S. Wallis and E. Femholz, *J. Am. Chem. Soc.*, **58**, 2446-2449 (1936).

^e E. S. Wallis and P. N. Chakravorty, *J. Org. Chem.*, **2**, 335-340 (1937).

^f J. C. E. Simpson and N. E. Williams, *J. Chem. Soc.*, 1937, 733-738.

^g A. Ichiba, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **28**, Nos. 605-606, 112-127 (1935).

dihydrobrassicasterol),²⁶⁰ as well as some of the isomers of the stellanols.¹¹⁶



According to the terminology suggested by Ruigh,¹¹² β -sitostanol may be properly called 24-dextro-ethylcholestanol, while γ -sitostanol is the 24-levo-ethylcholestanol. The formula in which the ethyl group attached to C_{24} is pictured below the side chain is arbitrarily considered as the dextro isomer, while the case where the ethyl group is placed above the side chain would connote the levo isomer. According to the terminology employed by Fieser and Fieser,¹¹⁶ the isomer in which the stanol form gives a dextro rotation is spoken of as the 24a-type; the group attached to the C_{24} in this case is represented by a dotted line. The epimer giving rise to the levo saturated alcohol is considered as the 24b form, and the group attached to C_{24} is represented by a solid line. The actual rotation on C_{24} is determined from the rotation of the aliphatic ketone produced by severing the side chain between C_{17} and C_{20} .¹¹²

Two additional sitosterols, δ and ϵ , have been isolated by Ichiba²⁶¹ and by Simpson and Williams,²⁶² respectively. The solubility of γ -sitosterol is quite low as compared with that of the α -form, while that of the β -isomer is

²⁶⁰ E. Fernholz and W. L. Ruigh, *J. Am. Chem. Soc.*, 62, 3346-3348 (1940).

²⁶¹ A. Ichiba, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 28, Nos. 605-606, 112-127 (1935).

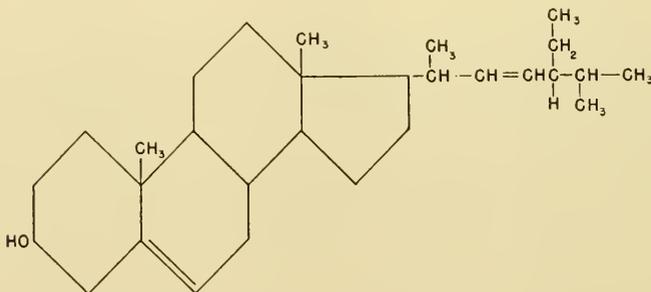
²⁶² J. C. E. Simpson and N. E. Williams, *J. Chem. Soc.*, 1937, 733-738.

intermediate. Comparative data on the δ - and ϵ -sitosterol are not at present available.

All three α -isomers have been shown to be present in wheat germ oil,^{253,254} while rye germ oil has been shown to contain α_1 - and α_3 -sitosterol, as well as the β - and γ -forms.²⁶³ Pure β -sitosterol has been prepared from cottonseed oil, which contains chiefly this type of sitosterol.²⁶⁴ The seeds of the sweet-scented shrub (*Calycanthus floridus*) also contain almost exclusively the β -isomer,²⁶⁵ while soybean and wheat germ oils contain principally γ -sitosterol. β -Sitosterol has been shown to be present in the kidney bean (*Phaseolus vulgaris*), along with stigmasterol.²⁶⁶ Chincol, which is a sterol from cinchona bark, appears to be a β -sitosterol.²⁶⁷

Sitosterol mixtures as found in plants crystallize from dilute alcohol with one molecule of water of crystallization. The shiny white leaf-like crystals are very similar to those of cholesterol. Fine white needles are produced when the sterol is crystallized from ether. The sitosterols give a difficultly soluble precipitate with digitonin. Characteristic products formed from sitosterols are the acetates, benzoates, and 3,5-dinitrobenzoates. The melting points and specific rotations of these esters show considerable variation in the different forms of the sitosterols (Table 6).

b'. Stigmasterol: Stigmasterol frequently occurs with sitosterol and is second in importance of the products classed as phytosterols. Only one



Stigmasterol

stigmasterol has been reported, and this has been shown²²³ to have an empirical formula of $C_{29}H_{48}O$, with double bonds at the 5,6 and 22,23 positions and with an ethyl group at C_{24} .²⁶⁸⁻²⁷⁰ The conversion of stigmasterol

²⁵³ S. W. Gloyer and H. A. Schuette, *J. Am. Chem. Soc.*, 61, 1901-1903 (1939).

²⁵⁴ E. S. Wallis and P. N. Chakravorty, *J. Org. Chem.*, 2, 335-340 (1937).

²⁵⁵ J. W. Cook and M. F. C. Paige, *J. Chem. Soc.*, 1944, 336-337.

²⁵⁶ A. C. Ott and C. D. Ball, *J. Am. Chem. Soc.*, 66, 489-491 (1944).

²⁵⁷ W. Dirscherl, *Z. physiol. Chem.*, 257, 239-245 (1939).

²⁵⁸ A. Guiteras, *Z. physiol. Chem.*, 214, 89-90 (1933).

²⁵⁹ E. Fernholz, *Ann.*, 507, 128-138 (1933).

²⁷⁰ E. Fernholz, *Ann.*, 508, 215-224 (1934).

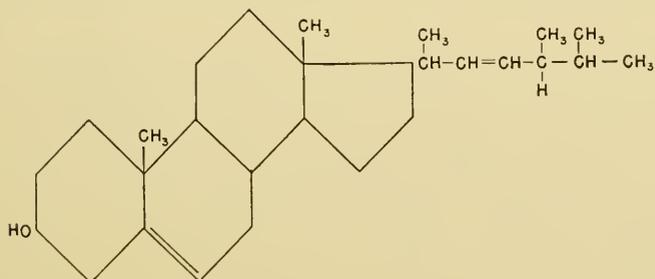
into the hormone, progesterone, offers further proof of the postulated structure.²⁷¹⁻²⁷³

Stigmasterol was first isolated as a component of the non-saponifiable fraction of the oil of the deadly calabar bean¹⁵⁵ (*Physostigma venenosum*). It is also a prominent component in the oil of the kidney bean (*Phaseolus vulgaris*),²⁶⁶ as well as of corn, coconut, rapeseed, rice bran, and soybean oils. The properties of some of its esters and their derivatives are included in Table 7.

TABLE 7
PROPERTIES OF SOME STIGMASTEROL DERIVATIVES

Stigmasteryl ester	Formula	M.p., °C.	Remarks
-acetate	$C_{29}H_{47}O \cdot OCCH_3$	144.0-144.6	White (Schaffer)
-acetate-dibromide	$C_{29}H_{47}Br_2O \cdot OCCCH_3$	132-135	White needles, more readily sol. than acetate. From acetate in $CHCl_3$ sol. with Br in cold
-acetate-tetrabromide	$C_{29}H_{47}Br_4O \cdot OCCCH_3$	205	Produced from acetate in acetic acid ether sol. with Br
-benzoate	$C_{29}H_{47}O \cdot OCC_6H_5$	160	From $CHCl_3$ Difficultly sol. in ethanol
-palmitate	$C_{29}H_{47}O \cdot OC(CH_2)_{14}CH_3$	99	Difficultly sol. in ethanol
-stearate	$C_{29}H_{47}O \cdot OC(CH_2)_{16}CH_3$	101	" " " "
-oleate	$C_{29}H_{47}O \cdot OC(CH_2)_7-CH:CH(CH_2)_7CH_3$	44	" " " "

e'. Brassicasterol: Brassicasterol is a doubly unsaturated sterol first isolated from oil of turnip (*Brassica rapa*) by Windaus and Welsch,²⁷⁴



Brassicasterol

which was first thought to be isomeric with stigmasterol,¹⁰⁸ even though the brassicasterol differs from stigmasterol.²⁷⁵ However, Fernholz and Stavely²⁷⁶ later reformulated the structure to the C_{28} order ($C_{28}H_{46}O$) and

²⁷¹ E. Fernholz and P. N. Chakravorty, *Ber.*, 67, 2021-2026 (1934).

²⁷² A. Butenandt, U. Westphal, and H. Cobler, *Ber.*, 67, 1611-1616 (1934).

²⁷³ E. Fernholz, *Ber.*, 67, 2027-2031 (1934).

²⁷⁴ A. Windaus and A. Welsch, *Ber.*, 42, 612-616 (1909).

²⁷⁵ E. Fernholz and H. E. Stavely, *J. Am. Chem. Soc.*, 61, 142-143 (1939).

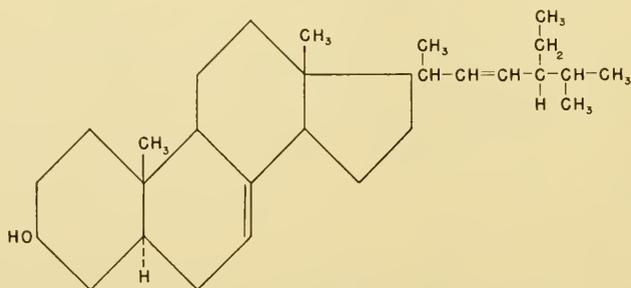
²⁷⁶ E. Fernholz and H. E. Stavely, *J. Am. Chem. Soc.*, 62, 428-430, 1875-1877 (1940).

ascribed the configuration, 5,6,22,23-ergostadienol to it. 22-Dihydrobrassicasterol is believed to be isomeric with campesterol, the only difference between the molecules being the isomerism on C₂₄.

The unsaturated linkages of brassicasterol are readily saturated with bromine. Like most of the other sterols isolated from natural sources, it also is readily precipitated with digitonin. The sterol crystallizes from alcohol in glistening hexagonal platelets which contain one molecule of water of crystallization. It melts at 148°C. and has a specific rotation of -64.5° in chloroform at 18°C.

Brassicasteryl acetate, C₂₈H₄₅O·OCCH₃, forms hexagonal platelets when crystallized from ethanol. It melts at 157–158°C. The benzoate ester, C₂₈H₄₅O·OCC₆H₅, when prepared from ethanol, forms long silk-like needles which are especially characteristic. They melt at 167°C. to a turbid liquid which clears at 169–170°C. This assumes a blue-green color on cooling.

d'. Spinasterol: Spinasterol is the name of a series of phytosterols which are closely related to the γ -sitosterols and which are isomeric with stigmasterol. Larsen and Heyl^{277, 278} isolated three isomers from spinach fat which were designated as α , β , and γ . On hydrogenation, the three forms all yield spinastanol, which is identical with stigmastanol.²⁷⁹ Fernholz and Ruigh²⁸⁰ first postulated that α -spinasterol is 8,14,22,23-stigmastadienol. Subsequent experimental evidence²⁸¹ based upon its oxidation products seemed to indicate that the cyclic double bond is located at position 8,9 instead of at 8,14 but Barton²³³ more recently proved that it is located in the 7,8 position. In view of the fact that the sterol has a low



α -Spinasterol

levo rotation, and since α -spinastadienone is not an α,β -unsaturated ketone, Simpson²⁸² has suggested that the usual unsaturated linkage present in

²⁷⁷ F. W. Heyl and D. Larsen, *J. Am. Chem. Soc.*, 56, 942–943 (1934).

²⁷⁸ D. Larsen and F. W. Heyl, *J. Am. Chem. Soc.*, 56, 2663–2665 (1934).

²⁷⁹ C. D. Larsen, *J. Am. Chem. Soc.*, 60, 2431–2434 (1938).

²⁸⁰ E. Fernholz and W. L. Ruigh, *J. Am. Chem. Soc.*, 62, 2341–2343 (1940).

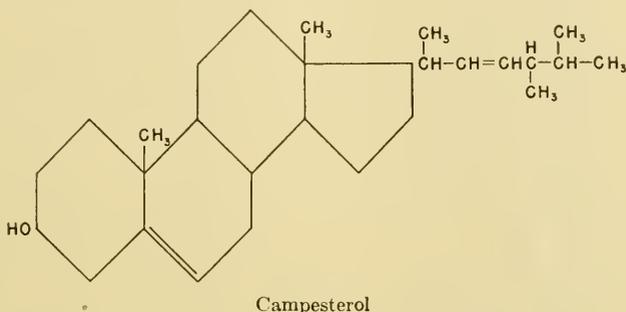
²⁸¹ H. E. Stavely and G. N. Bollenback, *J. Am. Chem. Soc.*, 65, 1600–1603 (1943).

²⁸² J. C. E. Simpson, *J. Chem. Soc.*, 1937, 730–733.

most sterols at the 5,6 position is absent. A second natural sterol, zymosterol, is also saturated at the 5,6 position.

The α -spinasterol has also been shown to be a component of alfalfa seed oil^{283,284} (*Medicago sativa*), as well as of seneca snake-root²⁸² (*Polygala senega*). In all probability, the medicagosterol II isolated by Dam *et al.*²⁸⁵ from alfalfa extracts is α -spinasterol.

e'. Campesterol: Campesterol is a mono-unsaturated sterol isolated by Fernholz and MacPhillamy²⁸⁶ from rapeseed (*Brassica campestris*). It melts at 157–158°C., and it has a specific rotation of -33° . It belongs to the C_{28} series and is believed by Fernholz and Ruigh²⁸⁷ to have the following structure in which an asymmetry around C_{24} is evident:



f'. Dihydrositosterol: Dihydrositosterol, $C_{29}H_{51}OH$, has been reported in a number of oils, including corn, wheat, rice, and rye, as well as in the so-called tall oil, which is a by-product of the paper manufacturing industry. This sterol forms colorless platelets which melt at 144°C. and which have a specific rotation of 28.0° at 18°C. The acetate, $C_{29}H_{51}O \cdot OCCH_3$, melts at 141°C. and has an $[\alpha]_D$ of $+12.72^\circ$. Because of the absence of any unsaturated linkage, dihydrositosterol does not respond to the Liebermann-Burchard or Salkowski tests for the sterols.

(f) *Occurrence and Properties of Mycosterols.* The sterols elaborated by the cryptogams differ markedly from those found in higher plants or animals, although the essential characteristics of the sterol nucleus are retained. Because of its widespread distribution, as well as because it is a precursor of vitamin D, ergosterol assumes the most importance in this group. Two other members, fucosterol and zymosterol, are also well known.

a'. Ergosterol: Tanret²⁸⁸ isolated ergosterol from the fungus ergot

²⁸³ L. C. King and C. D. Ball, *J. Am. Chem. Soc.*, **61**, 2910–2912 (1939).

²⁸⁴ E. Fernholz and M. L. Moore, *J. Am. Chem. Soc.*, **61**, 2467–2468 (1939).

²⁸⁵ H. Dam, A. Geiger, J. Glavind, P. Karrer, W. Karrer, E. Rothschild, and H. Salomon, *Helv. Chim. Acta.*, **22**, 310–313 (1939).

²⁸⁶ E. Fernholz and H. B. MacPhillamy, *J. Am. Chem. Soc.*, **63**, 1155–1156 (1941).

²⁸⁷ E. Fernholz and W. L. Ruigh, *J. Am. Chem. Soc.*, **63**, 1157–1159 (1941).

²⁸⁸ C. Tanret, *Ann. chim. phys.* [5], **17**, 493–512 (1879). Cited by C. E. Bills, *Physiol. Revs.*, **15**, 1–97 (1935).

TABLE 8
 OCCURRENCE OF ERGOSTEROL IN TRACES ADMIXED WITH TOTAL STEROL OF VARIOUS
 ANIMAL SOURCES BASED UPON SPECTROGRAPHIC DETERMINATION^a

Tissue	E:TS, parts/1000*	Ref.
Brain, unspecified	0.5	<i>b</i>
Brain, rabbit	0.7	<i>c</i>
Brain, Coptic mummy	+	<i>d</i>
Brain, human fetus	0.5	<i>e</i>
Brain, human infant	0.2	<i>e</i>
Brain, human adult	0.06	<i>e</i>
Blood, dog	0.1	<i>c</i>
Blood, man	+	<i>f</i>
Carcasses, mice	0.8	<i>c</i>
Carcasses, rats	0.3	<i>c</i>
Gallstones, man	+	<i>g</i>
Lymph, dog	0.3	<i>c</i>
Plankton, marine	+	<i>h</i>
Preen gland, birds	+	<i>i</i>
Sclerotic aortas, human	—	<i>j</i>
Skin, pig	+	<i>k</i>
Skin, infant	1.5	<i>l</i>
Skin, adult	4.2	<i>l</i>
Skin, man	+	<i>m</i>
Spinal cord, cattle	1.2	<i>g</i>
Butter	+	<i>n</i>
Cod-liver oil	+	<i>o</i>
Egg-yolk	1.0	<i>p</i>
Milk, cow	2.3	<i>q</i>
Venom, toad	0.0-13.6	<i>r,s</i>

* Ratio of ergosterol to total sterol in parts per thousand.

^a C. E. Bills, *Physiol. Revs.*, *15*, 1-97 (1935), p. 10.

^b O. Rosenheim and T. A. Webster, *Biochem. J.*, *21*, 389-397 (1927).

^c R. Schönheimer, H. v. Behring, and K. v. Gottberg, *Z. physiol. Chem.*, *208*, 77-85 (1932).

^d H. King, O. Rosenheim, and T. A. Webster, *Biochem. J.*, *23*, 166-167 (1929).

^e I. H. Page and W. Menschick, *Biochem. Z.*, *231*, 446-459 (1931).

^f L. H. Dejust, D. Van Stolk, and E. Dureiul, *Compt. rend.*, *187*, 311-313 (1928).

^g C. E. Bills, E. M. Honeywell, and W. A. MacNair, *J. Biol. Chem.*, *76*, 251-261 (1928).

^h G. Belloc, R. Fabre, and H. Simonnet, *Compt. rend.*, *191*, 160-162 (1930).

ⁱ H. C. Hon, *Chinese J. Physiol.*, *2*, 345-380 (1928).

^j R. Schönheimer, *Z. physiol. Chem.*, *211*, 65-68 (1932).

^k O. Rosenheim and T. A. Webster, *Lancet*, *2*, 622-625 (1927).

^l H. Hentschel and L. Schindel, *Klin. Wochschr.*, *9*, 262 (1930).

^m I. M. Heilbron, *Brit. J. Actinotherapy*, *2*, 210-215 (1928).

ⁿ A. Adam, *Klin. Wochschr.*, *7*, 1825-1828 (1928).

^o J. W. Woodrow, *Phil. Mag.* [7], *5*, 944-946 (1928).

^p R. Schönheimer and H. Dam, *Z. physiol. Chem.*, *211*, 241-245 (1932).

^q H. Hentschel and O. Bachmann, *Z. ges. exper. Med.*, *71*, 744-754 (1930).

^r K. K. Chen, H. Jensen, and A. L. Chen, *J. Pharm.*, *43*, 13-50 (1931); *47*, 307-320 (1933); *49*, 14-25 (1933).

^s K. K. Chen and A. L. Chen, *J. Pharm.*, *49*, 514-525, 526-542, 543-547 (1933).

(*Claviceps purpurea*). He noticed that the product was more strongly levorotatory than cholesterol, and he showed that it differed from both cholesterol and the phytosterols.^{127,128} In later investigations, Tanret prepared exceedingly pure ergosterol, uncontaminated by the other fungus sterols. He was thus able to determine the properties and to formulate the empirical composition^{289,290} of ergosterol as $C_{27}H_{42}O_2$. This formula, however, has been shown by the work of Windaus and Lüttringhaus²⁹¹ to be incorrect; the empirical formula established by the latter investigators is $C_{28}H_{44}O$. More recently this formula has been substantiated by a number of workers.

Gérard,²⁹²⁻²⁹⁵ after an extended study of the distribution of the sterols in the plant world, came to the conclusion that a taxonomic relationship obtains with the type of sterol present. He propounded a rule that phytosterol is the characteristic sterol in the phanerogams, while ergosterol is the predominant compound found in the cryptogams. Cholesterol had long been accepted as the characteristic animal sterol. Gérard's rule is the basis for the usual classification of sterols, but some exceptions to it do occur.

Ergosterol is found in such lower forms as brown algae, slime fungi, bacteria (*Staphylococci*), *Mucor* species, yeast, *Penicillia*, and the lichens. It is highly probable that the "adipocire" isolated by Braconnot¹²⁴ from mushrooms was also ergosterol, as was the "fungine" of Vauquelin¹²⁵ and the "agaricine" of Gobley.¹²⁶ The distribution of ergosterol in mushrooms is discussed at length by Zellner.²⁹⁶ It has been found to be present in some fungi as the palmitate ester.²⁹⁷

However, ergosterol is also definitely found in animal tissues. It is stated that the cholesterol of commerce contains about 0.03% of ergosterol as an impurity.¹¹⁷ Ergosterol has been reported in one variety of snail (*Arion empiricorium*), to the extent of 19-25% of the sterols,²¹⁵ and in the earthworm (order *Oligochaeta*) in a proportion of 22% of the total sterols.^{153,215,216} Its presence in traces in many other animal tissues (including those of the human) has been noted by a number of investigators. On the other hand, it accounts for 90-100% of the total sterols in yeast. Cottonseed oil contains 7.5-25% of the sterols as ergosterol. It is likewise present in corn, peanut, and linseed oils.²⁹⁸ The data on distribution are summarized in Table 8.

²⁸⁹ C. Tanret, *Compt. rend.*, 147, 75-77 (1908).

²⁹⁰ C. Tanret, *Ann. chim. phys.* [8], 15, 313-330 (1908).

²⁹¹ A. Windaus and A. Lüttringhaus, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse III*, 1932, 4-7.

²⁹² E. Gérard, *Compt. rend.*, 114, 1544-1546 (1892).

²⁹³ E. Gérard, *Compt. rend.*, 121, 723-726 (1895).

²⁹⁴ E. Gérard, *J. pharm. chim.* [6], 1, 601-608 (1895).

²⁹⁵ E. Gérard, *Compt. rend.*, 126, 909-911 (1898).

²⁹⁶ J. Zellner, *Chemie der höheren Pilze*, Engelmann, Leipzig, 1907, pp. 27-39.

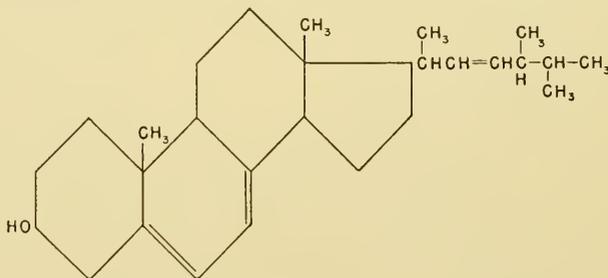
²⁹⁷ A. E. Oxford and H. Raistrick, *Biochem. J.*, 27, 1176-1180 (1933).

²⁹⁸ I. M. Heilbron, E. D. Kamm, and R. A. Morton, *Biochem. J.*, 21, 1279-1283 (1927).

Some bacteria, such as *Bacillus butyricus*²⁹⁹ and *Staphylococcus albus*,²⁹⁵ contain ergosterol, although the mycobacteria which most closely resemble the molds do not include it. *Mycobacterium tuberculosis* and certain other bacteria have no sterol whatsoever.³⁰⁰

It is, therefore, apparent that the presence of ergosterol is not an invariable occurrence in the lower forms, nor is it confined exclusively to the cryptogams. Bills¹⁵ suggests that Gérard's rule should rather state that ergosterol predominates in the lower orders of cryptogams.

(1') Structure of Ergosterol: Chuang³⁰¹ showed that the same tetracyclic nucleus is present in ergosterol as occurs in cholesterol, as evidenced by degradation in both cases to 3(β)-hydroxynorallocholanic acid. Ergosterol contains three double bonds, since six hydrogen atoms are absorbed in forming the saturated alcohol ergostanol,³⁰² C₂₈H₄₉OH. The original Rosenheim and King formula³⁰³ for ergosterol agrees with the present conception except that the hydroxyl group was placed on C₄ instead of on C₃. The positions of the double bonds and of the hydroxyl group were correctly postulated by Windaus *et al.*,³⁰⁴ who criticized the Heilbron formula because the double bonds were not in conjugate position as the reactions indicated.³⁰² The position of the third double bond in the side chain between C₂₂ and C₂₃, as well as of the methyl group at C₂₄, was readily deducible by the isolation of methyl isopropylacetaldehyde after ozonization.^{305,306}



Ergosterol

(2') Properties of Ergosterol: Ergosterol crystallizes from alcohol in small, white, glistening platelets; these platelets contain one to one and one-half molecules of water of crystallization, which can be removed only with difficulty. When ergosterol is crystallized from an ether-acetic acid mixture, needle-like crystals are formed which are anhydrous. It is soluble

²⁹⁹ A. Heiduschka and H. Lindner, *Z. physiol. Chem.*, **181**, 15-23 (1929).

³⁰⁰ R. J. Anderson and E. Chargaff, *J. Biol. Chem.*, **84**, 703-717 (1929).

³⁰¹ C. K. Chuang, *Ann.*, **500**, 270-280 (1933); *Chem. Abst.*, **27**, 2157 (1933).

³⁰² A. Windaus, H. H. Inhoffen, and S. v. Reichel, *Ann.*, **510**, 248-259 (1934).

³⁰³ O. Rosenheim and H. King, *J. Soc. Chem. Ind.*, **53**, 196-200T (1934).

³⁰⁴ A. Windaus and O. Linsert, *Ann.*, **465**, 148-166 (1928).

³⁰⁵ F. Reindel and H. Kipphan, *Ann.*, **493**, 181-190 (1932).

³⁰⁶ A. Guiteras, Z. Nakamiya, and H. H. Inhoffen, *Ann.*, **494**, 116-126 (1932).

with difficulty in cold methanol and oils, but it dissolves somewhat more easily in cold chloroform (0.2%) and to a much greater extent in the hot solvent (3.1%). It is also quite readily dissolved either by cold (2%) or by boiling ether (3.6%). Anhydrous ergosterol melts at 163°C., and the form containing one molecule of water melts between 166° and 183° C. The best preparations of the hydrated form melt at 168°C.³⁰⁷ Ergosterol can be distilled without decomposition under a high vacuum (0.4 mm.) at 250°C. The specific rotation of a 2% chloroform solution at 20°C. is -132°. On irradiation with ultraviolet light, it is destroyed. Ring B splits between C₉ and C₁₀ with formation of vitamin D₃ (see Chapter VIII for discussion of this reaction). Ergosterol is also decomposed by such oxidizing agents as concentrated halogens or potassium permanganate. Mineral acids cause an isomerization of the molecule. When it is hydrogenated in the presence of selenium, a hydrocarbon is formed^{308,309} which is identical with the compound produced when cholesterol is similarly treated.³¹⁰ Ergosterol also forms a difficultly soluble digitonide. Characteristic absorption bands, which are largely independent of the solvent, have peaks at 260, 269, 281, and 293 m μ , with the maximum at 281 m μ . Table 9 gives data on some of the derivatives.

TABLE 9
PROPERTIES OF SOME ERGOSTEROL ESTERS

Ergosteryl	M.P., °C.	$[\alpha]_D$	Remarks
Acetate (C ₂₈ H ₄₃ O·COCH ₃)	181	-90°	
Benzoate (C ₂₈ H ₄₃ O·COC ₆ H ₅)	168	-68°	Slightly sol. in cold ethanol, readily sol. in ethyl acetate and ether
Palmitate (C ₂₈ H ₄₃ O·CO(CH ₂) ₁₄ CH ₃)	107-108	-51°	Platelets, difficultly sol. in ethanol and ether
Allophanate (C ₂₈ H ₄₃ O·CONHCONH ₂)	250	-67°	Needles, difficultly sol. in most org. solvents except readily sol. in pyridine

b' Dihydroergosterol: 22,23-Dihydroergosterol (C₂₈H₄₅OH) assumes considerable importance, since it, also, is a provitamin (D₄). Although it is much less widely distributed in nature than is ergosterol, this product has been isolated from natural sources. Ruíz³¹¹ prepared it from ergot, and it occurs to the extent of 2-5% in yeast.¹¹⁵ Its structure is proved by its synthesis from ergosterol.³¹² On hydrogenation of this sterol, after pre-

³⁰⁷ C. E. Bills and E. M. Honeywell, *J. Biol. Chem.*, **80**, 15-23 (1928).

³⁰⁸ L. Ruzicka, M. W. Goldberg, and G. Thomann, *Helv. Chim. Acta*, **16**, 812-832 (1933).

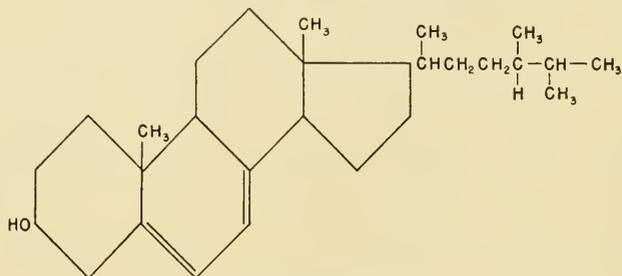
³⁰⁹ L. Ruzicka, M. W. Goldberg, G. Thomann, and E. Brandenberger, *Nature*, **132**, 643 (1933).

³¹⁰ O. Diels and A. Karstens, *Ann.*, **478**, 129-137 (1930).

³¹¹ A. S. Ruíz, *Anales real acad. farm.*, **3**, 201-231 (1941); *Chem. Abstr.*, **38**, 503 (1944). Cited by W. L. Ruigh, *Ann. Rev. Biochem.*, **14**, 232 (1945).

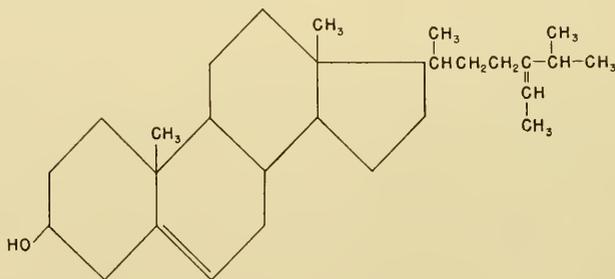
³¹² A. Windaus and R. Langer, *Ann.*, **508**, 105-114 (1933).

vious acetylation and treatment with maleic anhydride to form an addition product, dihydroergosterol can be prepared by removal of the protecting groups. Dihydroergosterol crystallizes in plates which melt at 174°C. and have a specific rotation of -20.1° (2% solution in chloroform at 14°C.). The two double bonds react with halogens.



Dihydroergosterol

c'. Fucosterol: Heilbron *et al.*³¹³⁻³¹⁵ isolated a doubly unsaturated sterol from algae. It has been found to be characteristic for all members of *Fucus* (rock weeds) and all other phaeophycetes (brown algae and sea weeds), bacillariophycetes (diatoms), and chrysophycetes (brown-orange algae).³¹⁶ The chlorophycetes (green, fresh-water algae) and rhodophycetes (red sea weed), which are more closely related to higher plants, usually contain sitosterol, while the more primitive myxophycetes (blue-green algae) have no sterols whatsoever. There appears to be a parallelism in the distribution of the carotenoid pigments and of the sterols, but the significance of this interrelationship is not clear. Thus, xanthophyll is found in the phanerogams, fucoxanthin in the cryptogams, and myxoxanthophyll in the myxophycetes.



Fucosterol

³¹³ P. W. Carter, I. M. Heilbron, and B. Lythgoe, *Proc. Roy. Soc. London*, B218, 82-109 (1939).

³¹⁴ D. H. Coffey, I. M. Heilbron, F. S. Spring, and H. R. Wright, *J. Chem. Soc.*, 1935, 1205-1207.

³¹⁵ I. M. Heilbron, R. F. Phipers, and H. R. Wright, *J. Chem. Soc.*, 1934, 1572-1576.

³¹⁶ I. M. Heilbron, *J. Chem. Soc.*, 1942, 79-89.

Fucosterol is isomeric with stigmasterol and yields stigmastanol on hydrogenation.³¹⁷ One unsaturated linkage is found between C₅ and C₆. The empirical formula is C₂₉H₄₈O. It melts at 124°C. and has a specific rotation of -38.4°. Fucosterol forms a difficultly soluble digitonide.

d'. Zymosterol: A second sterol³¹⁸ which occurs in yeast is zymosterol (C₂₇H₄₃OH),³¹⁹ which has two double bonds.³²⁰ It melts at 110°C. and has a specific rotation of +47.3°. It has been identified as Δ^{8,14,24,25}-cholesta-dienol.³²¹ It is unique in being one of the few sterols which have no double bond in the C₅-C₆ position. Additional information on the decomposition products is given by Wieland and Benend.³²²

Other mycosterols which have been isolated from yeast include ascosterol (C₂₉H₄₅OH)³²³ and several others named fecosterol, episterol, and neosterol. The last is probably the so-called isoergosterol, and it contains three unsaturated linkages. Still another yeast product which has been prepared by Wieland and Joost³²⁴ is cryptosterol. It is possible that this is another example of a triterpene, as it contains 30 carbons.

c. **Fatty Acids.** The fatty acids which are combined with the higher aliphatic alcohols or with the sterols in the waxes comprise many of those which are frequently found as components of the triglycerides. Thus, lauric (C₁₂), myristic (C₁₄), palmitic (C₁₆), and stearic (C₁₈) acids frequently occur as components of the waxes.

The larger proportion of acids present in the wax esters, however, are those having longer chains than C₁₈. Carnaubic acid (C₂₄), which is an isomer of lignoceric acid, and myricinic acid (C₃₀) are found in carnauba wax; lacceric acid (C₃₂) is a component of "stick-lac", geddic acid (C₃₄) occurs in ghedda wax, and carbocerotic acid (C₂₇H₅₄O₂) is reported as a component of Chinese insect wax. Cerotic acid, which occurs with montanic (C₂₉) and melissic (C₃₁) acids in beeswax, is a compound of disputed composition. Cerotic acid is now generally believed to be a C₂₆ saturated straight-chain acid, although a number of authors, including Francis, Piper, and Malkin,⁹⁴ consider that the cerotic acids already described are mixtures of C₂₄, C₂₆, and C₂₈ acids. With the exception of lacceric acid, all of the above long-chain fatty acids are classified as *iso*-acids, *i.e.*, acids having a forked chain.

Unsaturated acids play a relatively insignificant role in the case of waxes, in contrast to the important place they occupy in the case of the neutral

³¹⁷ D. H. Coffey, I. M. Heilbron, and F. S. Spring, *J. Chem. Soc.*, 1936, 738-741.

³¹⁸ I. Smedley-MacLean, *Biochem. J.*, 22, 22-26 (1928).

³¹⁹ H. Wieland, F. Rath, and W. Benend, *Ann.*, 548, 19-33 (1941).

³²⁰ F. Reindel and A. Weickmann, *Ann.*, 475, 86-100 (1929).

³²¹ B. Heath-Brown, I. M. Heilbron, and E. R. H. Jones, *J. Chem. Soc.*, 1940, 1482-1489.

³²² H. Wieland and W. Benend, *Ber.*, 75, 1708-1715 (1942).

³²³ H. Wieland, F. Rath, and H. Hesse, *Ann.*, 548, 34-49 (1941).

³²⁴ H. Wieland and E. Joost, *Ann.*, 546, 103-119 (1941).

fats. Those which have been identified contain 24 or fewer carbon atoms. The unsaturated acids are found only in the liquid waxes. Oleic acid is present in small amounts in the vegetable waxes, while larger proportions have been noted in marine oils. Linoleic acid and the more highly unsaturated linolenic acid accompany oleic acid in some of the fruit and flower waxes. Palmitoleic (C₁₆) and 9-eicosenoic (C₂₀) acids have been re-

TABLE 10
ESTERS OCCURRING IN NATURAL WAXES^{a, b}

Ester	Formula	M.p., °C.	Mol. wt.
Cetyl laurate	CH ₃ (CH ₂) ₁₄ CH ₂ O·OC(CH ₂) ₁₀ CH ₃	41	424.73
Lauryl myristate	CH ₃ (CH ₂) ₁₀ CH ₂ O·OC(CH ₂) ₁₂ CH ₃	32	396.68
Myristyl myristate	CH ₃ (CH ₂) ₁₂ CH ₂ O·OC(CH ₂) ₁₂ CH ₃	38	424.73
Cetyl myristate	CH ₃ (CH ₂) ₁₄ CH ₂ O·OC(CH ₂) ₁₂ CH ₃	48	452.78
Ceryl myristate (b)	C ₂₆ H ₅₃ O·OC(CH ₂) ₁₂ CH ₃	62	593.04
Cetyl palmitate	CH ₃ (CH ₂) ₁₄ CH ₂ O·OC(CH ₂) ₁₄ CH ₃	51.6, 53	480.83
Octadecyl palmitate	CH ₃ (CH ₂) ₁₆ CH ₂ O·OC(CH ₂) ₁₄ CH ₃	55	508.88
Ceryl palmitate (b)	C ₂₆ H ₅₃ O·OC(CH ₂) ₁₄ CH ₃	69	621.09
Myricyl palmitate	C ₃₀ H ₆₁ O·OC(CH ₂) ₁₄ CH ₃	73	676.85
Melissyl margarate	C ₃₁ H ₆₁ O·OC(CH ₂) ₁₅ CH ₃	79	705.25
Lauryl stearate	CH ₃ (CH ₂) ₁₀ CH ₂ O·OC(CH ₂) ₁₆ CH ₃	49	450.76
Cetyl stearate	CH ₃ (CH ₂) ₁₄ CH ₂ O·OC(CH ₂) ₁₆ CH ₃	55, 56.5	508.88
Stearyl stearate	CH ₃ (CH ₂) ₁₆ CH ₂ O·OC(CH ₂) ₁₆ CH ₃	58.5, 62	536.93
Ceryl stearate (b)	C ₂₆ H ₅₃ O·OC(CH ₂) ₁₆ CH ₃	73	649.14
Myricyl stearate	C ₃₀ H ₆₁ O·OC(CH ₂) ₁₆ CH ₃	76	705.25
Myricyl isobehenate	C ₃₀ H ₆₁ O·OCC ₂₁ H ₄₃	82	761.35
Ceryl carnaubate (b)	C ₂₆ H ₅₃ O·OCC ₂₃ H ₄₇	81	733.30
Ceryl lignocerate (b)	C ₂₆ H ₅₃ O·OC(CH ₂) ₂₂ CH ₃	79	733.30
Myricyl tetracosanate	C ₃₀ H ₆₁ O·OCC ₂₃ H ₄₇	83	789.40
Carnaubyl cerotate	C ₂₄ H ₄₉ O·OCC ₂₆ H ₅₁	78.5	733.30
Ceryl cerotate (b,b)	C ₂₆ H ₅₃ O·OCC ₂₅ H ₅₁	84	761.35
Myricyl cerotate (b)	C ₃₀ H ₆₁ O·OCC ₂₅ H ₅₁	87	817.46
Ceryl cerotate (c,c)	C ₂₇ H ₅₅ O·OCC ₂₆ H ₅₃	87	789.40
Montanyl cerotate (c)	C ₂₉ H ₅₉ O·OCC ₂₆ H ₅₃	88	817.46
Melissyl cerotate (c)	C ₃₁ H ₆₃ O·OCC ₂₆ H ₅₃	86	845.51
Tetracosyl neomontanate	C ₂₄ H ₄₉ O·OCC ₂₇ H ₅₅	84	761.35
Ceryl montanate (c)	C ₂₇ H ₅₅ O·OCC ₂₇ H ₅₅	87	817.46
Montanyl montanate	C ₂₉ H ₅₉ O·OCC ₂₈ H ₅₇	85, 89	845.51
Ceryl myricinate	C ₂₆ H ₅₃ O·OCC ₂₉ H ₅₉	87	817.46
Myricyl myricinate	C ₃₀ H ₆₁ O·OCC ₂₉ H ₅₉	87, 90.3	873.56
Ceryl melissate (c)	C ₂₇ H ₅₅ O·OCC ₃₀ H ₆₁	86	845.51
Melissyl melissate	C ₃₁ H ₆₃ O·OCC ₃₀ H ₆₁	90.5, 92.5	901.61
Myricyl lacerate	C ₃₀ H ₆₁ O·OCC ₃₁ H ₆₃	90, 92.5	901.61
Lacceryl lacerate	C ₃₂ H ₆₅ O·OCC ₃₁ H ₆₃	92.5, 95	929.66
Geddyl geddate	C ₃₄ H ₆₉ O·OCC ₃₃ H ₆₇	98	985.77

^a If the alcohol or acid group of the ester has 26 C atoms, it is referred to as (b); if 27 C atoms as (c).

^b Adapted from A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 33.

ported in jojoba oil, which has the properties of a wax. An unsaturated hydroxy dibasic acid, shellolic acid, is found in shellac.¹ Hydroxy mono-basic acids have likewise been demonstrated in some waxes, for example, aleuritic acid (9,10,16-trihydroxypalmitic acid) in shellac wax.

d. Esters in Natural Waxes. The natural waxes contain chiefly the alkyl esters of the higher aliphatic alcohols or sterols, and the fatty acids. However, free alcohol and free fatty acid may occur, as well as small amounts of hydrocarbons. Some of the commoner esters which are known to occur in natural waxes are listed in Table 10.

(2) *Properties of Natural Waxes*

The waxes are relatively inert substances which, in most cases, are solids at ordinary temperatures. Some of them, such as beeswax, have the property which permits them to be cut and shaped with ease. Beeswax melts at a low temperature and it mixes well with coloring matter; the consistency may be varied by admixture with inert material, or with fats or oils. For this reason it has found wide application in the preparation of wax figures and models.

Although waxes respond to saponification, as do other esters, the reaction is generally quite sluggish. According to Chibnall and collaborators,³⁵ the hydrolysis is particularly difficult in the case of insect waxes, and special procedures must be applied to obtain an effective breakdown. The difficulty is due in part to the insolubility of one of the hydrolysis products—the higher alcohol—in water, so that it is hard to determine when saponification has been completed. Moreover, the subsequent separation of the hydrolysis products is complicated by unexpected solubility relations. Although the higher alcohols can be readily separated from the water-soluble potassium and sodium salts of the lower fatty acids by their extraction with organic solvents, the separation becomes more difficult with the higher acids, since their sodium and potassium soaps do become appreciably soluble in the fat solvents.^{32b} The sodium and potassium salts of the C₂₆ to C₃₆ alcohols dissolve to some extent in boiling acetone, and readily in boiling benzene. On the other hand, the calcium soaps of these higher acids dissolve only sparingly in boiling acetone and alcohol, while they are only slightly soluble in boiling benzene. They are insoluble in boiling diethyl ether. Chibnall and co-workers³⁵ have developed a satisfactory method for the saponification of coccerin (a wax from *Coccus cacti*) which involves saponification in a benzene-alcohol solution of potassium hydroxide followed by a second saponification, by the use of sodium ethoxide, of the portion still unsaponified.

In spite of the relative stability of the ester linkage in the waxes, they

^{32b} S. H. Piper, A. C. Chibnall, and E. F. Williams, *Biochem. J.*, **28**, 2175–2188 (1934).

may undergo interesterification in the same way as is the case with the triglycerides (see Chapter III). A number of patents have been issued for the preparation of modified products by the reaction of montan wax with glycerol,³²⁶⁻³²⁸ as well as with ethylene glycol and with monohydric alcohols.³²⁹ Similar interesterification reactions have been applied to the liquid marine oils, using polyhydric alcohols.

Since most waxes consist of esters of saturated acids and saturated alcohols, they cannot be hydrogenated. However, the liquid waxes, which are mostly of animal origin, can absorb hydrogen to become solid. This is particularly true of the marine oils in which the alcohols contain unsaturated linkages.

(3) Description of Natural Waxes

A number of products are referred to as waxes largely because of the similarity in their physical properties to those of the true waxes. The principal group consists of the natural (or true) waxes, which are obtained both from animal and from vegetable sources. A second group includes fossil waxes, earth waxes, and lignite paraffins. The paraffin or petroleum waxes comprise the third category. These differ from the first group in being almost exclusively hydrocarbons rather than esters of monohydric alcohols. The synthetic waxes, which are considered separately, may have a composition which varies widely from the usual biochemical conception of the composition of waxes.

a. Natural Waxes of Insect Origin. There are two general types of wax-producing insects, namely the *Apidae*, which include the bees, and the *Coccidae*, from members of which the Chinese wax (*Coccus ceriferus*), and stick-lac (*Tachardia lacca*) are obtained.

(a) *Waxes from the Apidae.* There are three genera in this order which produce waxes. These include: (1) *Apis*; (2) *Melipona* (large, stingless bee), *Trigona* (dwarf stingless bee), and *Tetrasoma*; and (3) *Bombus* (humble bee).

a'. Beeswax: The beeswax of commerce is obtained from a number of species of the genus, *Apis*. The most important members of this group are the domesticated honey bee (*A. mellifica*), the giant bee (*A. dorsata*), and also *A. indica* (Asia) and the little bee (*A. florea*, East India).¹

There are many races of the common honey bee throughout the world,

³²⁶ E. Schliemanns Export-Ceresin-Fabrik, G.m.b.H., *German Patent* No. 244,786 (Apr. 17, 1912).

³²⁷ W. Pungs and M. Jahrstorfer (to I. G. Farbenindustrie), *U. S. Patent* No. 1,737,975 (Dec. 3, 1929); *German Patent* No. 563,394 (Nov. 8, 1932).

³²⁸ F. W. Guthke and W. Pungs (to I. G. Farbenindustrie), *U. S. Patent* No. 1,834,056 (Dec. 1, 1931); *German Patent* No. 558,437 (Sept. 7, 1932). J. Y. Johnson, *Brit. Patent* No. 296,145 (Oct. 17, 1928).

³²⁹ J. Y. Johnson (to I. G. Farbenindustrie), *Brit. Patent* No. 376,276 (June 27, 1932).

but the wax obtained from them differs little in physical or chemical characteristics. Although the production of beeswax is especially widespread, the principal sources are the Portuguese colonies, where the yearly export is reported as 1,213,000 tons.³³⁰ Vansell and Bisson³³¹ have given the following chemical and physical constants for pure beeswax: acid value, 17.0; iodine number (Hanus), 5.8; saponification value, 84.4; ash content, near 0; melting point, $64.0^{\circ} \pm 0.9^{\circ}\text{C}.$; solidifying point, $63.5^{\circ} \pm 0.9^{\circ}\text{C}.$; and refractive index, 1.4402. Beeswax is readily soluble in ether, chloroform, and carbon tetrachloride, partially soluble in cold benzene and carbon disulfide, and slightly soluble in cold alcohol. Beeswax is insoluble in water. It dissolves in triglycerides, but not in mineral oils.

The approximate chemical composition of beeswax is given in Table 11.

b'. Ghedda Wax: Ghedda or gedda wax was formerly known as East Indian beeswax, and is now considered to be a wax produced by any bee other than the honey bee, *Apis mellifica*. The wax comes chiefly from three species, *i.e.*, *A. indica* F., *A. florea* F., and *A. dorsata* F. Its production is centered in India and the East Indian region, although Japan, Korea, and the Philippines also produce it. Warth¹ has listed the following constants for ghedda wax: acid value, 3.5–10.5; iodine number (Hübl), 4.8–11.4; saponification value, 86–130; ester value, 69–123; melting point, 60.4 – $66.4^{\circ}\text{C}.$; solidification point, $60^{\circ}\text{C}.$; refractive index ($80^{\circ}\text{C}.$), 1.4404; and specific gravity ($15^{\circ}\text{C}.$), 0.956–0.973. The chemical composition of ghedda wax is given in Table 12.

(b) *Waxes from Melipona and Trigona*. The so-called "stingless" bee or dammar bee belongs to the *Melipona* or *Trigona* species. These were the only types in America prior to the introduction of the *Apis* variety after the discovery of America. These species are still favored in Mexico and in Central American countries. Stingless bee wax is also available from Brazil, Trinidad, and India. Warth¹ has indicated an average composition of stingless bee wax as follows:

<i>Alkyl esters of monobasic acids</i> (myricyl palmitate, ceryl hydroxymargarate, myricyl cerotate)	35%
<i>Saponifiable sticky resinous matter</i>	14%
<i>Free fatty acids</i> (cerotic, myricinic, unidentified unsaturated).	12.3%
<i>Hydrocarbons</i> (hentriacontane, unidentified).	7.3%
<i>Cellulosic, scleroprotein, and mineral matter</i>	31.5%

³³⁰ C. Lepierre and A. Carvalho, *Chimie & Industrie*, 29, Special Nos. 1087–1093 (1933); *Chem. Abst.*, 28, 356 (1934). Cited by A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 41.

³³¹ G. H. Vansell and C. S. Bisson, *U. S. Dept. Agr., Bur. Entomol., Plant Quarantine*, E495, 1–11, Feb., 1940. Cited by A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 44; *Chem. Abst.*, 34, 3118 (1940).

TABLE 11
APPROXIMATE COMPOSITION OF YELLOW BEESWAX^a

Constituent	Formula	M.p., °C.	Per cent
Alkyl esters.....		63.5	72
Myricyl palmitate.....	$\text{CH}_3(\text{CH}_2)_{23}\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_{14}\text{CH}_3$	—	33
Laeceryl palmitate.....	$\text{CH}_3(\text{CH}_2)_{30}\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_{14}\text{CH}_3$	—	9
Myricyl palmitoleate.....	$\text{CH}_3(\text{CH}_2)_{23}\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_5\text{CH}:\text{CH}(\text{CH}_2)_7\text{CH}_3$	38	12
Myricyl hydroxypalmitate.....	$\text{CH}_3(\text{CH}_2)_{23}\text{CH}_2\text{O}\cdot\text{OC}(\text{OH})\text{C}_{13}\text{H}_{25}$	—	6
Myricyl cerotate.....	$\text{CH}_3(\text{CH}_2)_{23}\text{CH}_2\text{O}\cdot\text{OCC}_{25}\text{H}_{51}$	—	12
Cholesterol esters.....		40	0.8
Cholesteryl palmitoleate.....	$\text{C}_{27}\text{H}_{45}\text{O}\cdot\text{OC}(\text{CH}_2)_5\text{CH}:\text{CH}(\text{CH}_2)_7\text{CH}_3$	41-42	0.6
Lactones.....		77.5-79	13-13.5
ω -Myristolactone.....	—	77.8	—
Wax acids.....		82.5	—
Neocerotic.....	$\text{C}_{25}\text{H}_{50}\text{O}_2$	86.8	—
Cerotic (c) ^b	$\text{C}_{27}\text{H}_{54}\text{O}_2$	90	—
Montanic.....	$\text{C}_{29}\text{H}_{58}\text{O}_2$	—	12-12.5
Melissic.....	$\text{C}_{31}\text{H}_{62}\text{O}_2$	—	1.5
Hydrocarbons.....		63.5	—
Nonacosane.....	$\text{CH}_3(\text{CH}_2)_{27}\text{CH}_3$	68.7	11
Heptacosane.....	$\text{CH}_3(\text{CH}_2)_{25}\text{CH}_3$	—	1-2
Moisture.....		—	—

^a Adapted from A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 49.

^b For explanation cf. Table 10.

(c) *Humble Bee Wax*. Humble bee wax is produced by two species: *Bombus terrestris*, which lives in carded moss, and *B. lapidarius*, which is found in cavities among rocks. The quantity of wax produced by either type of bee is exceedingly small, and it is obtained only with difficulty. It contains an unusual C₃₂ alcohol called psylyl, while myricyl alcohol, and palmitic and cerotic acids are absent.³³² The Finnish worker Sundwik³³² also reported the presence of a C₃₃ or C₃₄ alcohol, melting at 69–70°C., and probably corresponding to *incarnatyl alcohol*.

TABLE 12
APPROXIMATE COMPOSITION OF GHEDDA WAX^a

Constituent	Formula	M.p., °C.	Per cent
Alkyl esters.....			87
Ceryl myristate.....	CH ₃ (CH ₂) ₂₄ CH ₂ O·OC(CH ₂) ₁₂ CH ₃	59	—
Ceryl palmitate.....	CH ₃ (CH ₂) ₂₄ CH ₂ O·OC(CH ₂) ₁₄ CH ₃	69	20
Ceryl stearate.....	CH ₃ (CH ₂) ₂₄ CH ₂ O·OC(CH ₂) ₁₆ CH ₃	73	—
Ceryl hydroxymargarate..	CH ₃ (CH ₂) ₂₄ CH ₂ O·OC(OH)C ₁₆ H ₃₂	—	4
Melissyl margarate.....	C ₃₁ H ₆₃ O·OC(CH ₂) ₁₅ CH ₃	79	—
Free fatty acids.....			5
Cerotic.....	C ₂₅ H ₅₁ COOH	76–77	1
Geddic.....	C ₃₃ H ₆₇ COOH	95	2
Margaric.....	CH ₃ (CH ₂) ₁₅ COOH	61.3	2
Hydrocarbons.....			7
Heptacosane.....	CH ₃ (CH ₂) ₂₅ CH ₃	59–59.5	5
Hentriacontane.....	CH ₃ (CH ₂) ₂₉ CH ₃	68–68.5	1
Tritriacontane.....	CH ₃ (CH ₂) ₃₁ CH ₃	71.8	1

^a Adapted from A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 53.

(d) *Waxes from the Coccidae*. A number of insects of this family produce wax, but only two of these waxes are of commercial importance. One is Chinese insect wax, which is secreted by *Coccus ceriferus*, while the other well-known member is stick-lac wax (or shellac wax in refined form), which is produced by *Tachardia lacca*. The wax secreted by the “coccids” is a shell housing separated from the body. The character of the wax varies somewhat with the host plant on which the insect feeds. In a sub-group, *Coccinae*, the “scale” is merely the thickened surface of the insect.

a'. Chinese Insect Wax: This wax is produced chiefly by the scale insect (*Coccus ceriferus* Fabr.). This insect infests the twigs of the Chinese ash (*Fraxinus chinensis* Roxburgh), and becomes imbedded in the waxy material. This is scraped off the branches along with the insects, and is freed from them by melting and straining. Warth¹ has reported the average composition of Chinese insect wax as follows:

³³² E. E. Sundwik, *Z. physiol. Chem.*, 26, 56–59 (1898); 53, 365–369 (1907); 72, 455–458 (1911).

<i>Esters of monobasic acids</i>	95-97%
Myricyl lignocerate.....	2%
Neomontanyl lignocerate	
Ceryl lignocerate	
Ceryl neomontanate.....	20%
Ceryl cerotate.....	20%
<i>Resins</i>	<1%
<i>Free wax acids (cerotic)</i>	0.5-1%
<i>Free aliphatic alcohols (myricyl)</i>	<1%
<i>Hydrocarbons (heptacosane)</i>	<1%

Chinese wax finds use in candle-making, as an insulating agent, in treating silk and cotton fabrics, in sizing and glazing of papers, and in the shoe-polish industry.

b'. Shellac Wax: This is produced by the lac insect (*Carteria lacca*), together with resins and other non-waxy substances. As in the case of Chinese wax, the insects are attached to branches of trees, from which the crude stick-lac is removed; it is refined to produce shellac. The refined wax melts at 74-78°C. and has a saponification value of 100 to 126. The specific gravity at 15.5°C. is 0.971-0.980. The following composition has been reported by Warth:¹

<i>Esters of wax acids</i>	60-62%
Ceryl lignocerate	
Ceryl cerotate	
Lacceryl laccerate.....	10-12%
Ceryl aleuritrate.....	<1%
<i>Free wax acids (lacceric)</i>	1%
<i>Free wax alcohols (neoceryl, lacceryl)</i>	35-36%
<i>Hydrocarbons</i>	2-6%
Pentacosane.....	2%
Hentriacontane	

b. Natural Waxes from Animals. Both land and sea animals produce waxes. Wool wax, or lanolin, is the principal wax of commercial importance from the terrestrial animals. The marine waxes may be solid, as is the case with spermaceti, or liquid as with sperm oil.

(a) *Wool Wax*. Wool wax, also known as wool fat, is obtained when wool is cleaned and processed. The yield of fat obtained commercially depends upon the nature of the wools. New Zealand wool is reported to give 16.6% fat, that from Australia 16%, that from South America 13.2%, while Russian wool yields only 6.6% of fat.³³³ The employment of modern centrifugation methods has improved the yield of fat from 25% to 65% of that present.

The physical constants of wool fat vary somewhat with its source. The

³³³ J. Lewkowitsch, *Chemical Technology of Oils, Fats and Waxes*, 6th ed., Vol. II, Macmillan, London, 1922, p. 901.

melting point is given as 36–41°C.³³⁴ while the wax solidifies at about 30°C.³³³ The refractive index of Yorkshire grease is 1.4781 at 40°C.³³⁴ and 1.465 at 60°C. It is optically active, showing a specific rotation of +6.70° at 35°C.³³³ Some of the chemical constants are as follows:

Wool fat:

Acid value.....	0.5+
Saponification value.....	82–127
Iodine number (Wijs).....	15–47
Reichert-Meißl number.....	4.7–6.9
Acetyl value.....	23.3

Mixed acids:

Melting point.....	41.8°C.
Iodine number.....	17
Molecular weight.....	327.5

Mixed alcohols:

Melting point.....	33.5°C.
Iodine number.....	26–36
Acetyl value.....	144
Molecular weight.....	239

The term lanolin is generally reserved for the refined wax. It was early recognized that lanolin possessed many characteristic differences in composition as compared with other waxes. In the first place, it was demonstrated that the alcohol components were mainly cyclic alcohols, and cholesterol was found to be the chief one. The so-called “ischolesterol,” which comprises an appreciable fraction of the wax, is now known to have a structure distinctly different from that of cholesterol³³⁵; it consists of at least two separate alcohols, which are triterpenes. These are discussed in the section on triterpenes (see Section 2).

Another unique feature in the composition of lanolin is the fact that the saturated acids differ considerably from the isomeric aliphatic acids. The so-called lano series, *i.e.*, lanomyristic, lanopalmitic, lanostearic, lanoarachidic, and lanocerotic, are believed to be *iso* or forked-chain compounds with one or more side chains. This structural difference from the aliphatic series accounts for their optical isomerism. The methyl side chain cannot be situated solely on the terminal (or ω) carbon, as asymmetry would not be established by this arrangement. If more than one carbon side chain occurs in such an acid, this would not preclude a side chain on the terminal carbon. For a discussion of the *iso* and *ante-iso* acids of wool fat, see pages 36 and 37.

Another characteristic group of acids in wool fat are the hydroxy acids, which contain one or two hydroxy groups. The asymmetry of the carbon atoms to which the hydroxyl or methyl groups are attached accounts for

³³⁴ F. Utz, *Rev. Fett-Harz. Ind.*, 13, 249–250, 275–277 (1906); *Chem. Abst.*, 1, 492–493 (1907).

³³⁵ L. Ruzicka, E. Rey, and A. C. Muhr, *Helv. Chim. Acta*, 27, 472–489 (1944).

the optical isomerism. The composition of neutral anhydrous wool fat is summarized in Table 13.

TABLE 13
CHEMICAL COMPOSITION OF ANHYDROUS WOOL FAT^a

Constituent	Formula	M.p., °C.	Per cent
<i>Esters of cholesterol and fatty alcohols</i>			73
Lano acids.....		48-55	7.1
Lanomyristic.....	C ₁₃ H ₂₇ COOH	58.5-59.5	—
Lanopalmitic.....	C ₁₅ H ₃₁ COOH	44.5-46	—
Lanostearic.....	C ₁₇ H ₃₅ COOH	54.0-56.0	—
Lanoarachidic.....	C ₁₉ H ₃₉ COOH	56.8-58.4	—
Lanocerotic.....	C ₂₅ H ₅₁ COOH	78	—
Monohydroxy acids.....			36.2
Lanopalmic.....	C ₁₆ H ₃₀ (OH)COOH	86-87	—
Lanoarachic.....	C ₁₉ H ₃₈ (OH)COOH	—	—
Lanocerinic.....	C ₂₅ H ₅₀ (OH)COOH	—	—
Dihydroxy acids.....			2.5
Lanocerie.....	C ₂₉ H ₅₇ (OH) ₂ COOH and/or C ₃₁ H ₆₁ (OH) ₂ COOH	102.5 103-104	— —
Zoosterols.....			10.5
Cholesterol.....	C ₂₇ H ₄₆ OH	145-148	—
7,8-Dihydrocholesterol.....	C ₂₇ H ₄₇ OH	128-129	—
Oxycholesterol.....	C ₂₇ H ₄₄ (OH) ₂	—	—
Unsatd. alcohols.....			8
Decenyl.....	C ₁₀ H ₁₉ OH	—	—
Hendecenyl.....	C ₁₁ H ₂₁ OH	—	—
Lanolin alcohol.....	C ₁₂ H ₂₃ OH	—	—
Lano-octadecyl.....	C ₁₈ H ₃₅ OH	42-43	—
Satd. alcohols.....			8
Cetyl.....	C ₁₆ H ₃₃ OH	50	—
Carnaubyl.....	C ₂₂ H ₄₅ OH	68-69	—
Dihydric alcohol.....			1.8
Lanyl.....	C ₂₁ H ₄₀ (OH) ₂	78.5	—
<i>Free fatty acids</i>			1
Lanopalmic.....	C ₁₅ H ₃₀ (OH)COOH	86-87	—
Lanocerotic.....	C ₂₅ H ₅₁ COOH	78	—
<i>Free Alcohols</i>			25
Ceryl alcohol.....	C ₂₆ H ₅₃ OH	—	—
Triterpene alcohols.....			5
Agnosterol.....	C ₃₀ H ₄₇ OH	—	—
γ-Lanosterol.....	C ₃₀ H ₄₉ OH	—	—
Cholesterol.....	C ₂₇ H ₄₆ OH	—	1
<i>Lactone</i>			<1
Lanocerin.....	C ₃₀ H ₅₈ O ₃	88	—
<i>Mineral matter (K₂O)</i>			<1
<i>Hydrocarbons</i>			1-2

^a Adapted from A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, pp. 78, 79.

(b) *Spermaceti*. Spermaceti represents a solid wax which is obtained from marine animals. The U. S. Pharmacopoeia³³⁶ officially defines this product as a peculiar, concrete, fatty substance, obtained from the head of the sperm whale (*Physeter macrocephalus* Linné) which occurs in "white, somewhat translucent, slightly unctuous masses having a crystalline fracture and a pearly lustre, with a very faint odor and a bland, mild taste."

Spermaceti occurs in the head cavities and blubber, mixed with the sperm oil. In the live whale, the wax is dissolved in the sperm oil. However, when the whale dies, the spermaceti separates as a white spongy mass admixed with sperm oil, from which it can be separated mechanically. In addition to being present in the sperm whale, it has been reported as a component of the bottlenose whale (*Balaena rostrata*), as well as of some other cetaceans.³³⁷

Spermaceti melts between 42° and 44°C., although an especially pure sample has been reported to melt at 45.8°C.³³⁸ Spermaceti is insoluble in water, and practically so in cold ethyl alcohol, but it dissolves in boiling alcohol as well as in ether, chloroform, and other common fat solvents. The specific gravities of 15 samples were found to vary³³⁹ between 0.905 and 0.945 at 15°C.

The approximate chemical composition has been reported by Warth¹ as follows:

<i>Esters of monobasic acids</i>	98-98.5%
Saturated	
Lauryl myristate.....	1-2%
Cetyl palmitate.....	90%
Lauryl stearate.....	3-4%
Cetyl stearate.....	1.1%
Unsaturated	
Unidentified.....	1-2%
<i>Free monobasic acids</i>	0.4%
Lauric acid	
<i>Free monohydric alcohols</i>	1-1.5%
Cetyl, stearyl, and oleyl	

Spermaceti is used as a demulcent, in cosmetics, for the finishing and lustering of linens, in laundry wax, and in special soaps and emulsifying agents. It likewise finds some application in candle making, as it decreases the brittleness.

(c) *Sperm Oil*. A number of liquid animal waxes differ from the fatty oils in that they are practically free from glycerides. They consist chiefly

³³⁶ U. S. Pharmacopoeia, XIII, Mack Printing Co., Easton, Pa., 1947.

³³⁷ E. Thorpe, *Dictionary of Applied Chemistry*, Revised ed., Longmans, Green & Co., London, Vol. VII, 1927, pp. 454, 455.

³³⁸ A. H. Warth and E. M. Hanzely, *Unpublished report*. Cited by A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 82.

³³⁹ L. F. Kebler, *Am. J. Pharm.*, 68, 7-10 (1896); 69, 104-107 (1897).

of esters of unsaturated monatomic alcohols with unsaturated acids. They may be solidified by elaidinization with nitrous acid, whereby the *cis* form of the unsaturated acid is changed to the *trans* modification. The most common member of this group is sperm oil. However, a bird oil from the mutton-bird (*Aestrelata lessoni*),³⁴⁰ and a vegetable wax (jojoba wax) also possess a similar composition.

True sperm oil is a pale-colored liquid wax from the head oil of the sperm whale (*Physeter macrocephalus*), which is also the source of spermaceti. This product is sometimes referred to as spermaceti oil or cachalot oil.¹ Arctic sperm oil is a closely related product obtained from *Hyperoodon rostratus*. The latter product is also known as bottlenose oil and doegling oil. Dolphin oil, obtained from *Delphinus delphis*, is also a liquid wax but it contains considerable amounts of triglycerides admixed with it.

Sperm oil has a low specific gravity; the value at 15°C. is 0.880–0.883.³⁴¹ A saponification value of 123–133, and iodine numbers of 81–84 have been reported. Table 14 shows the approximate composition of a typical oil.

TABLE 14
COMPOSITION OF SPERM OIL^a

Component	Formula	Per cent
Myristyl caprate	$\text{CH}_3(\text{CH}_2)_{12}\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_8\text{CH}_3$	4.5
Cetyl laurate	$\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_{10}\text{CH}_3$	20.0
Hexadecenyl lauroleate	$\text{C}_{16}\text{H}_{31}\text{O}\cdot\text{OCC}_{11}\text{H}_{21}$	7.0
Oleyl myristate	$\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_7\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_{12}\text{CH}_3$	13.0
Oleyl myristoleate	$\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_7\text{CH}_2\text{O}\cdot\text{OCC}_{13}\text{H}_{25}$	18.0
Cetyl palmitate	$\text{C}_2\text{H}_3(\text{CH}_2)_{14}\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_{14}\text{CH}_3$	9.0
Hexadecenyl palmitoleate	$\text{C}_{16}\text{H}_{31}\text{O}\cdot\text{OCC}_{15}\text{H}_{29}$	3.0
Oleyl oleate	$\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_7\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_7\text{CH}_3$	11.0
Stearyl stearate	$\text{CH}_3(\text{CH}_2)_{16}\text{CH}_2\text{O}\cdot\text{OC}(\text{CH}_2)_{16}\text{CH}_3$	2.5
Eicosenyl eicosenate	$\text{C}_{20}\text{H}_{39}\text{O}\cdot\text{OCC}_{19}\text{H}_{37}$	7.0
Eicosenyl eicosdienoleate	$\text{C}_{20}\text{H}_{39}\text{O}\cdot\text{OCC}_{19}\text{H}_{35}$	2.0
Undetermined	.	3.0

^a Adapted from A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 87.

In addition to the constituents reported in Table 14, sperm and Arctic sperm oil contain small proportions of the glyceryl ethers, batyl, chimyl, and selachyl alcohols (see page 392).

c. Natural Waxes from Plants. The waxes serve as protective agents on plant leaves. The outer wall of the epidermis of an adult plant leaf

³⁴⁰ L. H. Smith, *J. Soc. Chem. Ind.*, 30, 405 (1911).

³⁴¹ G. Martin, *Animal, Vegetable Oils, Fats and Waxes*, Crosby, Lockwood & Sons, London, 1920.

has a thickened portion consisting of cutin, which is a wax-like substance impermeable to water. Plants indigenous to arid or desert sections have especially well-developed cutinized areas on their leaves. Ordinarily, the greatest development of the wax coat is on the under surfaces of the leaves, where the stomata or pores are most abundant. The wax apparently serves largely to reduce the loss of moisture by transpiration. Where the rays of the sun are particularly intense, resins as well as waxes may serve as protective agents. This is the case with the Coville creosote bush (*Larrea tridentata* Vail.), and many other desert xerophytes.

Wax sometimes forms over the whole leaf, as in the bog bilberry (*Vaccinium uliginosum* L.), or sometimes only on the lower surface, as in the bog rosemary (*Andromeda polifolia* L.), the small cranberry (*Vaccinium oxycoccus* L.), and many other shrubs and grasses.

In some cases wax may be secreted over the entire plant to protect it from water or saltiness, as is the case with the red swamp maple (*Acer rubrum* L.). A wax coating is a prevailing feature of a number of halophytes (salt-water plants). This provides a glaucous or mat blue-green coating for their leaves. Examples of this last category are the sea pea (*Lathyrus japonicus maritimus* L.), the sea holly (*Eryngium maritimum* L.), the sea bluebell (*Mertensia maritima* L.), and the yellow horn poppy (*Glaucium flavum* Crantz).

Another common site for the deposition of wax is on the cuticles of fruits and vegetables. It is particularly noticeable in grapes and plums, where it is sometimes called the bloom. It may be present in the form of grains, rods, or crusts. The function seems to be to prevent absorption of water as it flows over the surface of the fruit.

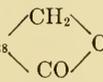
(a) *Waxes from Plant Leaves and Stems.* One may subdivide this group into the following 5 subgroups: (1) waxes of palms, where the wax is more abundant on the leaves than on other parts, as in the case of *Copernicia cerifera* Mart., the source of carnauba wax; (2) waxes of shrubs and herbs in which the wax is distributed over the entire plant, as in the wax slipper flower, *Pedilanthus pavonis* Boiss., the source of candelilla wax; a wax also called candelilla, but differing in constants, is obtained from the wax euphorbia (*Euphorbia antisiphilitica*); (3) waxes of grasses and sedges, in which the wax is principally on the stem, as in *Saccharum officinarum* L., from which sugar-cane wax is derived; (4) waxes of broad-leaf trees, which contain only small amounts; and (5) waxes of narrow-leaf trees such as conifers, from the essential oils of which waxes can be recovered.

a'. *Carnauba Wax:* One of the best known of the plant waxes is obtained from the leaves of an American species of the palm, known as *Copernicia cerifera* Martius. The natural habitat for this palm is Brazil where it has a wide distribution through the semi-arid Brazilian Northeast. Several varieties of carnauba palm abound throughout this area. The so-

called carnauba wax is separated from the dried leaves by hand after the wax-containing ribs have been split apart and the webs opened.

Carnauba wax has a specific gravity of about 1.000 at 15°C. (0.990 to 1.0008); its reported melting values vary from 78° to 84° C.; the saponification numbers which have been found are from 79 to 95; and the iodine numbers cited in the literature range from 7 to 14.¹ The acetyl value is extremely high (54.8–55.2), which indicates the presence of a number of free hydroxyl groups. Carnauba wax is sparingly soluble in organic solvents at room temperature, but it dissolves fairly well in these solvents at temperatures above 45°C.¹

TABLE 15
COMPOSITION OF CARNAUBA WAX^a

Component	Formula	M.p., °C.	Per cent
<i>Alkyl esters of wax acids</i>			80–81
Myricyl carnaubate.....	C ₃₀ H ₆₁ O·OCC ₂₃ H ₄₇	78	—
Myricyl cerotate.....	C ₃₀ H ₆₁ O·OCC ₂₅ H ₅₁	87	75
Ceryl cerotate.....	C ₂₇ H ₅₅ O·OCC ₂₅ H ₅₁	84	1
Ceryl octacosanate.....	C ₂₇ H ₅₅ O·OCC ₂₇ H ₅₅	86	1
Ceryl ω-hydroxyuncosanate..	C ₂₇ H ₅₅ O·OCC ₂₀ H ₄₀ OH	84	—
<i>Free wax acids</i>			1–1.5
Montanic.....	C ₂₉ H ₅₈ O ₂ (C ₂₈ , C ₃₀)	83	—
Melissic.....	C ₃₁ H ₆₂ O ₂ (C ₃₀ , C ₃₂)	90	—
Lacceroic.....	C ₃₂ H ₆₄ O ₂	—	—
<i>Lactones</i>			3–5
ω-1-Lactone of medullic acid.	C ₁₉ H ₃₈ 	103.5	—
<i>Free monohydric alcohols</i>			9–10
Carboceryl.....	C ₂₇ H ₅₅ OH(C ₂₆ , C ₂₈)	80–82.5	—
Octacosyl.....	C ₂₈ H ₅₇ OH	83.2	—
Myricyl.....	C ₃₀ H ₆₁ OH	86.4–86.8	—
Lacceryl.....	C ₃₂ H ₆₃ OH	89.4	6
<i>Free polyhydric alcohols</i>			1–2
Dihydric pentacosanol.....	HOCH ₂ (CH ₂) ₂₃ CH ₂ OH	103.6	—
Oxyalcohol.....	?	—	—
<i>Hydrocarbons</i>			<1
Heptacosane.....	CH ₃ (CH ₂) ₂₅ CH ₃	59.2	—
<i>Resins</i>			3–4

^a Adapted from A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 104.

Carnauba wax is characterized by containing a number of alcohols and acids not found in other waxes. These include carboceryl alcohol (C₂₇), octacosanol (C₂₈),³⁴² triacontanol (C₃₀), 1-dotriacontanol (C₃₂),³⁴² and a C₂₅ dihydric alcohol. Carnaubic acid (C₂₄) derives its name from its origin in

³⁴² S. D. Koonce and J. B. Brown, *Oil & Soap*, 21, 231–234 (1944).

this product. A normal C_{27} acid was first thought to be present, but it is now believed to be a mixed dimer of C_{26} and C_{28} acids. A C_{27} hydrocarbon, heptacosane, and a ω -lactone have also been reported. A comparative study of its composition was made by Liebermann,³⁴³ and a more complete analysis was later carried out by Koonce and Brown.³⁴² Table 15 gives a summary of the composition of carnauba wax.

Carnauba wax is of considerable value as a "melting point booster" for other waxes. It is widely used in floor polishes, and there is no satisfactory substitute for it. When mixed with montan wax, it constitutes a satisfactory medium for recording phonograph records. It is used as a hardener in leather dressings, for candles, and in the manufacture of carbon papers. It is also employed as a constituent of shoe creams, in photographic films, in chalk, in matches, in soap, and in dry batteries.

b'. Palm Wax: Another type of wax is obtained from the lofty wax palm (*Cerorylon andicola* Humb.), which grows in the South American Andes. Instead of occurring on the leaves, this palm wax is found on the trunk of the tree in layers from one-sixth to one inch in thickness. About 25 pounds can be obtained from a single tree. Palm wax contains about two-thirds resin and one-third true wax, and its composition is reported to be quite similar to that of carnauba wax.

c'. Ouricuri Wax: The tall *Attalea* palm (*Scheelia martiana* (*Attalea excelsa*)), sometimes confused with the true cohune palm (*Orbignya cohune*), produces the so-called ouricuri wax found on the under surface of the leaves. It is also known as uricuri, uricury, oricury, and ouricoury wax.¹ A machine-processed product is reported to have a melting point of 87°C ., a saponification value of 110, and an iodine number of 17.2.¹ It is used as a substitute for carnauba wax in floor polishes, in shoe creams, and in other polishes. Another palm wax is that obtained from the dried leaves of the Madagascar raffia palm (*Raphia pedunculata* (*ruffia* Mart.)), which is called raffia wax.

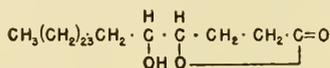
(b) *Waxes from Herbs and Shrubs.* a'. Candelilla Wax: The most important wax in the category of herbs and shrubs is that derived from a weed, *i.e.*, the candelilla. This is obtained from slipper flowers, or spurge, *Pedilanthus pavonis* Boiss. and *P. aphyllus*, both of which grow in the semi-arid regions of northern Mexico, Southern Texas, Arizona, and Southern California. The wax coats the entire surface of the shrubs, except for the root. Various samples of candelilla wax have been reported to melt between 64° and 71°C . Iodine numbers range between 13 and 37; saponification values from 46 to 67 have been noted.¹

Sanders³⁴⁴ found that the candelilla wax contains as much as 77% of

³⁴³ C. Liebermann, *Ber.*, 18, 1975-1983 (1885).

³⁴⁴ J. M. Sanders, *Proc. Chem. Soc.*, 27, 250 (1911). Cited by A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 116.

non-saponifiable material, of which the hydrocarbons comprise 48.6%. Hentriacontane, $\text{CH}_3(\text{CH}_2)_{29}\text{CH}_3$, occurs³⁴⁵ to the extent of 40%, while tritriacontane, $\text{CH}_3(\text{CH}_2)_{31}\text{CH}_3$, is likewise present.¹ Sitosterol, $\text{C}_{29}\text{H}_{49}\text{OH}$, is found combined with dihydroxymyricinoleic acid, $\text{C}_{30}\text{H}_{62}\text{O}_4$, to the extent³⁴⁶ of 20%, while a second ester is myricyl dihydroxymyricinoleate. A lactone, identified as δ -hydroxy- γ -myricinolactone, probably originates from dihydroxymyricinoleic acid, and is present to the extent of 5-6%.



δ -Hydroxy- γ -myricinolactone

Because of its high melting point, candelilla wax is employed to harden other waxes. Like carnauba wax, it is used in shoe polishes, floor pastes, for phonograph records, sealing wax, candles, electrical insulators, waterproof boxes, and in the textile industry.

b'. Other Related Waxes: Alcoocer wax is obtained from the plant stems of *Euphorbia cerifera* Alcoocer ("jerba"). It is almost identical with candelilla wax. Madagascar waxes are obtained from the stems of a wild desert plant, the swallow wort (*Cyanchum messeri* Aselep.), the flat-stem euphorbia (*Euphorbia xylophyloides* Brongn.), and the "rhimba" tree (*E. stenoclada*).³⁴⁷ The wax is especially hard (m.p., 88°C.); it possesses considerable luster, and includes the sterol sitosterol¹ in its composition.

Snow-brush wax can be obtained in large yield from the surface of the *Ceanothus velutinus* Douglas. It is widely distributed in California forests, and its waxy covering causes it to be a fire menace. It contains 5 to 6% of a triterpenoid, which is α - or β -amyrin isovalerate. Flax wax is present on the outer epidermal layer of *Linum usitatissimum* L. It contains the alcohols neoceryl, ceryl, and myricyl, a hydrocarbon triacontane, and the acids cerotic, stearic, palmitic, and linoleic.³⁴⁸

Cotton wax is obtained from the cotton fiber of *Gossypium* species. It contains the alcohols montanyl, $\text{C}_{28}\text{H}_{57}\text{OH}$, ceryl, carnaubyl, and one discovered by Fargher and Probert³⁴⁹ for which they proposed the name gossypyl alcohol. It has the formula $\text{C}_{30}\text{H}_{61}\text{OH}$; the γ -form has likewise been reported in the corms of the Indian jack-in-the-pulpit (*Arisaema triphyllum* (L.) Schott.). A number of acids, including palmitic, stearic, cerotic (C_{26}), montanic (C_{28}), gossypic (C_{30}), and a C_{34} acid, occur in the free

³⁴⁵ G. S. Fraps and L. R. Rafter, *Ind. Eng. Chem.*, 2, 454-455 (1910).

³⁴⁶ R. Berg, *Chem.-Ztg.*, 38, 1162-1163 (1914); *Chem. Abst.*, 9, 728 (1915).

³⁴⁷ A. Hébert and F. Heim, *Bull. l'office col.*, 8, No. 86, 96-101 (1915); *J. Soc. Chem. Ind.*, 34, 1061 (1915).

³⁴⁸ M. M. Chilikin and L. D. Kamolova, *Khim. Referat. Zhur.*, U. S. S. R., 1939, No. 6, 117; *Chem. Abst.*, 34, 3939 (1940).

³⁴⁹ R. G. Fargher and M. E. Probert, *J. Textile Inst.*, 14, 49-65T (1923); *Chem. Abst.*, 17, 1890-1891 (1923).

state. Sitosterol and stigmasterol are present, as well as several triterpenoids, α - and β -amyryns and lupeol.

Hemp wax from the fiber of *Cannabis sativa* L., broom wax from Scotch broom (*Cytisus scoparius* L.) and from Algerian or weaver's broom (*Spartium junceum* L.), Pisang wax from the leaves of the Pisang banana (*Musa simiarum* Rumphius), and tea wax from the leaves of several species of tea: *Camelia sinensis* L. (common tea), *C. japonica* L. (Japanese rose), or *C. sasanqua* Nois (*Sasanqua camelia*) are all well-known representatives of this group.

(c) *Waxes from Grasses and Sedges.* a'. Sugar-Cane Wax: Cane wax, also referred to in earlier terminology as cerosie, and known more recently as cerosin, is present on the periphery of the stem of the sugar cane (*Saccharum officinarum* L.). Various samples have been investigated, including East Indian, South African, Philippine, and Louisiana cane waxes; they are quite similar in properties and composition. Highly refined wax melts at 82°C. and solidifies at 80°C. The specific gravity has been reported as 0.961 at 10°C. It burns readily with a white flame, like spermaceti. It is partially soluble in 95% ethanol (5.9 grams per 100 milliliters) at 26°C. and much more so in hot ethanol. Ethylene dichloride dissolves it only sparingly at 37°C. (1.6 grams per 100 milliliters), as does cold ether. It is somewhat more soluble in boiling ether and is readily soluble in chloroform.

Mitsui³⁵⁰ has reported that stigmasterol constitutes 2% of the total wax of sugar cane and that sitosterol accounts for an additional 0.77%. Sitossterolin, or 22-dihydrostigmasterol, also, has been reported in small amounts. Related steroids are a diol (α -saccharostanediol)³⁵¹ and a ketone (β -saccharostenone). The composition of a semi-refined sugar-cane wax is given by Warth¹ as follows:

<i>Wax esters</i>		70-72%
Myricyl palmitate.....	20%	
Myricyl myricinate.....	1-2%	
Stigmasteryl palmitate.....	37-38%	
Phytosteryl dihydroxypalmitate.....	13%	
<i>Glycerides</i>		Present
<i>Free acids</i>		14%
Palmitic.....	Large amount	
Myricinic.....	Small amount	
Palmitoleic.....	Small amount	
<i>Alcohols</i>		12-13%
Monohydric (myricyl).....	8%	

³⁵⁰ T. Mitsui (Mitui), *J. Agr. Chem. Soc. Japan*, 13, 494-501 (1937); 14, 342-348 (1938); *Chem. Abst.*, 31, 8240 (1937); 32, 6254-6255 (1938).

³⁵¹ T. Mitsui (Mitui), *J. Agr. Chem. Soc. Japan*, 15, 356, 526-530 (1939); *Bull. Agr. Chem. Soc. Japan*, 16, 144-145 (1940); *Chem. Abst.*, 34, 383-384 (1940); 35, 4390-4391 (1941).

Cyclic and derivatives		
Sitosterol.....	0.8%	
α -Saccharostanediol.....	4%	
β -Saccharostenone.....	0.1-0.2%	
Hydrocarbons.....		3-5%
Hentriacontane.....	1.5-3%	
Pentatriacontane.....	1.5-2%	

Cane wax finds application in the polish and electrical industries³⁵² and as a replacement for carnauba wax, beeswax, and montan wax.

b'. Other Related Waxes: Mountain bamboo leaf wax, obtained from *Sasa (Arundinaria) paniculata* Makino, is a hard, brittle wax obtained from Japan. It contains myricyl alcohol and myricinic acid, but no appreciable amount of sterols.³⁵³ Esparto wax is a product of esparto-grass or needle-grass, which grows in Libya and North Africa. Two varieties contain appreciable amounts of wax, *Stipa tenacissima* L. and *Lygeum spartium* L. They are dewaxed prior to use in the manufacture of paper. This is a high-melting wax (78°C.). It contains the usual wax acids and alcohols. Esparto is one of the best substitutes for carnauba wax. Fiber wax is the name of a somewhat similar product made from grass straw in Spain. Lachryma wax is also similar to esparto wax; it is obtained from a Japanese and East Indian grass called Job's tears (*Coix lachryma-jobi* L.). The waxes from many other leaf blades have been studied, such as cocksfoot or orchard grass (*Dactylis glomerata* L.), wheat (*Triticum aestivum* L.), lucerne leaf (*Medicago sativa* L.), henequen (*Agave fourcroydes*), and the related sisal-hemp (*A. sisalana* Mill.).

(d) *Waxes from Leaves of Broad-Leaf Trees.* The principal source of wax in this category is from certain species of the eucalyptus. A wax melting at 55-56°C. has been isolated from the "red gum" or cider eucalyptus of Tasmania (*Eucalyptus gunni acervula* Hook.) which contains principally the hydrocarbons, heptacosane and nonacosane.

The leaves of the white sandalwood (*Santalum album* L.) are another source of wax. They contain 44% of a ketone, palmitone, as well as 6% of a hydroxy-ketone, *d*-10-hydroxypalmitone.³⁵⁴ Other members of the *Santalales* family which have waxes are the Pacific-American mistletoe, *Phoradendron villosum*, and the balanophoraceae.

(e) *Waxes from Leaves of Narrow-Leaf Trees.* The waxes obtained from the conifers have been investigated by Bougault and Bourdier.³⁵⁵ The acids present were hydroxy-acids, which combined with each other in ester linkage to form etholides (see page 27). Juniperic acid (C₁₆H₃₂O₃) is present

³⁵² M. Rindle, *S. African J. Ind.*, 5, 513-518 (1922); *Chem. Abst.*, 17, 646-647 (1923).

³⁵³ M. Tuzimoto, *J. Soc. Chem. Ind. Japan*, 42, suppl., 396 (1939); *Chem. Abst.*, 34, 2197 (1940).

³⁵⁴ A. C. Chibnall, S. H. Piper, H. A. el Mangouri, E. F. Williams, and A. V. V. Iyengar, *Biochem. J.*, 31, 1981-1986 (1937).

³⁵⁵ J. Bougault and L. Bourdier, *Compt. rend.*, 147, 1311-1314 (1908).

not only in the savin shrub (*Juniperus sabina*), but also in the Norway spruce (*Picea abies (excelsa)* Wall.) and the Northern white cedar or arborvitae (*Thuja occidentalis*). Another hydroxy-acid, sabinic, is an isomer of hydroxylauric acid ($C_{12}H_{24}O_3$). Sitosterol occurs in the wax of the slash pine (*Pinus caribaea* Morelet) while the wax of the white pine (*P. strobus* L.) contains an ester of 17-ketohexatriacontanol and 11-ketotriacontanoic acid.

(f) *Waxes from Roots and Rhizomes.*—A number of waxes have been isolated from roots and similar structures. That from the root of the common dandelion (*Taraxacum officinale* Weber or *Leontodon taraxacum* L.) has been shown to contain the sterols stigmasterol and β -sitosterol. In the case of alkanet root (*Anchusa tinctoria* Lam.), Betrabet and Chakravorti¹⁴ have demonstrated that the wax is composed chiefly of carnaubyl cerotate.

(g) *Waxes from Barks.* Most barks contain waxes, but generally the quantities are quite small. The Douglas fir bark (*Pseudotsuga taxifolia* Britt.) is apparently an exception, as fairly large quantities of wax can be prepared from the bark. Many bark waxes have been shown to contain considerable quantities of the phytosterols. The components which have been identified in some of the tree-bark waxes are listed in Table 16.

TABLE 16
COMPOSITION OF SOME TREE BARK WAXES

Sources of bark waxes	M.P., °C.	Components
Privet tree bark ^a <i>Ligustrum vulgare</i> L.	—	Ceryl palmitate; palmitic, arachidic, and behenic acid esters, phytosterol, ceryl alcohol, $C_{26}H_{44}O$ or $C_{25}H_{42}O$ alcohol, platanolic acid
Red beech bark ^b <i>Fagus sylvatica</i> L.	64	Ceryl alcohol, arachidyl alcohol, phytosterol, carnaubic acid
Cork tree (oak) ^{c,d} <i>Quercus suber</i>	—	Cerin, ^{e,d} friedelin (ketone, $C_{30}H_{50}O$) ^d , phytosterol, ^a phellonic acid (22-hydroxy-tetracosanic acid), ^e suberinic acid (ricinoleic), ^e phloionic acid ^e
Douglas fir bark ^f <i>Pseudotsuga taxifolia</i> Lamb.	63	Phytosterol, melissic acid
Oleander bark (sweet-scented) ^f <i>Nerium indicum</i> Aiton	97	Tetraatriacontane, cocccic acid, carnaubyl alcohol
Ocotillo or candlewood ^f <i>Fouquieria splendens</i> Englemann	84	Melissyl alcohol, cerotic acid
Plane tree or sycamore maple ^a <i>Acer pseudoplatanus</i> L.	—	Ceryl alcohol, phytosterol, palmitic acid, stearic acid
Hawthorn ^a <i>Crataegus oxyacantha</i> L.	—	Ceryl alcohol, phytosterol, stearic acid, palmitic acid
English elm ^a <i>Ulmus procera</i> Salisb.	—	Phytosterol, other unidentified alcohols

^a J. Zellner, *Monatsh.*, 46, 309–331 (1925); 47, 151–177; 659–679 (1926).

^b S. Clotofski, H. Weikert, and H. Nick, *Ber.*, B74, 299–307 (1941).

^c F. Scurti and G. Tammasi, *Gazz. chim. ital*, 46, II, 159–168 (1916); *Chem. Abst.*, 11, 1157 (1917).

^d N. L. Drake and R. P. Jacobsen, *J. Am. Chem. Soc.*, 57, 1570–1574 (1935).

^e N. L. Drake, H. W. Carhart, and R. Mozingo, *J. Am. Chem. Soc.*, 63, 617–620 (1941).

^f A. H. Warth, *The Chemistry and Technology of the Waxes*, Reinhold, New York, 1947.

(h) *Waxes from Fruits and Berries.* a'. Japan Wax: The fat contained, in the form of a greenish coating, between the kernel and the outer skin of the berries of the small sumac-like wax tree, *Toxicodendron succedaneum* (*Rhus succedanea* L.) yields a wax which is known as Japan wax. The berries are about the size of navy beans, and are produced in amounts as high as 150 pounds per year in an adult tree. *T. succedaneum* is cultivated in both Japan and China as a source of the wax. The crude wax melts at about 51°C., although a recently fused wax may melt at a lower temperature. It solidifies at 41°C. On standing, the melting point gradually increases. Warth¹ states that it has a specific gravity at 15°C. of 0.990, a saponification value of 206.6–237.5, and an iodine number of 4.5 to 12.8. The approximate composition of Japan wax is given in Table 17.

TABLE 17
COMPOSITION OF JAPAN WAX^a

Component	Formula	Per cent
<i>Triglycerides of monobasic acids</i>		87
Arachidic	CH ₃ (CH ₂) ₁₈ COOH	1
Palmitic	CH ₃ (CH ₂) ₁₄ COOH	71
Stearic	CH ₃ (CH ₂) ₁₆ COOH	4
Oleic and Linoleic	CH ₃ (CH ₂) ₇ CH:CH(CH ₂) ₇ COOH } CH ₃ (CH ₂) ₂ CH:CHCH ₂ CH:- } CH(CH ₂) ₇ COOH }	11
<i>Dibasic acids (Japanic acids)</i>		5.2–7.1
(Combined as mixed triglycerides with oleic acid)		
Nonadecanedioic	HOOC·(CH ₂) ₁₇ ·COOH	—
Eicosanedioic	HOOC·(CH ₂) ₁₈ ·COOH	—
Japanic	HOOC·(CH ₂) ₁₉ ·COOH	—
Docosanedioic	HOOC·(CH ₂) ₂₀ ·COOH	—
Tricosanedioic	HOOC·(CH ₂) ₂₁ ·COOH	4–6
<i>Free monobasic acids</i>		3–5
Pelargonic	CH ₃ (CH ₂) ₇ COOH	—
Palmitic	CH ₃ (CH ₂) ₁₄ COOH	—
Oleic	CH ₃ (CH ₂) ₇ CH:CH(CH ₂) ₇ COOH	—
<i>Free monohydric alcohols</i>		1–2
Pelargonyl	CH ₃ (CH ₂) ₇ CH ₂ OH	—
Ceryl	CH ₃ (CH ₂) ₂₄ CH ₂ OH	—
Myricyl	CH ₃ (CH ₂) ₂₈ CH ₂ OH	—
<i>Sterols</i>		<1
Phytosterol	—	—

^a Data adapted from A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1947, p. 155.

Japan wax finds wide application in Japan in the manufacture of candles. It is also used in the vulcanization of rubber, as well as for the preparation

of pomades, polishes, and leather dressings. Because of its high triglyceride content, it is used in the manufacture of soap.

b'. Apple Cuticle Wax: The wax from the cuticle of the apple, *Malus pumila* (*Pyrus malus* L.) is one of the best known fruit waxes. According to Sando,³⁵⁶ the proportion of wax obtained varies with the species of apple. One hundred grams of fresh tissue yields 140 to 175 milligrams of wax, in the form of a greenish yellow powder which is extremely water-repellent and which melts at 80°C.

The composition of apple wax has been extensively studied by Markley and collaborators³² as well as by Chibnall and his co-workers.²² The principal hydrocarbon was proved, by Markley *et al.*,³² to be *n*-nonacosane, $\text{CH}_3(\text{CH}_2)_{27}\text{CH}_3$, instead of triacontane, $\text{C}_{30}\text{H}_{62}$, which had previously been reported.^{356,357} The hydrocarbon was identified by its x-ray diffraction pattern, which corresponded with that reported by Piper and collaborators,³⁵⁸ and by its melting point.^{22,359} Heptacosane, $\text{C}_{27}\text{H}_{56}$, has likewise been reported as a hydrocarbon in apple wax.

In addition to the hydrocarbons, several alcohols have been found in apple cuticle wax. The chief one is apparently a secondary alcohol,^{22,32} 10-nonacosanol, $\text{CH}_3(\text{CH}_2)_{18}\text{CHOH}(\text{CH}_2)_8\text{CH}_3$, instead of 14-heptacosanol as previously reported by Sando.³⁵⁶ The confusion apparently arose because the two alcohols have the same melting points as do their acetate esters. Octacosanol and triacontanol have also been reported as minor constituent alcohols. α -Ursolic acid, $\text{HOC}_{29}\text{H}_{46}\text{COOH}$, has likewise been found as an important constituent of apple cuticle wax.

c'. Pear Cuticle Wax: Markley and collaborators³⁶⁰ reported a comprehensive investigation of the wax from the skin of the common pear (*Pyrus communis* L.). As in the apple, the predominant ether-soluble component was found to be *n*-nonacosane. The usual C_{20} - C_{20} primary alcohols, including those usually designated as lignoceryl and ceryl, were found. Ursolic acid was identified by its elementary composition, melting point, and by its oxidation product, ursonic acid, $\text{C}_{28}\text{H}_{45}\cdot\text{CO}\cdot\text{COOH}$. However, neither 10-nonacosanol nor any other secondary alcohols were found in pear wax, in contradistinction to apple cuticle wax.

d'. Waxes from the Cherry: Markley and Sando³⁶¹ have investigated the composition of the wax obtained from the skins of Bing sweet cherries (*Prunus avium* L.). In addition to such acids as palmitic, stearic, oleic,

³⁵⁶ C. E. Sando, *J. Biol. Chem.*, **56**, 457-468 (1923).

³⁵⁷ F. B. Power and V. K. Chestnut, *J. Am. Chem. Soc.*, **42**, 1509-1526 (1920).

³⁵⁸ S. H. Piper, A. C. Chibnall, S. J. Hopkins, A. Pollard, J. A. B. Smith, and E. F. Williams, *Biochem. J.*, **25**, 2072-2094 (1931).

³⁵⁹ K. S. Markley and C. E. Sando, *J. Biol. Chem.*, **101**, 431 (1933).

³⁶⁰ K. S. Markley, S. B. Hendricks, and C. E. Sando, *J. Biol. Chem.*, **111**, 133-146 (1935).

³⁶¹ K. S. Markley and C. E. Sando, *J. Biol. Chem.*, **119**, 641-645 (1937).

and linoleic, an acid higher than C_{18} was present. The only hydrocarbons found were nonacosane and one with a longer chain length, while *d*-glucosidylsitosterol and ursolic acid were likewise identified. The low content of waxes as compared with that in pears and apples may explain the less efficient protection afforded by the surface coating of the cherry.

Power and Moore³⁶² have studied the composition of a closely allied member of the *Prunus* family, *P. serotina* Ehrh. (wild black cherry). These workers have reported the hydrocarbons hentriacontane, $C_{31}H_{64}$, and pentatriacontane, $C_{35}H_{72}$, ceryl alcohol, and palmitic, stearic, linoleic, isolinoleic, and ursolic acids.

e'. Other Fruit Waxes: Grape pomace wax obtained from Concord grapes (*Vitis labruscana* Bailey) has been shown to contain³⁶³ nonacosane, and hentriacontane, $CH_3(CH_2)_{29}CH_3$, sitosterol, and oleanolic acid, $C_{30}H_{48}O_3$. The last compound occurs in place of ursolic acid, which is present in fairly large amounts in the cuticle waxes of the apple and pear.

Cranberry wax is derived from the skins of the *Vaccinium oxycoccus* or *V. macrocarpum* Aiton, which are the usual types of edible cranberry (small and large, respectively). The wax serves as a water repellent and is present to the extent of 0.15%. Markley and Sando³⁶⁴ have analyzed the ether-soluble constituents of cranberry pomace. In addition to nonacosane, $C_{29}H_{60}$, and hentriacontane, $C_{31}H_{64}$, the solid acids of the C_{16} to C_{26} series were found, together with oleic, linoleic, and linolenic acid. Free ursolic acid was likewise identified.

A number of citrus waxes have been investigated. In general they are soft and have low melting points. They may occur in the pulp as well as in the peel. The constituents of the wax of the Florida grapefruit (*Citrus paradisi*) include nonacosane, $C_{29}H_{60}$, and hentriacontane, $C_{31}H_{64}$, phytosterol, $C_{28}H_{47}OH$, oleic, linoleic, and linolenic acids, and a C_{32} saturated acid, a triterpene ketone, $C_{30}H_{52}CO$, and a new product, $C_9H_6O_3$, called umbelliferone.³⁶⁵

(i) *Seed Waxes*. Most seeds contain waxes in the hulls, but they are found mixed with the triglyceride oils when the oil is expressed or extracted. The waxes occur in smaller quantities than do the triglycerides. Sunflowerseed wax³⁶⁶ (*Helianthus annuus* L.) contains chiefly ceryl cerotate, while corn wax from *Zea mays* L. is composed of hentriacontane, myricyl tetracosanate and myricyl isobehenate, sitosterol, and stigmasterol.

³⁶² F. B. Power and C. W. Moore, *J. Chem. Soc.*, 97, 1099-1112 (1910); *Proc. Chem. Soc.*, 26, 124 (1910); *Chem. Abst.*, 4, 2182 (1910).

³⁶³ K. S. Markley, C. E. Sando, and S. B. Hendricks, *J. Biol. Chem.*, 123, 641-654 (1938).

³⁶⁴ K. S. Markley and C. E. Sando, *J. Biol. Chem.*, 105, 643-653 (1934).

³⁶⁵ K. S. Markley, E. K. Nelson, and M. S. Sherman, *J. Biol. Chem.*, 118, 433-441 (1937).

³⁶⁶ A. Barenther, *Chem. Umschau Fette, Öle, Wachse Harze*, 30, 117-119 (1923); *Chem. Abst.*, 17, 2790 (1923).

Soybean wax (*Glycine soja*), celery seed (*Apium graveolens* L.), and sesame seed (*Sesamum indicum* L.) all contain waxes, which have been studied.

a'. Jojoba Wax: The jojoba seeds or goat-nut (*Simmondsia chinensis californica*) Nutt.) are unique in having as their lipid component an oil which has a composition closely resembling that of wax. The seeds are produced by an evergreen shrub which grows in Western Mexico, Arizona, and California. The jojoba nuts, which are also called "goat nuts," contain more than 50% of oil.³⁶⁷ It has a density of 0.8642 at 25°C., a refractive index at the same temperature of 1.4648, an iodine value (Hanus) of 81.7, and a saponification number of 92.2.² The chemical composition is reported¹ as follows: saturated acids, 1.64%; palmitoleic acid, 0.24%; oleic acid, 0.66%; eicosenoic acid, 30.3%; docosenoic (erucic) acid, 14.2%; eicosenol, 14.6%; docosenol, 33.7%; and hexacosenol, 2.0%. The chief acid has been identified as 11-eicosenoic acid, by Green, Hilditch, and Stainsby,⁴⁷ while the principal alcohols are 11-eicosenol and 13-docosenol. 11,12-Dihydroxyeicosenoic acid has also been reported as a constituent of jojoba wax.

(j) Flower Waxes. Wax-like extracts have been prepared from a variety of flowers. Rogerson³⁶⁸ was able to identify hentriacontane, C₃₁H₆₄, incarnatyl alcohol, C₃₄H₆₉OH, a phytosterol "trifolianol," and palmitic, stearic, oleic, linoleic, and isolinolenic acids from the wax of the crimson clover flower (*Trifolium incarnatum* L.). The waxes of roses, violets (*Viola odorata* L.), acacia and silver-green wattle flowers (*Acacia cavenia* and *A. decurrens* var. *dealbata*), the grape hyacinth flower (*Muscari botryoides* Mill.), the jasmine flower (*Jasminum odoratissimum* L.), and other flowers have been investigated.¹

d. Waxes from Microorganisms. Tubercle bacilli contain a large proportion of waxy materials, which have been extensively investigated. In 1901 Kresling³⁶⁹ was the first to demonstrate the high proportion of alcohols in the lipid extracts of tubercle bacilli. The principal acids are mycolic and the forked-chain compounds tuberculostearic and phthioic³⁰⁰ acids. The normal saturated fatty acids, palmitic, stearic,³⁰⁰ and hexacosanoic acids occur, as well as some unsaturated acids which yield hexacosanoic acid on hydrogenation. For a discussion of these acids, see Chapter II. All tubercle wax fractions contain an alcohol, phthiocerol, C₃₅H₇₂O₃.

2. Triterpenes

The triterpenes are a group of plant constituents which contain 30 carbon atoms. They may occur as the free triterpene or combined with sugars in glucoside linkage to form a saponin. The aglycones formed on hydrolysis of

³⁶⁷ L. N. Markwood, *U. S. Dept. Commerce, Domestic Commerce*, 30, No. 11, 20-21 (Sept. 10, 1942).

³⁶⁸ H. Rogerson, *J. Chem. Soc.*, 97, 1004-1015 (1910); *Proc. Chem. Soc.*, 26, 112 (1910).

³⁶⁹ H. Kresling, *Zentr. Bakt. Parasitenk.*, 1, 30, 897-909 (1901).

TABLE 18
EMPIRICAL FORMULAS AND SOURCES OF SOME TRITERPENES^a

Name	Formula	Source
Agnol (agnosterol)	C ₃₀ H ₄₈ O	Wool fat ^b
α -Amyrin	C ₃₀ H ₅₀ O	Manila elemi resin from Java almond (<i>Canarium commune</i>), and resin and latex of many other plants ^c ; shea nut oil (<i>Butyrospermum parkii</i>) ^{d,e}
β -Amyrin	C ₃₀ H ₅₀ O	Accompanies α -amyrin
Basseol	C ₃₀ H ₅₀ O	Shea nut oil (<i>Butyrospermum parkii</i>) ^{d,e}
Betulin	C ₃₀ H ₅₀ O ₂	Pigment of white birch bark (<i>Betula pendula alba</i>) ^f
α -Boswellic acid	C ₃₀ H ₄₈ O ₃	As acetate in olibanum, or Bible frankincense resin (<i>Boswellia carteri</i>) ^g
β -Boswellic acid	C ₃₀ H ₄₈ O ₃	Accompanies α -boswellic acid
Cryptol (cryptosterol)	C ₃₀ H ₅₀ O	Yeast (<i>Saccharomyces</i>) ^h
Echinocystic acid	C ₃₀ H ₄₈ O ₄	As saponin in California big-root, or man-root (<i>Megarrhiza californica</i> or <i>Echinocystis fabacea</i>) ⁱ
Elemadienolic acid (elemic, α -elemolic)	C ₃₀ H ₄₈ O ₃	Manila elemi resin (<i>Canarium commune</i>) ^j
Elemadienonic acid (α -elemic, β -elemonic)	C ₃₀ H ₄₆ O ₃	Accompanies elemadienolic acid
ErythrodioI	C ₃₀ H ₅₀ O ₂	As monostearate in fruit of the cocaine tree (<i>Erythroxylum novoguianense</i>) ^k
Glycyrrhetic acid	C ₃₀ H ₄₆ O ₄	As saponin in licorice root (<i>Glycyrrhiza glabra</i>) ^l
Gypsogenin	C ₃₀ H ₄₆ O ₄	As saponin in wild soapwort (<i>Gypsophila</i>) ^m ; in Fuller's herb or "bouncing Bet" (<i>Saponaria officinalis</i>) ⁿ
Hederagenin	C ₃₀ H ₄₈ O ₄	As saponin in ivy leaves (<i>Hedera helix</i>) ^o ; soapberry (<i>Sapindus saponaria</i>) ^p ; leaves of the Chinese angelica tree (<i>Fatsia japonica</i>) and of the ornamental tree (<i>Kalopanax pictus</i> (<i>riciniifolius</i>)) ^q
Lanol (lanosterol)	C ₃₀ H ₅₀ O	Wool fat ^b ; yeast ^r
Lupeol	C ₃₀ H ₅₀ O	With amyris in bresk (gutta-percha-like material from <i>Alstonia costulata</i>) ^s ; shea nut oil (<i>Butyrospermum parkii</i>) ^d
Oleanolic acid	C ₃₀ H ₄₈ O ₃	As saponin in guaiac bark or lignumvitae (<i>Guaiacum officinale</i>) ^t ; sugar beet (<i>Beta vulgaris</i> (<i>alba</i>)) ^u ; calendula flowers (<i>Calendula officinalis</i>) ^v ; leaves of the angelica (<i>Fatsia japonica</i>) ^q and of <i>Kalopanax pictus</i> ^q
		Free in olive leaves (<i>Olea europaea</i>) ^w ; clove buds (<i>Syzygium aromaticum</i> or <i>Eugenia aromatica</i>) ^x ; mistletoe leaves (<i>Viscum album</i>) ^y ; grape skins (<i>Vitis</i>) ^r

Name	Formula	Source
Quillaic acid	$C_{30}H_{46}O_3$	As saponin in soap bark (<i>Quillaja saponaria</i>) ^{aa}
Quinovic acid	$C_{30}H_{46}O_3$	As glycoside in quinine (<i>Cinchona</i>) ^{ab}
Stearinolic acid	$C_{30}H_{48}O_4$	Stiam gum benzoin from the Siam snowbell (<i>Styrax benzoides</i>) ^{ac}
Sumaresinolic acid	$C_{30}H_{48}O_4$	Sumatra gum benzoin from the Sumatra snowbell (<i>Styrax benzoin</i>) ^{ad}
Ursolic acid	$C_{30}H_{48}O_3$	Widely distributed in wax-like coatings of leaves and fruits as apple, cherry, bearberry (<i>Arctostaphylos uva-ursi</i>), ^{ae} and cranberry (<i>Vaccinium</i> spp.) ^{af}
		^a C. R. Noller, <i>Ann. Rev. Biochem.</i> , 14 , 383-406 (1945).
		^b A. Windaus and R. Tschesche, <i>Z. physiol. Chem.</i> , 190 , 51-61 (1930).
		^c K. A. Vesterberg, <i>Ann.</i> , 428 , 243-246 (1922).
		^d I. M. Heilbron, G. L. Moffet, and F. S. Spring, <i>J. Chem. Soc.</i> , 1934 , 1583-1585.
		^e K. H. Bauer and H. Moll, <i>Fette u. Seifen</i> , 46 , 560-563 (1939); <i>Chem. Abst.</i> , 35 , 2350 (1941).
		^f K. A. Vesterberg, <i>Ber.</i> , 56 , 845 (1923).
		^g A. Winterstein and G. Stein, <i>Z. physiol. Chem.</i> , 199 , 64-74 (1931); 208 , 9-25 (1932).
		^h H. Wieland, H. Pasodach, and A. Ballauf, <i>Ann.</i> , 529 , 68-83 (1937).
		ⁱ I. Bersteinsson and C. R. Noller, <i>J. Am. Chem. Soc.</i> , 56 , 1403-1405 (1934).
		^j L. Ruzicka, E. Eichenberger, M. Furter, M. W. Goldberg, and R. L. Wakeman, <i>Helv. Chim. Acta</i> , 15 , 681-693 (1932).
		^k J. Zimmermann, <i>Rec. trav. chim.</i> , 51 , 1200-1203 (1932).
		^l L. Ruzicka and H. Leuenberger, <i>Helv. Chim. Acta</i> , 19 , 1402-1406 (1936).
		^m L. Ruzicka and G. Giacomello, <i>Helv. Chim. Acta</i> , 19 , 1136-1140 (1936).
		ⁿ G. A. R. Kon and H. R. Soper, <i>J. Chem. Soc.</i> , 1940 , 617-620.
		^o A. W. Van der Haar, <i>Arch. pharm.</i> , 250 , 424-435 (1912).
		^p W. A. Jacobs, <i>J. Biol. Chem.</i> , 63 , 621-629 (1925).
		^q A. Winterstein and G. Stein, <i>Z. physiol. Chem.</i> , 211 , 5-18 (1932).
		^r H. Wieland and W. Benend, <i>Z. physiol. Chem.</i> , 274 , 215-222 (1942).
		^s N. H. Cohen, <i>Rec. trav. chim.</i> , 28 , 368-390 (1909).
		^t E. Wedekind and W. Schlicke, <i>Z. physiol. Chem.</i> , 198 , 181-184 (1931).
		^u A. W. Van der Haar, <i>Rec. trav. chim.</i> , 46 , 775-792 (1927).
		^v Z. Kitasato and C. Sone, <i>Acta Phytochim. Japan</i> , 6 , 179-225 (1932).
		^w F. B. Pover and F. Tutin, <i>J. Chem. Soc.</i> , 93 , 891-904 (1908).
		^x F. D. Dodge, <i>J. Am. Chem. Soc.</i> , 40 , 1917-1939 (1918).
		^y A. Winterstein and W. Hämmerle, <i>Z. physiol. Chem.</i> , 199 , 56-64 (1931).
		^z K. S. Markley, C. E. Sando, and S. B. Hendricks, <i>J. Biol. Chem.</i> , 123 , 641-654 (1938).
		^{aa} D. F. Elliott and G. A. R. Kon, <i>J. Chem. Soc.</i> , 1939 , 1130-1135.
		^{ab} H. Wieland and M. Furter, <i>Helv. Chim. Acta</i> , 15 , 472-482 (1932).
		^{ac} L. Ruzicka and M. Zinke, <i>Monatsh.</i> , 39 , 219-230 (1918).
		^{ad} H. Lieb and A. Zinke, <i>Monatsh.</i> , 39 , 219-230 (1918).
		^{ae} C. E. Sando, <i>J. Biol. Chem.</i> , 90 , 477-495 (1931).
		^{af} K. S. Markley and C. E. Sando, <i>J. Biol. Chem.</i> , 105 , 643-653 (1934).

TABLE 19
EMPIRICAL FORMULAS AND SOURCES OF SOME PROBABLE TRITERPENES^a

Name	Formula	Source
Amriol	$C_{30}H_{50}O_2$	Flowers of mountain arnica (<i>Arnica montana</i>), colt's foot (<i>Trussilago farfara</i>), sunflower (<i>Helianthus annuus</i>) ^b
Basic acid	$C_{30}H_{46}O_5$	As saponin in mowrah meal from the Indian mowrah butter-tree (<i>Madhuca longifolia</i>) and shea nut press cake (<i>Butyrospermum parkii</i>) ^c and seeds of other <i>Sapotaceae</i> ; the saponilla (<i>Achras zapota</i>), rivan seed from the evergreen tree (<i>Mimusops hexandra</i>), <i>Dumoria heckeli</i> Pierre, and <i>Pajena lucida</i> ^d
Breïn	$C_{30}H_{50}O_2$	Manila elemi resin from the Java almond (<i>Canarium commune</i>) ^e
Caoutchicol	$C_{30}H_{50}O$	Jelutong (acetone extract of crude rubber) ^f
Cerin	$C_{30}H_{50}O_2$	Cork (<i>Quercus suber</i>) ^g
Faradiol	$C_{30}H_{50}O_2$	Accompanics amidiol ^b
Friedelin	$C_{30}H_{50}O$	Accompanics cerin ^g
Germanicol	$C_{30}H_{50}O$	Dried latex of lettuce opium (<i>Lactuca virosa</i>) ^h
Gratiolone	$C_{30}H_{44}O_3$	Hedge hyssop (<i>Gratiola officinalis</i>) ⁱ
Mamilladiol	$C_{30}H_{50}O_2$	Accompanics breïn from Manila elemi from Java almond ^e
Onocerin	$C_{30}H_{43}O_2$	Roots of rest harrow (<i>Ononis spinosa</i>) ^j
Pachymic acid	$C_{30}H_{44}O_5$	Bukuryo or tuckshoe (<i>Poria cocos</i>) ^k
Parkeol	$C_{30}H_{50}O$	Shea nut butter (<i>Butyrospermum parkii</i>) ^l
Platygodigenin	$C_{30}H_{48}O_7$	As saponin in roots of the balloonflower (<i>Platycodon grandiflorum</i>) ^m
Senegenin	$C_{30}H_{46}O_3$	As saponin in roots of the Seneca snake root (<i>Polygala senega</i>) ⁿ
Skinmiol	$C_{30}H_{50}O$	The ornamental evergreen shrub (<i>Stimmia japonica</i>) ^o
Skimmione	$C_{30}H_{48}O$	" "
Soysapogenol-A	$C_{30}H_{50}O_4$	As saponins in soybeans ^p
Soysapogenol-(B + D)	$C_{30}H_{50}O_3$	" "
Soysapogenol-C	$C_{30}H_{50}O_2$	" "

Name	Formula	Source
Taraxol	C ₃₀ H ₄₆ O ₃	Dandelion roots (<i>Taraxacum officinale</i>) ^a
Taraxerol	C ₃₀ H ₅₀ O	" "
Taraxasterol	C ₃₀ H ₅₀ O	" "
ψ-Taraxasterol	C ₃₀ H ₅₀ O	" "
Vanguerigenin	C ₃₀ H ₄₆ O ₃	As saponin in voavanga (<i>Vangueria tomentosa</i>) ^r
Zeorin	C ₃₀ H ₅₂ O ₂	Lichens (<i>Parmelia leukotryliza</i>) ^s

^a C. R. Noller, *Ann. Rev. Biochem.*, **14**, 383-406 (1945).

^b J. Zimmermann, *Helv. Chim. Acta*, **26**, 642-647 (1943).

^c B. J. Heywood, G. A. R. Kon, and L. L. Ware, *J. Chem. Soc.*, **1939**, 1124-1129.

^d B. J. Heywood and G. A. R. Kon, *J. Chem. Soc.*, **1940**, 713-720.

^e I. M. Morice and J. C. E. Simpson, *J. Chem. Soc.*, **1942**, 198-203.

^f R. E. Marker and E. L. Whittle, *J. Am. Chem. Soc.*, **61**, 585-586 (1939).

^g N. L. Drake and R. P. Jacobsen, *J. Am. Chem. Soc.*, **57**, 1570-1574 (1935).

^h J. C. E. Simpson, *J. Chem. Soc.*, **1944**, 283-286.

ⁱ K. Maurer, K. Meier, and G. Reiff, *Ber.*, **72**, 1870-1873 (1939).

^j J. Zimmermann, *Helv. Chim. Acta*, **21**, 853-859 (1938); **23**, 1110-1113 (1940).

^k S. Nakamisi, M. Yamamoto, and H. Ikeda, *J. Pharm. Soc. Japan*, **59**, 725-729 (in English) 273-276 (1939); *Chem. Abst.*, **34**, 1025 (1940).

^l K. H. Bauer and H. Moll, *Fette u. Seifen*, **46**, 560-563 (1939); *Chem. Abst.*, **32**, 2350 (1941).

^m M. Tuzimoto and R. Senzuyu, *J. Agr. Chem. Soc. Japan*, **15**, 857-861, 862-864 (1939); *Chem. Abst.*, **34**, 767-768 (1940).

ⁿ W. A. Jacobs and O. Isler, *J. Biol. Chem.*, **119**, 155-170 (1937).

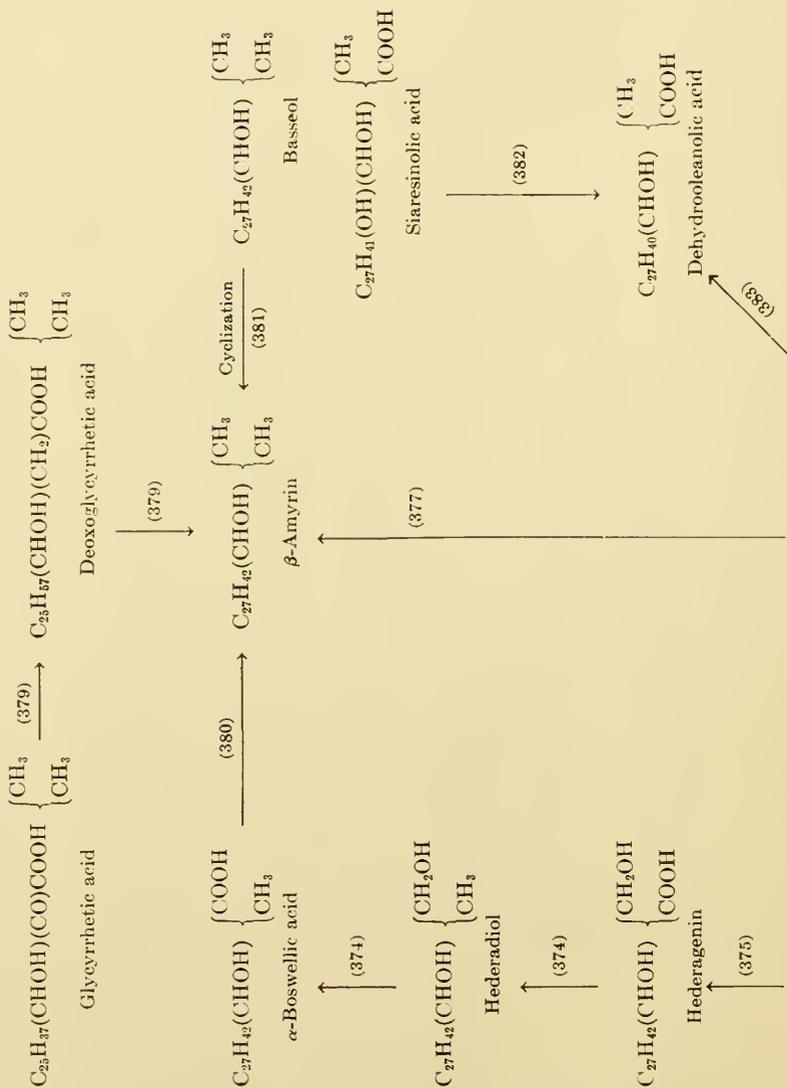
^o K. Takeda, *J. Pharm. Soc. Japan*, **61**, 63-65, 117-123 (1941); *Chem. Abst.*, **36**, 444 (1942).

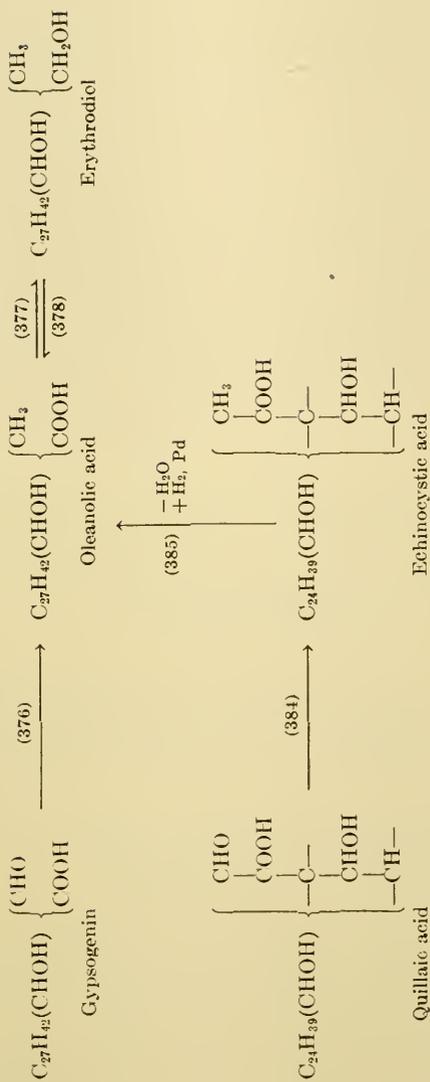
^p K. Tsuda and S. Kitagawa, *Ber.*, **71**, 790-797 (1938).

^q S. Burrows and J. C. E. Simpson, *J. Chem. Soc.*, **1938**, 2042-2047.

^r K. W. Merz and H. Tschubel, *Ber.*, **72**, 1017-1028 (1939).

^s Y. Asahina and H. Akagi, *Ber.*, **71**, 980-985 (1938).

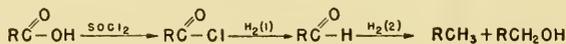


Fig. 1. Interconversions of the β -amyrin series of triterpenes.³⁷³

these saponins are frequently spoken of as triterpene sapogenins. Although practically all the triterpenes have been isolated only from plant sources, at least two of them, lanol (lanosterol) and agnol (agnosterol), which were formerly called isocholesterol, were first isolated from wool fat, where they are associated with the zoosterol cholesterol.²³¹ Lanol has also been isolated from yeast, in addition to another similar triterpene, cryptol (cryptosterol).³⁷⁰ The triterpenes have been obtained from all parts of different plants, but only in the sunflower have they been found in more than one part of a single plant.³⁷¹ Zimmermann³⁷² found that, whereas the steryl glycosides or hydrocarbons isolated from fruits and flowers are always the same, the triterpene is related to the type of pigment. Triterpendiols occur when carotenoid pigments are present, while the triterpene hydroxy-acids are present when the pigments are anthocyanins.

Noller,³⁷³ in a comprehensive review of the triterpenes, has listed the empirical formulas and sources of some triterpenes (see Tables 18 and 19).

In some instances the triterpenes are acids, and in other cases they are alcohols. The relationship between them can be demonstrated by the series of reactions given here, when a carboxyl group is converted to a methyl group:



(1) Rosemund reduction

(2) Wolff-Kishner reduction

The interrelation of the various triterpenes is indicated in Figure 1.

There appear to be five groups of triterpenes which have different basic structures. The acyclic group in which squalene is the only member is dis-

³⁷⁰ H. Wieland, H. Pasedach, and A. Ballauf, *Ann.*, 529, 68-83 (1937).

³⁷¹ J. Zimmermann, *Helv. Chim. Acta*, 26, 642-647 (1943).

³⁷² J. Zimmermann, *Helv. Chim. Acta.*, 27, 332-334 (1944).

³⁷³ C. R. Noller, *Ann. Rev. Biochem.*, 14, 383-406 (1945).

³⁷⁴ L. Ruzicka and A. Marxer, *Helv. Chim. Acta*, 23, 144-152 (1940).

³⁷⁵ L. Ruzicka and G. Giacomello, *Helv. Chim. Acta*, 20, 299-309 (1937).

³⁷⁶ L. Ruzicka and G. Giacomello, *Helv. Chim. Acta*, 19, 1136-1140 (1936).

³⁷⁷ L. Ruzicka and H. Schellenberg, *Helv. Chim. Acta*, 20, 1553-1556 (1937).

³⁷⁸ J. Zimmermann, *Helv. Chim. Acta*, 19, 247-253 (1936).

³⁷⁹ L. Ruzicka and A. Marxer, *Helv. Chim. Acta*, 22, 195-201 (1939).

³⁸⁰ L. Ruzicka and W. Wirz, *Helv. Chim. Acta*, 23, 132-135 (1940).

³⁸¹ J. H. Beynon, I. M. Heilbron, and F. S. Spring, *J. Chem. Soc.*, 1937, 989-991.

³⁸² P. Bilham, G. A. R. Kon, and W. C. J. Ross, *J. Chem. Soc.*, 1942, 540-544.

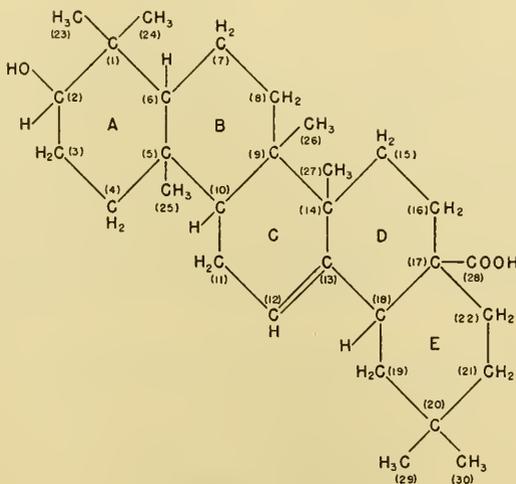
³⁸³ L. Ruzicka, A. Grob, and F. Van der Sluys-Veer, *Helv. Chim. Acta*, 22, 788-792 (1939).

³⁸⁴ D. F. Elliott, G. A. R. Kon, and H. R. Soper, *J. Chem. Soc.*, 1940, 612-617.

³⁸⁵ D. Frazier and C. R. Noller, *J. Am. Chem. Soc.*, 66, 1267-1268 (1944).

cussed under hydrocarbons. The β -amyrin group includes oleanolic acid, quillaic acid, α -boswellic acid, and a number of other members indicated in Figure 1. The third group is the α -amyrin group which includes ursolic acid³⁸⁶ and β -boswellic acid.³⁸⁷ The fourth group is centered on the monohydric terpene lupeol³⁸⁸; this group also includes betulin. The elemi acids, along with agnol (agnosterol), cryptol (cryptosterol), and lanol (lanosterol), form the last group.^{335, 389, 389a} The β - and α -amyrin groups, as well as luteol, are pentacyclic, while the elemi acids contain a tetracyclic group.

The proposed structure³⁹⁰ of the β -amyrin group is indicated by the structural formula for oleanolic acid, which has been strongly supported by Ruzicka *et al.*^{391, 392} The formula for α -amyrin closely resembles that of β -amyrin, but probably it is not merely a stereoisomer. However, both α - and β -amyrin give the same product on dehydrogenation with selenium.^{393, 394} Lupeol has the structure indicated here:



Oleanolic acid (a β -amyrin)

³⁸⁶ J. A. Goodson, *J. Chem. Soc.*, 1938, 999-1001.

³⁸⁷ L. Ruzicka and W. Wirz, *Helv. Chim. Acta*, 22, 948-951 (1939).

³⁸⁸ L. Ruzicka and M. Brenner, *Helv. Chim. Acta*, 22, 1523-1528 (1939).

³⁸⁹ L. Ruzicka, R. Denss, and O. Jeger, *Helv. Chim. Acta*, 28, 759-766 (1945).

^{389a} H. Schulze, *Z. physiol. Chem.*, 238, 35-53 (1936).

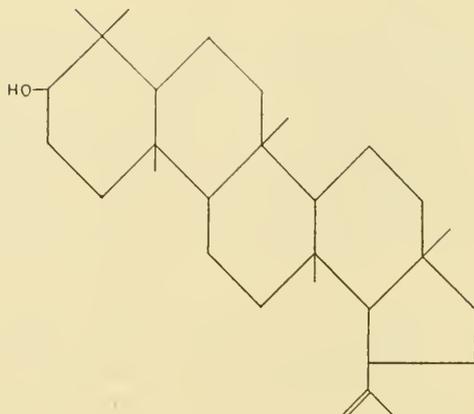
³⁹⁰ R. D. Haworth, *Ann. Repts Progress Chem., Chem. Soc. London*, 34, 327-342 (1937).

³⁹¹ L. Ruzicka, F. C. Van der Sluys-Veer, and O. Jeger, *Helv. Chim. Acta*, 26, 280-288 (1943).

³⁹² L. Ruzicka, O. Jeger, and W. Ingold, *Helv. Chim. Acta*, 26, 2278-2282 (1943).

³⁹³ O. Brunner, H. Hofer, and R. Stein, *Monatsh.*, 61, 293-298 (1932).

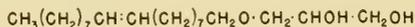
³⁹⁴ F. S. Spring and T. Vickerstaff, *J. Chem. Soc.*, 1937, 249-252.



Lupeol

3. Glyceryl Ethers

The glyceryl ethers consist of the three alcohols: batyl, chimyl, and selachyl. In these compounds, the component aliphatic alcohol (palmityl, stearyl, or oleyl) is combined with glycerol at the α -position in an ether linkage which forms a stable non-hydrolyzable union. Selachyl alcohol is found most abundantly in nature, while chimyl alcohol appears least frequently and in the smallest amount. Selachyl alcohol is the ether of oleyl alcohol and glycerol, while batyl alcohol can only be the corresponding stearyl ether, since it originates upon the hydrogenation of selachyl alcohol.



Selachyl alcohol



Batyl alcohol

Chimyl alcohol, $\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\cdot\text{O}\cdot\text{CH}_2\cdot\text{CHOH}\cdot\text{CH}_2\text{OH}$, is the lower homologue of batyl alcohol in which cetyl (palmityl) alcohol replaces the stearyl alcohol. Like the corresponding oleyl alcohol and triolein, selachyl alcohol is liquid at room temperature. Also in line with the melting points of cetyl and stearyl alcohols (as well as of tripalmitin and tristearin), chimyl and batyl alcohols have relatively high melting points (60–61.5°C. and 70–71°C., respectively).³⁹⁵

³⁹⁵ T. P. Hilditch, in H. Schönfeld, *Chemie und Technologie der Fette und Fettprodukte*, Vol. I, Springer, Vienna, 1936, pp. 60–110.

The glyceryl ethers were discovered by Tsujimoto and Toyama³⁹⁶ to be components of the non-saponifiable extract of liver oils of the elasmobranch fishes, such as the shark and the ray. Their presence in these oils was later confirmed by Nakamiya.³⁹⁷ Whereas the total non-saponifiable fraction is low in the liver fats of the skate (*Raia maculata*) and of several other fishes (2% and under, consisting mostly of cholesterol), Hilditch²⁸ points out the high content of this fraction (10%) in the liver oil of the grey dogfish (*Squalus acanthias*); in the sharks examined by Tsujimoto,³⁹⁸ the non-saponifiable portion consisted mainly of selachyl with some chimyl and batyl alcohols. Almost all of the non-saponifiable fraction of ratfish liver oil (*Chimaera monstrosa*), which amounts to 37% of the total fat,²⁸ consists of selachyl alcohol; small amounts of the other glyceryl ethers are also present. In the case of the shark, where the unsaponifiable matter totals anywhere from 50 to 80%, considerable amounts of selachyl, batyl, and chimyl alcohols are found, but the major component is squalene. However, the occurrence of the glyceryl ethers is not limited to the elasmobranch family, as they have been reported in the Japanese crab (*Paralithoides camtschatica* Tilesius).⁴⁶

The importance of batyl alcohol is further emphasized by recent discoveries of its wide distribution in nature. Kind and Bergmann³⁹⁹ have reported its presence in the unsaponifiable matter of *Plexaura flexuosa*, which is a reef-building gorgonia (coral). The compound referred to as "astrol," which was isolated from star fish by Kossel and Edbacher,²⁴⁰ has been shown to be batyl alcohol.⁴⁰⁰ Several recent reports have indicated the presence of batyl alcohol in higher animals. This glycerol ether has been isolated from the bone marrow of cattle,⁴⁰¹ the spleen of pigs,⁴⁰² and from arteriosclerotic arteries of human beings.⁴⁰³

The structure of the component alcohol groups was clarified by the demonstration of Toyama in 1924 that the correct empirical formula for batyl alcohol is $C_{21}H_{44}O_3$; that for chimyl alcohol was shown shortly thereafter to be $C_{19}H_{40}O_3$. Since Tsujimoto and Toyama had demonstrated that selachyl can be converted to batyl alcohol on hydrogenation,

³⁹⁶ M. Tsujimoto and Y. Toyama, *Chem. Umschau, Fette, Öle, Wachse Harze*, 29, 27-29, 35-37, 43-45 (1922). Y. Toyama, *ibid.*, 29, 237-240, 245-247 (1922); 31, 13-17, 61-67, 153-155 (1924).

³⁹⁷ Z. Nakamiya, *Bull. Inst. Phys. Chem. Research Tokyo*, 17, No. 11, 837-852 (1938); *Chem. Abst.*, 33, 8175-8176 (1939).

³⁹⁸ M. Tsujimoto, *Chem. Umschau, Fette, Öle, Wachse Harze*, 39, 50-52 (1931). Cited by T. P. Hilditch, *The Chemical Constitution of the Natural Fats*, 2nd ed., Wiley, New York, 1947; *J. Soc. Chem. Ind.*, 51, 317-323T (1932).

³⁹⁹ C. A. Kind and W. Bergmann, *J. Org. Chem.*, 7, 424-427 (1942).

⁴⁰⁰ W. Bergmann and H. A. Stansbury, *J. Org. Chem.*, 8, 283-284 (1943).

⁴⁰¹ H. N. Holmes, R. E. Corbet, W. B. Geiger, N. Kornblum, and W. Alexander, *J. Am. Chem. Soc.*, 63, 2607-2609 (1941).

⁴⁰² V. Prelog, L. Ruzicka, and P. Stein, *Heb. Chim. Acta*, 26, 2222-2242 (1943).

⁴⁰³ E. Hardegger, L. Ruzicka, and E. Tagmann, *Heb. Chim. Acta*, 26, 2205-21 (1943).

it was fairly simple to establish the formula for selachyl alcohol as $C_{21}H_{42}O_3$. Although Toyama proved that selachyl acetate gave rise to oleyl alcohol on dry distillation, the decision as to the nature of the third oxygen atom awaited the experiments of Weidemann.⁴⁰⁴ He found that hydriodic acid acts on batyl alcohol to produce methyl iodide. This proves that the third oxygen is present in a methoxy group, which fact indicates that an ether linkage exists between palmityl (cetyl) alcohol and the glycerol.

A number of experimental observations indicate that the connection between these higher alcohols and glycerol occurs through the α -hydroxyl group of the triatomic alcohol. In the first place, the proof was obtained by synthesis of the α -ether of glycerol and stearyl alcohol and by comparison of the synthetic with the natural product. To accomplish this, octadecyl iodide, $CH_3(CH_2)_{16}CH_2I$, was condensed with sodium allyl oxide to form the octadecylallyl iodide.⁴⁰⁵ The latter compound when oxidized with perhydrol gave α -octadecylglyceryl ether. This ether had the same melting point ($70-71^\circ C.$) as natural batyl alcohol. However, it did show a slight but definite depression in melting point when mixed with the natural product. That this does not indicate that the natural product is a β -ether was shown by the fact that the β -octadecyl glyceryl ether (m.p., $62-63^\circ C.$) showed a considerable depression in melting point when mixed with natural batyl alcohol.⁴⁰⁶ Similar variations were noted between the synthetic β -cetyl glyceryl ether and natural chimyl alcohol. The β -ethers were synthesized by a condensation of α , α' -benzylidene glycerol with octadecyl or cetyl iodide followed by hydrolysis of the resulting product. Further proof that natural glyceryl ethers were the α -forms was afforded by the demonstration that the surface film activity of the natural product was identical with that of the α -ether, and that it showed marked variations from that exhibited by the synthetic β -form.^{406, 407}

A third experimental finding also confirms the fact that an α -ether exists naturally. On oxidation of batyl alcohol with lead tetraacetate, which, according to Criegee,⁴⁰⁸ is a reagent specific for an α,β -glycol, glycol aldehyde octadecyl ether (m.p., $51^\circ C.$) and formaldehyde were obtained.⁴⁰⁶ This reaction indicates that the α' - and β -positions must have been free and leaves no alternative to the supposition that the glyceryl ethers are α -ethers.

Finally, the optical properties furnish further evidence that the combination must be an asymmetric one. Although Toyama³⁹⁶ originally did not observe optical activity, the free alcohol was later shown to possess this

⁴⁰⁴ G. Weidemann, *Biochem. J.*, **20**, 685-691 (1926).

⁴⁰⁵ G. G. Davies, I. M. Heilbron, and W. M. Owens, *J. Chem. Soc.*, 1930, 2542-2546.

⁴⁰⁶ W. H. Davies, I. M. Heilbron, and W. E. Jones, *J. Chem. Soc.*, 1933, 165-167, 1934, 1232-1235.

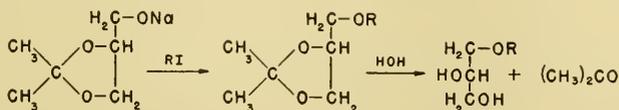
⁴⁰⁷ B. C. J. G. Knight, *Biochem. J.*, **24**, 257-261 (1930).

⁴⁰⁸ R. Criegee, *Ber.*, **64**, 260-266 (1931).

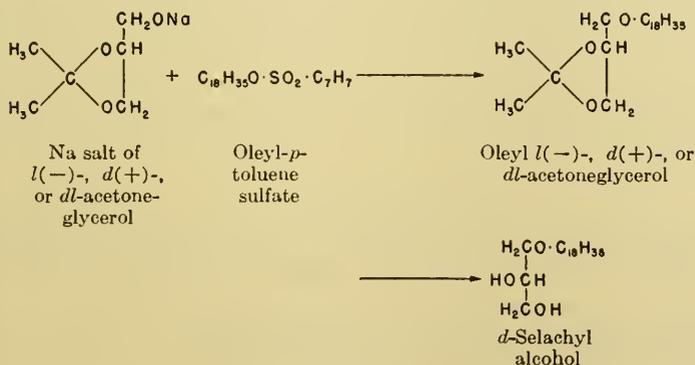
property. The specific rotation, $[\alpha]_{5461}^{20}$, in chloroform, was found to be $+2.6^\circ$ (conc., 0.95), while the similar value for batyl acetate in the same solvent has been reported to be -8.5° (conc., 2.63). Since optical activity could exist only when the higher alcohol was attached to the α -carbon of glycerol, this would seem to afford an indisputable proof of the α -ether structure. Under such conditions, the synthetic octadecyl glyceryl ether would be a racemic product which would possess somewhat different physical properties than the optically active natural form. Such an hypothesis offers an adequate explanation for the slight discrepancy in mixed melting point between the natural and the synthetic products.

The optical properties were further investigated by Baer and Fischer,⁴⁰⁹ who synthesized batyl and chimyl alcohol with *l*(-)-acetoneglycerol. The synthetic and the natural products were identical, and hence it is concluded that these glyceryl ethers belong to the *d*-series. Although selachyl alcohol was not synthesized in the original work, it was also assigned to the *d*-series because of its close relationship to batyl alcohol. The terminology for the optical isomers conforms to an earlier recommendation of Baer and Fischer⁴¹⁰ that the principle used for the classification of α -monoglycerides and glycerophosphates be followed.

The reaction used for the synthesis of the glyceryl ethers is indicated in the following equation:



However, more recently Baer, Rubin, and Fischer⁴¹¹ have synthesized the *d*-, *l*-, and *dl*-isomers of selachyl alcohol by the following reaction:



⁴⁰⁹ E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **140**, 397-410 (1941).

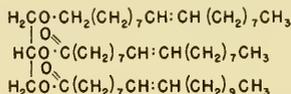
⁴¹⁰ E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **123**, 475-489, 491-500 (1939).

⁴¹¹ E. Baer, L. J. Rubin, and H. O. L. Fischer, *J. Biol. Chem.*, **155**, 447-457 (1944).

Whereas natural selachyl alcohol has a specific rotation of -4.5° ($[\alpha]_D^{15}$) according to Toyama and Ishikawa,⁴¹² the synthetic *d*-isomer was found to have a value of -4.35 at 55°C . The natural and synthetic *d*-acetates also showed a good agreement ($[\alpha]_D^{15} = -8.6^\circ$,⁴¹² and $[\alpha]_D^{23} = -8.6^\circ$,³⁹⁶ respectively).

The failure of Toyama³⁹⁶ in his original work to observe an optical rotation for batyl alcohol is partly to be ascribed to the varying effect of concentration on this property. It was found⁴¹² that the negative rotation observed at a high concentration gradually diminishes on dilution of the alcohol or chloroform solution until no rotation is observable at a 10% concentration. When the concentration is further decreased, a dextro-rotation actually obtains. The *d*- and *l*-selachyl alcohols were found to melt at 48.5 to 49.5°C . This could not be compared with the natural product, since it has been prepared only in the form of an oil.

Although Tsujimoto and Toyama³⁹⁶ demonstrated that there were two hydroxyl groups in the free alcohols which could be acetylated, these are actually esterified with long-chain fatty acids in the liver fat.^{413,414} Hil-ditch²³ pictured a possible di-acyl ester of ratfish liver oil as follows:



Oleyl, gadoleyl ester of
selachyl alcohol

The role which the glyceryl ethers play is not fully understood. They do not possess any growth-promoting action, nor do they have an antirachitic action.⁴⁰⁴ They seem to represent an intermediate between the true glycerides and the waxes. Lovern⁴¹⁵ has shown, also, that the appearance of glyceryl ethers is always accompanied by a subnormal degree of unsaturation in the fatty acids of the oil. Such a tendency toward saturation is exhibited not only by a reduction of the ester carbonyl group but also by the lowering of the mean unsaturation of the fatty acids as determined by the iodine number.

4. Colored Fats

The so-called "colored fats" are sometimes known also as the colored waxes (*Farbwachse*) or the lipochrome esters.⁴¹⁶ According to Zech-

⁴¹² Y. Toyama and I. Ishikawa, *J. Chem. Soc. Japan*, 59, 1367-1374 (1938).

⁴¹³ E. André and A. Bloch, *Compt. rend.*, 195, 627-629 (1932).

⁴¹⁴ E. André and A. Bloch, *Bull. soc. chim.* [5], 2, 789-802 (1935); *Compt. rend.*, 196, 618-620 (1933).

⁴¹⁵ J. A. Lovern, *Biochem. J.*, 31, 755-763 (1937).

⁴¹⁶ L. Zechmeister, in H. Schönfeld, *Chemie und Technologie der Fette und Fettprodukte*, Vol. I, Springer, Vienna, 1936, pp. 149-193.

meister,⁴¹⁶ three classes of fats are possible, one of which is colorless while two are colored. These are as follows:

1. Colorless alcohols esterified with colorless acids. *Examples:* Usual fats, waxes, lecithin, and sterol esters; *colorless.*
2. Colored acids combined with colorless alcohols, such as phytol ($C_{20}H_{39}OH$). *Example:* Chlorophyll; *colored.*
3. Colorless acids combined with colored alcohols, such as xanthophyll. *Example:* Colored waxes; *colored.*

The group which is of primary interest to us at present is group 3, since these esters are made up of the usual fatty acids combined with higher dihydric alcohols. Because such alcohols are insoluble in water and because they are found in the non-saponifiable extract after hydrolysis of the colored fats, it is evident that they are analogous to the waxes. However, instead of having only one hydroxyl group, as is true with the higher alcohols in most waxes, the colored fats appear to be esters of dihydric carotenoid alcohols.

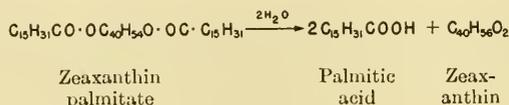
A most interesting question arises as to whether the lipochromes are lipids in which the pigments are mechanically mixed or whether an actual combination obtains between the chromogenic material and some component of the lipid. According to Zechmeister,⁴¹⁷ Krukenberg advanced the idea in 1882 that the fats and the pigments are merely "associated," although he did not furnish any clear-cut evidence to support his hypothesis. One might readily accept the fact that the hydrocarbons, α -, β -, and γ -carotene, as well as lycopene, would be present as mixtures in fats, since there are no groups available for combination with fatty acids.

However, Zechmeister and Cholnoky⁴¹⁷ were the first to point out that some carotenoid pigments are chemically bound to fatty acids. They obtained an almost quantitative separation of the pigment for the coral-red berries of the boxthorn, or matrimony vine (*Lycium halimifolium*, *Solanaceae*), in beautiful crystals, by simple extraction of the berries. The molecular weight of the crystalline material was between 900 and 1000. This was higher than any value previously obtained for carotenoids except one called *physalien*, which had previously been prepared from the winter ground cherries (*Physalis alkekengi*) and the Chinese lantern plant (*Physalis francheti*), by Kuhn and Wiegand.⁴¹⁸ The *Lycium* pigment was shown to be identical with *physalien*. However, when the berry extract was saponified in the cold with a potassium hydroxide-methyl alcohol mixture, the pigment gradually passed into the methyl alcohol layer, and a quantitative separation of the pigment was again obtained. In this case, however, the crystalline pigment which was obtained melted at a temperature more than 100°C. higher than did the *physalien*, and had a molecular weight between

⁴¹⁷ L. Zechmeister and L. v. Cholnoky, *Ann.*, *481*, 42-56 (1930).

⁴¹⁸ R. Kuhn and W. Wiegand, *Helv. Chim. Acta*, *12*, 499-506 (1929).

500 and 600. It turned out to be an isomer of lutein (xanthophyll) which had an empirical composition of $C_{40}H_{56}O_2$. In spite of the great variation in properties from those of physalinen, it had an almost identical spectrum. It was shown to be identical with zeaxanthin, which had been previously isolated by Karrer, Salomon, and Wehrli.⁴¹⁹ A further study of the pure lycium-physalinen preparation showed that, when the pigment was dissolved in ether and saponified in the cold by standing for 48 hours with methyl-alcoholic potash, two components could be isolated from the reaction mixture. The first consisted of a pigment identical with zeaxanthin, while the second product was a saturated fatty acid melting at $62^\circ C$. The latter proved to be palmitic acid. From the relative amounts of zeaxanthin, it was believed that there were two moles of palmitic acid to one of the carotenol. This was strong evidence that lycium and physalinen were actually dipalmitates of zeaxanthin. The empirical formula for this ester is $C_{72}H_{116}O_4$, and it breaks down on hydrolysis:



That the combination of zeaxanthin with palmitic acid is an ester linkage rather than an ether linkage, as first assumed for the oxygen-containing carotenoids, seems certain in view of the proof furnished by Karrer *et al.*⁴²⁰ that the oxygen was present in hydroxyl groups. Further experimental confirmation⁴¹⁷ of the nature of the zeaxanthin combination was afforded by the separation and purification of perhydrophysalinen, which was prepared by treating physalinen with perbenzoic acid. This compound was a colorless wax which, on hydrolysis, gave rise to exactly two moles of palmitic acid to one of perhydrozeaxanthin. These data are explicable only on the assumption that physalinen and lycium are actually dipalmitic acid esters of zeaxanthin.

A further demonstration of the varied nature of the natural carotenoid esters is evident from the later studies of Zechmeister and Cholnoky⁴²¹ on capsanthin. This pigment is the red compound present in the skin of the ripe paprika; these investigators had on all previous attempts over a period of 5 years failed to obtain it in definite crystalline form. However, the crude pigment was obtained in small, red, needle-like crystals, from an acetone solution. A cool alkaline methyl alcohol saponification of this purified product gave rise to a pigment and fatty acids. These results were taken to indicate that capsanthin was present as an ester. However, it

⁴¹⁹ P. Karrer, H. Salomon, and H. Wehrli, *Helv. Chim. Acta*, **12**, 790-792 (1929).

⁴²⁰ P. Karrer, A. Helfenstein, and H. Wehrli, *Helv. Chim. Acta*, **13**, 87-88 (1930).

⁴²¹ L. Zechmeister and L. v. Cholnoky, *Ann.*, **487**, 197-213 (1931).

was found that the acid hydrolyzed from the ester was not exclusively palmitic acid, as had been the case with physalien, but was a mixture of saturated and unsaturated acids. A fractionation of the acid showed that 45% was unsaturated, and that this contained exclusively oleic acid. From the saturated acids (55%) pure preparations of carnaubic, myristic, and palmitic acids were prepared, the last two in the preponderate amount. Only minor amounts of stearic acid were combined with capsanthin, although it could be isolated on saponification of the mother liquor from the colorless esters.

Zechmeister and Cholnoky⁴²¹ prepared a number of synthetic fatty acid esters of capsanthin by the method employed by Kuhn *et al.*⁴²² for zeaxanthin and by Karrer and Ishikawa for lutein (xanthophyll).^{423,424} When the purified capsanthin was treated with the acyl chlorides of a number of acids in pyridine, esterification ensued. When methyl alcohol was added, the ester precipitated. It was found that two acyl groups were present per molecule of capsanthin, since it contained two hydroxyl groups. The following products were synthesized:

	M.p. °
Capsanthin diacetate.....	145-146
Capsanthin dipropionate.....	140
Capsanthin dicaprate.....	102
Capsanthin dimyristate.....	88
Capsanthin dipalmitate.....	85
Capsanthin distearate.....	84
Capsanthin dioleate.....	Liquid
Capsanthin dibenzoate.....	121-122

The melting point falls as the fatty acid chain is lengthened, probably because it approaches that of the fatty acid as this element becomes more predominant than the capsanthin. The solubility in methanol is also decreased when the fatty acid chains of the esters are lengthened. The esters are readily obtained in crystalline form.

It would thus seem that carotenols, as well as the sterols, higher alcohols, or glycerol, may serve for the esterification of fatty acids. The fatty acids react with the molecule available at the spot where synthesis occurs. There does not seem to be much selectivity exerted, although it is believed by some that cholesterol favors a combination with the unsaturated acids. The colorless vitamin A esters are in the same category as the colored esters. These will be considered in the chapter on vitamin A.

An interesting unexplored field is concerned with the nature and distribution of mixed esters of the carotenols. Although the dipalmitate

⁴²² R. Kuhn, A. Winterstein, and W. Kaufmann, *Ber.*, *B63*, 1489-1497 (1930).

⁴²³ P. Karrer and S. Ishikawa, *Helv. Chim. Acta*, *13*, 709-713 (1930).

⁴²⁴ P. Karrer and S. Ishikawa, *Helv. Chim. Acta*, *13*, 1099-1102 (1930).

would appear to be the sole representative in physalien, capsanthin poses a different problem. With the variety of fatty acids present in the latter ester, it is almost certain that capsanthin is composed of a number of different mixed esters.

5. Hydrocarbons

Although hydrocarbons are frequently considered to be compounds without biological significance in higher animals, there is an increasing amount of evidence that they play an important role in the plant kingdom, as well as in many marine forms. In contradistinction to the fatty acids and higher alcohols, which are invariably composed of an even number of carbon atoms, many of the hydrocarbons contain an odd number of carbon atoms. Moreover, they frequently contain forked chains, which is a type of compound unusual in the other lipids.

(1) Squalene

Squalene is a highly unsaturated hydrocarbon with an empirical formula of $C_{30}H_{50}$. An unsaturated hydrocarbon was first isolated from the liver oil of a black shark of the genus *Zameus*⁴²⁵; this was later shown to be squalene.⁴²⁶ It was found to be present in the liver oils of 16 of the 36 species of elasmobranch fish, from Japanese waters, which were examined,⁴²⁷ including the basking shark (*Cetorhinus maximus*).⁴²⁸ According to Hilditch²⁸ it is chiefly present in the liver oils of some of the family *Squalidae* (sharks) as well as in some representatives of the other shark families, *Cetorhinidae* (basking shark), *Chlamydoselachidae* (frilled shark), *Dalatiidae*, and the *Scylliorhinidae* (spotted dog fish). It was found in the eggs of two species in which it was also present in the liver oil. In this connection Heilbron *et al.*⁴²⁹ have shown that it may be present in the comparatively undeveloped eggs of *Etmopterus spinax*, which is a member of the *Squalidae*; however, it was not found in the mature eggs. This would seem to suggest that it plays an important role in embryological development. Although Channon⁴³⁰ reported squalene in three of the *Squalidae* (*Spinax niger*, *Dalatis licha* (*Scymnorhinus licha*), and *Lepidorhinus squamosus*), it was not present in two other representatives of this family or in 11 members of different elasmobranch families. Channon also failed to find squalene in any of 14 species of teleostid fishes or in any of a number of phyto- and

⁴²⁵ M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, 9, 953 (1906). Cited by M. Tsujimoto, *Ind. Eng. Chem.*, 8, 890 (1916).

⁴²⁶ M. Tsujimoto, *Ind. Eng. Chem.*, 8, 889-896 (1916).

⁴²⁷ M. Tsujimoto, *Ind. Eng. Chem.*, 12, 63-73 (1920).

⁴²⁸ M. Tsujimoto, *Bull. Chem. Soc. Japan*, 10, 144-148, 149-153 (1935); *J. Chem. Soc. Japan*, 55, 702-741 (1934); *Chem. Abst.*, 28, 6484 (1934).

⁴²⁹ I. M. Heilbron, E. D. Kamm, and W. M. Owens, *J. Chem. Soc.*, 1926, 1630-1644.

⁴³⁰ H. J. Channon, *Biochem. J.*, 22, 51-59 (1928).

which decomposes on treatment with water to carbon dioxide, formaldehyde, acetone, succinic, and levulinic acids, and, in addition, to two undefined acids with the formulas $C_6H_{10}O_5$ and $C_5H_{14}O_6$.^{439,440} On dry distillation of squalene, isoprene and cyclodihydromyricin are formed; this is taken to indicate that it is a dihydroterpene. Heilbron *et al.*⁴²⁹ were also able to separate a series of hemiterpenes, monoterpenes, sesquiterpenes, and diterpenes by the distillation of squalene. These reactions would all seem to offer justification for defining this hydrocarbon as a class of triterpenes which is non-cyclized.

(2) Pristane

Pristane, $C_{18}H_{38}$, is another hydrocarbon which has been separated by Tsujimoto^{428,441} from the liver oil of the basking shark, where it was present to the extent of 6–10%. According to Toyama,⁴⁴² pristane is present in most liver oils which contain squalene, but only in small amounts. Toyama and Tsuchiya⁴⁴³ have found it also in sardine, herring, sperm whale, and other oils. It is presumably a branched-chain, saturated hydrocarbon, but the structure is unknown. It is a colorless liquid which boils at 296°C.

(3) Other Hydrocarbons

Gadusene, $C_{18}H_{32}$, has been isolated from wheat germ oil by Drummond *et al.*,⁴⁴⁴ which is probably the same as the material separated earlier by Tsujimoto⁴⁴⁵ from the liver oil of the Japanese ishinagi (*Stereolepis ishinagi*): Nakamiya⁴⁴⁶ isolated gadusene from the unsaponifiable fraction of rice germ oil, soybean oil, and some fish liver oils. It had the same absorption spectrum as the samples that Tsujimoto⁴⁴⁵ and Drummond *et al.*⁴⁴⁴ had prepared from other sources; thus, it is apparently identical with them. Oleastane, $C_{21}H_{36}$, has been isolated from the fruit of the olive,⁴⁴⁷ while another octadecylene called zamene, $C_{18}H_{36}$, has been prepared from the liver oil of the basking shark.⁴²⁸ Marcelet⁴⁴⁸ reported that the non-saponifiable fraction of peanut oil contains hypogene, $C_{15}H_{30}$, and arachidene, $C_{19}H_{38}$. The following hydrocarbons were identified from olive oils:

⁴³⁹ B. Kubota, *J. Chem. Soc. Tokyo*, 39, 879–907 (1918); *Chem. Abst.*, 13, 441 (1919).

⁴⁴⁰ R. Majima and B. Kubota, *Japanese J. Chem.*, 1, 19–33 (1922).

⁴⁴¹ M. Tsujimoto, *Ind. Eng. Chem.*, 9, 1098–1099 (1917).

⁴⁴² Y. Toyama, *Chem. Umschau Fette, Öle, Wachse Harze*, 30, 181–187 (1923); *Chem. Abst.*, 17, 3616 (1923).

⁴⁴³ Y. Toyama and T. Tsuchiya, *J. Soc. Chem. Ind. Japan*, 38, suppl., 254–258 (1935).

⁴⁴⁴ J. C. Drummond, E. Singer, and R. J. MacWalter, *Biochem. J.*, 29, 456–471 (1935).

⁴⁴⁵ M. Tsujimoto, *Bull. Chem. Soc. Japan*, 6, 237–239 (1931); *Chem. Abst.*, 26, 612–613 (1932).

⁴⁴⁶ Z. Nakamiya, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 28, 16–26 (1935).

⁴⁴⁷ G. Sani, *Atti accad. Lincei*, 12, 238–242 (1930).

⁴⁴⁸ H. Marcelet, *Bull. soc. chim.* [5], 3, 1156–1160, 2055–2057 (1936); *Compt. rend.*, 202, 867–869, 1809–1811 (1936).

$C_{13}H_{24}$ (oleatridecadiene), $C_{16}H_{30}$ (oleahexadecadiene), $C_{19}H_{36}$ (oleanonadecadiene), $C_{23}H_{42}$ (oleatricosatriene), $C_{24}H_{50}$ (oleatetracosane), $C_{26}H_{54}$ (oleahexacosane), and $C_{28}H_{50}$ (oleaoctacosatetraene). Shea butter, which is the seed fat from *Butyrospermum parki*, contains 5–8% of a non-saponifiable residue. This includes chiefly a hydrocarbon "Kariten" which melts at 64°C., and has an empirical formula of $(C_5H_8)_n$.⁴⁴⁹ It is believed to be identical with "Illipen," $C_{32}H_{56}$ (m.p., 64.5°C.), isolated by Kobayashi⁴⁵⁰ from illipe butter, which is a seed fat from *Madhuca indica*.

A number of paraffin hydrocarbons have been found associated with fruit and vegetable waxes. These are listed in Table 20.

TABLE 20

PARAFFIN HYDROCARBONS THAT HAVE BEEN SEPARATED FROM FRUITS AND VEGETABLES

Hydrocarbon	Formula	M.p., °C.	Source	Ref.
<i>n</i> -Eicosane	$C_{20}H_{42}$	69	Red-berry bryonia oil	<i>a</i>
			Laurel berry fat	<i>b</i>
			Parsley seed oil	<i>c</i>
<i>n</i> -Heptacosane	$C_{27}H_{56}$	63.5	Apple cuticle wax	<i>d</i>
<i>n</i> -Nonacosane	$C_{29}H_{60}$		Cabbage lipids	<i>e, f</i>
			Apple peel wax	<i>g</i>
			Pear wax	<i>h</i>
			Grapefruit peel wax	<i>i</i>
			Bing cherry skin wax	<i>j</i>
			Brussels sprouts	<i>k</i>
<i>n</i> -Hentriacontane	$C_{31}H_{64}$	67.6–67.8	Brussels sprouts	<i>k</i>
			Cabbage lipids	<i>l</i>
			Chinese insect wax	<i>m</i>
			Grapefruit peel wax	<i>i</i>
			Spinach lipids	<i>f</i>
<i>n</i> -Pentatriacontane	$C_{35}H_{72}$		Sugar cane wax	<i>n</i>

^a A. Étard, *Compt. rend.*, *114*, 364–366 (1892).

^b H. Matthes and H. Sander, *Arch. Pharm.*, *246*, 165–177 (1908).

^c H. Matthes and W. Heintz, *Ber. deut. pharm. Ges.*, *19*, No. 9, 325–328 (1909).

^d A. C. Chibnall, S. H. Piper, A. Pollard, J. A. B. Smith, and E. F. Williams, *Biochem. J.*, *25*, 2095–2110 (1931); (with P. N. Sahai), *28*, 2189–2208 (1934).

^e H. J. Channon and A. C. Chibnall, *Biochem. J.*, *23*, 168–175 (1929).

^f D. L. Collison and I. Smedley-Maclean, *Biochem. J.*, *25*, 606–613 (1931).

^g K. S. Markley and C. E. Sando, *J. Biol. Chem.*, *101*, 431 (1933).

^h K. S. Markley, S. B. Hendricks, and C. E. Sando, *J. Biol. Chem.*, *111*, 133–146 (1935).

ⁱ K. S. Markley, E. K. Nelson, and M. S. Sherman, *J. Biol. Chem.*, *118*, 433–441 (1937).

^j K. S. Markley and C. E. Sando, *J. Biol. Chem.*, *119*, 641–645 (1937).

^k P. N. Sahai and A. C. Chibnall, *Biochem. J.*, *26*, 403–412 (1932).

^l J. Ozaki, *J. Agr. Chem. Soc. Japan*, *6*, 773–782 (1930); *Chem. Abst.*, *25*, 2754 (1931).

^m F. J. E. Collins, *J. Soc. Chem. Ind.*, *54*, 33–35T (1935).

ⁿ N. L. Vidyarthi and M. Narasingarao, *J. Indian Chem. Soc.*, *16*, 135–143 (1939).

⁴⁴⁹ K. H. Bauer and G. Ulmbach, *Ber.*, *65*, 859–862 (1932).

⁴⁵⁰ S. Kobayashi, *J. Soc. Chem. Ind. Japan*, *25*, 1188–1196 (1922); *Chem. Abst.*, *17*, 3106 (1923).

A number of ketones are also found to be associated with the waxes; these may be related to the hydrocarbons. They include a di-*n*-tetradecyl ketone⁴⁵¹ in cabbage, as well as *n*-nonacosane-15-one, which could be expected because of the high content of nonacosane⁴⁵¹ in this vegetable. The latter ketone has also been separated from the lipids of brussels sprouts by Sahai and Chibnall.¹⁷ Markley *et al.*³⁶⁵ have also reported a ketone with a formula of $C_{30}H_{52}O$ in grapefruit peel wax, but they believe that this belongs to the polyterpenes.

⁴⁵¹ H. J. Channon and A. C. Chibnall, *Biochem. J.*, **23**, 168-175 (1929).

CHEMISTRY OF THE PHOSPHATIDES AND CEREBROSIDES

A. PHOSPHATIDES

1. Introduction

The phosphatides represent a group of combined or conjugated lipids which have the common property of containing a phosphate radical in their molecules. In the case of the phosphatidic acids, phosphoric acid is the only component in addition to the glycerol and fatty acids ordinarily present in the neutral fats. However, the chief phosphatides or phospholipids also contain one or two nitrogenous components. These may be divided into the compounds in which the N:P ratio is 1:1 (mono-aminophosphatides), and a second group in which a 2:1 ratio exists between nitrogen and phosphorus (di-aminophosphatides). In the case of lipositol, a carbohydrate-like compound appears to be present in the molecule.

It is interesting that the fats as well as the carbohydrates should have phosphate derivatives of such great importance. In the case of the sugars, phosphorylation seems in some way to be a prerequisite for enabling the molecule to undergo the transformations which occur in ordinary metabolism. The same function may be assigned to phosphorylation in the case of the lipids; there seems to be little doubt that a larger proportion of a phosphatide such as lecithin is always found in those organs in which active metabolic changes in fat are presumably taking place, while the phosphatides are almost completely absent from the fat depots whose chief function is the storage of this foodstuff. Moreover, the phosphorylated fats (principally lecithin and cephalin) are present, also, in increased amounts whenever fat is being transported to or from the fat depots of the body.

However, in some cases phosphatides may not necessarily represent a form of fat which is undergoing metabolic alteration or is in the process of transport from one site to another. Such compounds may occur as a portion of the structural element of an organ. It is generally believed that the high concentration of sphingomyelin and even of lecithin in brain and other nervous tissue is not related to its metabolic function under such conditions. In fact, there is ample evidence that carbohydrate is the principal source of energy for the nervous tissue. Possibly, the chemical make-up of the phosphorylated fats renders them especially satisfactory

for enabling the normal metabolic reactions of the carbohydrates to take place in nervous tissue.

The analogy between the phosphorus-containing carbohydrates and lipids is not too close in other respects. For example, only the phosphate ion is attached to the sugars, and this is in an ester linkage. This allows the phosphate to be easily detached or to be transferred to other compounds by simple chemical or biochemical means. Such carbohydrates can readily act as phosphate acceptors or phosphate donors. In the case of all the phosphatides except the phosphatidic acids, a second non-fatty component is combined with the phosphoric acid in such a way that these compounds cannot effectively act in phosphate transfer without hydrolysis of the molecule. It is true that the glycerol phosphate, which may be an intermediate, may act as the sugar phosphate compound.

Thudichum¹ originally assigned the name *phosphatide* to the compounds now more frequently referred to as *phospholipids*. He included in this group those substances which contained phosphoric acid, glycerol, higher fatty acids, and an organic base. Leathes² coined the term *phospholipines* for these products, and this terminology was also adopted by H. and I. S. MacLean,³ although the latter workers dropped the terminal "e." However, Thierfelder and Klenk⁴ prefer to retain the original Thudichum usage, since this includes the nitrogen-free phosphatidic acids, along with the nitrogen-containing phospholipids. In line with the preponderant current American practice, the term *phospholipid*⁵ will be used to connote all phosphorus-containing lipids, including the phosphatidic acids.

Specific monographs dealing entirely or largely with the phospholipids include the volume entitled *Lecithin and Allied Substances* by MacLean and MacLean,³ and *Die Chemie der Cerebroside und Phosphatide* by Thierfelder and Klenk.⁴ Among the various monographs on lipids, those of Bloor⁶ and of Schönfeld-Hefter⁷ present especially good discussions of the

¹ J. L. W. Thudichum, *Researches on the Chemical Constitution of the Brain*, Report of the Medical Officer of the Privy Council and Local Government Board [n.s.], 3, No. 5, 113-247 (1874). Cited by H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930, p. 63.

² J. B. Leathes, *The Fats*, Longmans, Green & Co., 1st ed., London (1910). Cited by H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930. 2nd ed., with H. S. Roper, London, 1925, p. 61.

³ H. MacLean and I. S. MacLean, *Lecithin and Allied Substances*, 2nd ed., Longmans, Green & Co., London, 1927.

⁴ H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*.

⁵ Although the majority of publications employ this spelling, some authors prefer to include a terminal "e" (*phospholipide*). Since this revised spelling would appear to offer no additional clarity of expression, the shorter term has been employed here.

⁶ W. R. Bloor, *Biochemistry of the Fatty Acids and Their Compounds, the Lipids*, Reinhold, New York, 1943.

⁷ A. Grün, in H. Schönfeld and G. Hefter, *Die Chemie und Technologie der Fette und Fettprodukte*, Vol. I, Springer, Vienna, 1936, pp. 456-516.

phosphatides. Other summaries include the classical and comprehensive review of Levene and Rolf,⁸ which deals with the structure and significance of the phosphatides. The review of Working and Andrews⁹ is confined to a discussion of structure, while the latest treatise of Thannhauser and Schmidt¹⁰ on lipins and lipidoses contains an excellent description of our present chemical concept of structure and classification. The application of labelling agents in the study of phospholipid metabolism is the subject of an interesting report by Chaikoff.¹¹

2. Historical Development

The first proof of the existence of complex fatty compounds is generally credited to Fourcroy,¹² whose experiments were reported as early as 1793; Vauquelin,¹³ however, was the initial investigator to prove the presence of bound phosphorus in the fat-like material of the brain. The work of Gobley¹⁴⁻¹⁶ was particularly outstanding, since it demonstrated for the first time the presence of a phosphatide in egg-yolk. This substance was later christened *lecithin* after the Greek equivalent of egg-yolk, *λεκιθος*. A further advance in our appreciation of the composition of at least one phosphatide, lecithin,¹⁷ followed the demonstration, in Hoppe-Seyler's laboratory in Tübingen, by Diakonow,^{18,19} that choline is its nitrogenous component. Strecker²⁰ had prepared this compound several years earlier from hog bile. Diakonow^{18,21} and Strecker²² were also able to show that the lecithin molecule was composed of two molecules of fatty acid, either similar or different; these were esterified with glycerol, which was also identified. Thudichum²³ was the first to differentiate cephalin from lecithin by separation of the two phosphatides in alcohol. Cephalin is

⁸ P. A. Levene and I. P. Rolf, *Physiol. Revs.*, **1**, 327-393 (1921).

⁹ E. B. Working and A. C. Andrews, *Chem. Revs.*, **29**, 245-256 (1941).

¹⁰ S. J. Thannhauser and G. Schmidt, *Physiol. Revs.*, **26**, 275-317 (1946).

¹¹ I. L. Chaikoff, *Physiol. Revs.*, **22**, 291-317 (1942).

¹² E. Fourcroy, *Ann. chim.*, **16**, 282-322 (1793). Cited by E. G. Working and A. C. Andrews, *Chem. Revs.*, **29**, 245 (1941).

¹³ M. Vauquelin, *Ann. chim.*, **81**, 37-51 (1812).

¹⁴ M. Gobley, *J. pharm. chim.* [3], **9**, 1-15, 81-91, 161-174 (1846).

¹⁵ M. Gobley, *J. pharm. chim.* [3], **11**, 409-417 (1847).

¹⁶ M. Gobley, *J. pharm. chim.* [3], **12**, 1-13 (1847).

¹⁷ C. Diakonow, *Med.-Chem. Untersuch.*, (F. Hoppe-Seyler), **2**, 221-227 (1867); **3**, 405-411 (1868).

¹⁸ C. Diakonow, *Zentr. Med. Wiss.*, **6**, No. 1, 2-3 (1868).

¹⁹ C. Diakonow, *Zentr. Med. Wiss.*, **6**, No. 7, 97-99 (1868).

²⁰ A. Strecker, *Ann.*, **123**, 353-360 (1862).

²¹ C. Diakonow, *Zentr. Med. Wiss.*, **6**, No. 28, 434-435 (1868).

²² A. Strecker, *Ann.*, **148**, 77-90 (1868).

²³ J. W. L. Thudichum, *A Treatise on the Chemical Constitution of Brain*, Baillière, Tindall and Cox, London (1884). Cited by H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930, pp. 65 ff.

insoluble in the latter, while lecithin readily dissolves in it. This same investigator also recognized the existence of a third phospholipid, now known as sphingomyelin, which separates from a warm alcohol extract of brain on cooling; this contains no glycerol, but is a diaminophosphatide with a N:P ratio of 2:1. The phosphatidic acids were first described as their calcium salts by Chibnall and Channon.²⁴

Folch²⁵ subsequently demonstrated that the compound formerly described as "cephalin" is actually a mixture of several components, two of which have been identified as phosphatidylethanolamine and phosphatidylserine. Folch,²⁵ as well as Folch and Woolley,²⁶ proved that a third cephalin fraction contains inositol. A new group of phosphorus-containing lipids, called *plasmalogens*, has been described by Feulgen and Bersin²⁷; these are shown to be acetals of fatty aldehydes with colamine glycerophosphate.

3. The Lecithins

(1) Structure of the Lecithins

Apparently a number of lecithins exist in nature which differ in the component fatty acids or in the position of such fatty acids in the molecule. Diakonow¹⁸ and Strecker²² were in agreement that lecithin contains two fatty acids in ester combination with glycerol, while a phosphoric acid molecule is attached both to the third hydroxyl of glycerol and to a choline molecule. These workers disagreed as to the method of combination of the phosphoric acid and choline, Diakonow¹⁸ insisting that the combination was in the nature of a salt and took place through the hydroxyl group on the choline nitrogen, while Strecker²² believed that the union occurred through the ethanol hydroxide. The results of Hundeshagen²⁸ and of Gilson²⁹ indicated that the latter ester form was the correct one.

Two varieties of lecithin occur, depending upon whether the phosphate is attached to the α - or to the β -carbon of glycerol. These are referred to as α - and β -lecithins, respectively. That ordinary lecithin is α -lecithin is indicated by the fact that most natural samples are optically active.³⁰ This is due to the unsymmetrical arrangement of the fatty acids on glycerol. Thus, it has been shown that an optically active glycerophosphoric acid can be isolated from the hydrolysis products of lecithin.³¹ That the β -variety of lecithin likewise occurs naturally was indicated by Fournneau

²⁴ A. C. Chibnall and H. J. Channon, *Biochem. J.*, **21**, 233-246 (1927).

²⁵ J. Folch, *J. Biol. Chem.*, **146**, 35-44 (1942).

²⁶ J. Folch and D. W. Woolley, *J. Biol. Chem.*, **142**, 963-964 (1942).

²⁷ R. Feulgen and T. Bersin, *Z. physiol. Chem.*, **260**, 217-245 (1939).

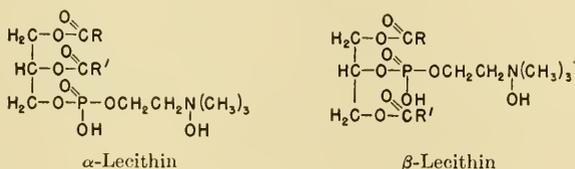
²⁸ F. Hundeshagen, *J. prakt. Chem.*, **28**, 219-255 (1883).

²⁹ E. Gilson, *Z. physiol. Chem.*, **12**, 585-602 (1888).

³⁰ C. Ulpiani, *Gazz. chim. ital.*, **31**, 47-61 (1901).

³¹ R. Willstätter and K. Lüdecke, *Ber.*, **37**, 3753-3758 (1904).

and Peittre,³² who were able to separate two calcium salts of glycerophosphoric acid from lecithin; these were identified as α - and β -salts. Bailly³³ further demonstrated that a crystalline sodium salt of β -glycerophosphoric acid and an amorphous sodium salt of α -glycerophosphoric acid could be isolated both from egg and from brain lecithin. Finally, Karrer and Salomon³⁴ presented a method for the separation of α - and β -glycerophosphoric acid; they were able to obtain both compounds from lecithin.



Since R and R' in the lecithin formulas represent different fatty acids, it is evident that many possible variations exist in specific lecithins, depending upon the particular fatty acids represented and their position in the molecule. If one includes the possibility of β - as well as of α -lecithins, then the number of opportunities for the various types of lecithin is greatly increased.

(2) Distribution of the Lecithins and Other Phospholipids

Although some phospholipids have been reported in such vegetable oils as corn, rapeseed, and soybean, the principal source of these compounds is found in animal tissues. The phosphatides are not present as components of the depot fat, but they make up a considerable proportion of the liver and brain lipids, as well as of blood fat.

Both α - and β -forms of lecithin and cephalin occur in animal tissue. By the use of a method of fractionation of α - and β -lecithins based upon the differential solubility of their cadmium salts in hot and cold acetone, Welch³⁵ studied the distribution of the α - and β -isomers of both lecithin and cephalin in a number of tissues from several species of animals. Although the α -form of lecithin is usually present in a considerably larger amount than the β -form, it is true that the β -compound is invariably found. The higher levels of the α -form in the liver and heart are in accordance with the results of Yoshinaga,³⁶ while the predominance of the α -lecithin in the brain has also been reported by Rae.³⁷ These data are summarized in Table 1.

³² E. Fourneau and M. Peittre, *Bull. soc. chim.* [4], 11, 805-810 (1912).

³³ O. Bailly, *Ann. chim.* [9], 6, 96-154 (1916).

³⁴ P. Karrer and H. Salomon, *Helv. Chim. Acta*, 9, 3-23 (1926).

³⁵ E. A. Welch, *J. Biol. Chem.*, 161, 65-69 (1945).

³⁶ T. Yoshinaga, *J. Biochem. Japan*, 27, 81-90 (1938).

³⁷ J. J. Rae, *Biochem. J.*, 28, 152-156 (1934).

TABLE I
 FRACTIONATION DATA ON NORMAL ANIMAL TISSUE^{a,b}

Tissue	Animal	No. of animals	Phospholipid fractionation			
			α -C	β -C	α -L	β -L
Liver	Beef	5	39.0-41.2 ^c	48.2-50.0 ^c	—	—
	Rat	8	9.8-10.7	25.8-31.0	29.9-31.1	13.5-15.6
	Guinea pig	13	11.9-13.1	23.9-26.6	26.9-32.3	19.1-20.1
Heart	Beef	5	28.8-29.7 ^c	57.3-63.0	—	—
	Cat	4	9.6-10.7	23.1-24.0	39.3-43.1	21.4-22.0
Brain	Rat	8	19.8-21.2	11.6-14.2	33.1-37.0	20.9-23.2
	Beef	5	58.6-60.2 ^c	27.9-30.1 ^c	70.9-75.0 ^c	19.9-21.5 ^c

^a Data, for which the extreme values in each series are given, are expressed as percentages of the total phospholipid except where indicated otherwise. C = cephalin, L = lecithin.

^b E. A. Welch, *J. Biol. Chem.*, **161**, 65-69 (1945), p. 68.

^c Expressed as percentage of lecithin or cephalin sample.

Taurog, Entenman, and Chaikoff³⁸ have found that the phospholipids of the blood plasma of dog and man consist almost entirely of choline-containing substances (lecithin and sphingomyelin). Only 5% or less of the total phospholipid failed to contain choline. The low values for cephalin in human plasma were substantiated by Hack,³⁹ while Sinclair⁴⁰ reports that the cephalin content of dog, beef, and pig serum, and of human plasma, varied between 3 and 8% of the total phospholipids. However, cephalin was shown to comprise 20% of the total phospholipids in turkey sera (*Meleagris gallopavo*).

The low values for cephalin in plasma recently reported are at variance with earlier figures found in the literature. These vary from 42%⁴¹ to 30-20%⁴²⁻⁴⁶ of the total phospholipid. Chaikoff *et al.*³⁸ attribute the above discrepancies to inadequate analytical technics. These difficulties have been overcome by a new procedure⁴⁷ which involves adsorption of the mixture on magnesium oxide. Under these conditions the non-choline fraction is retained, and the choline-containing compounds are eluted. The sphingomyelin content of plasma has been reported as 11%,⁴¹ 17%,⁴⁶ and 19%⁴² of the total plasma phospholipid, while Sinclair⁴⁰ has stated that sphingomyelin comprises 15 to 32% of the total phospholipid in human plasma.

³⁸ A. Taurog, C. Entenman, and I. L. Chaikoff, *J. Biol. Chem.*, **156**, 385-391 (1944)

³⁹ M. H. Hack, *J. Biol. Chem.*, **169**, 137-143 (1947).

⁴⁰ R. G. Sinclair, *J. Biol. Chem.*, **174**, 343-353 (1948).

⁴¹ S. J. Thannhauser, J. Benotti, and H. Reinstein, *J. Biol. Chem.*, **129**, 709-716 (1929).

⁴² B. N. Erickson, I. Avrin, E. M. Teague, and H. H. Williams, *J. Biol. Chem.*, **135**, 671-684 (1940).

⁴³ C. Artom, *J. Biol. Chem.*, **139**, 65-70 (1941).

⁴⁴ G. Brante, *Biochem. Z.*, **305**, 136-144 (1940).

⁴⁵ G. Blix, *Biochem. Z.*, **305**, 129-135 (1940).

⁴⁶ A. D. Marenzi and C. E. Cardini, *J. Biol. Chem.*, **147**, 371-378 (1943).

⁴⁷ A. Taurog, C. Entenman, B. A. Fries, and I. L. Chaikoff, *J. Biol. Chem.*, **155**, 19-25 (1944).

(3) *Preparation of the Lecithins*

Practically all of the methods for the preparation of lecithin employ its precipitation from alcoholic solution as the cadmium salt. The chief procedures are those of MacLean³ and of Levene and Rolf^{48,49} for the separation of lecithin from egg-yolk, and of Levene and Rolf⁴⁹ for the preparation of this phospholipid from such organs as brain and liver.

MacLean extracts the lecithin from the diced and pulverized egg-yolk with alcohol; then the extract is treated with alcoholic cadmium chloride solution to precipitate the lecithin. The precipitate is washed with alcohol and is stirred up with about 15 volumes of ether containing a trace of alcohol. The cadmium salt is separated by centrifugation, washed with ether, dried, and decomposed with alcoholic ammonium carbonate; the alcoholic solution of lecithin is then filtered hot. Further treatment consists in the concentration of the alcohol, the residue being taken up with ether, and in the precipitation of the lecithin with an excess of acetone. It can be further purified through emulsification with water and precipitation with acetone. This precipitate is dissolved in alcohol and is again precipitated as the double salt by cadmium chloride, and this is again recrystallized from a mixture of 2 parts of ethyl acetate and 1 part of 80% alcohol. The cadmium salt is again decomposed with ammonium carbonate and the lecithin is reprecipitated from aqueous solution by acetone. By the use of this procedure, MacLean obtained a preparation of lecithin with a N:P ratio of 1:1 which contained choline as the sole nitrogenous component.

For the preparation of lecithin from egg-yolk, Levene and Rolf⁴⁸ treat the sample first with acetone, to remove some of the other phosphatides which are otherwise separated with difficulty from lecithin. The acetone suspension is allowed to stand for at least 24 hours at 0° C., and the different crystalline residues are filtered off without letting the temperature exceed 10° C. This undissolved mass consists largely of fat and lecithin with a small amount of cephalin. The residue is taken up in 2 volumes of alcohol, and is allowed to stand for some time at 0° C. The fats remain undissolved, and are separated by filtration. After concentration of the alcoholic filtrate to about one-half the volume, under reduced pressure, lecithin is precipitated as the cadmium salt, with alcoholic cadmium chloride. The pulverized precipitate of the cadmium salt is dissolved in toluene, in which it forms a clear solution on warming, especially if the solvent contains a small amount of water. Any cerebrosides present cause an opalescence. They may be removed by centrifugation. Four volumes of cold ether containing 1% water are added, the lecithin is slowly precipitated, and the precipitate is removed by centrifugation. It is then washed with ether,

⁴⁸ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, 46, 193-207 (1921).

⁴⁹ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, 72, 587-590 (1927).

and is finally suspended in acetone to remove the toluene. The final purification involves its precipitation as the cadmium salt, after which the cadmium is removed and the lecithin is recrystallized. Levene and Rolf obtained 80–100 g. of the pure cadmium salt from 11 kg. of dried egg-yolk. Lecithin has also been prepared from liver⁵⁰ and brain⁵¹ by the application of this method. Levene and Rolf,⁴⁹ in later work, used a cold saturated methyl alcohol solution of cadmium chloride for the precipitation of egg-yolk lecithin. In the preparation of this phosphatide from liver, the lecithin is extracted from the diced, finely macerated liver by alcohol (28 liters for 20 kg.). The extract is then concentrated to one-third its volume and is allowed to stand at 0°C. overnight. After filtration of the precipitate, the lecithin is precipitated by methyl alcoholic cadmium chloride solution. In the case of brain, Levene and Rolf⁴⁹ employ two preliminary acetone extractions to remove the bulk of contaminants (16 liters each for 20 kg. of hashed brain previously dried under a vacuum). Following this treatment, the lecithin is extracted with 24 liters of alcohol. The subsequent treatment from this point on is the same. The method of Levene and Rolf for the preparation of lecithin has been somewhat simplified by Pangborn.⁵²

(4) Separation of α - and β -Lecithins

Methods for the separation of the α - and β -forms of lecithins and cephalins have been worked out by Suzuki, Yokoyama, and Nishimoto,^{53–56} and these have been modified by Welch.³⁵ The microestimation method of Welch³⁵ involves the separation of the phospholipids from the tissues by Bloor's procedure,⁵⁷ with the exception that the alcoholic extract is concentrated *in vacuo*.

The ether solution of the phospholipids is allowed to stand overnight in the refrigerator, and the insoluble material is then removed by centrifugation. The phospholipids are reprecipitated from ether by acetone, to remove any adhering fat and cholesterol. The lecithin and cephalin are generally separated by treatment of the residue obtained on evaporation of the ether extract with cold absolute ethyl alcohol and by allowing the solution to remain in the refrigerator overnight. This is then centrifuged cold and the supernatant is drawn off. The residue is again mixed with more absolute alcohol, boiled, chilled, and centrifuged cold. The absolute

⁵⁰ P. A. Levene and T. Ingvaldsen, *J. Biol. Chem.*, **43**, 359–378 (1920).

⁵¹ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, **46**, 353–365 (1921).

⁵² M. C. Pangborn, *J. Biol. Chem.*, **137**, 545–548 (1941).

⁵³ B. Suzuki and Y. Yokoyama, *Proc. Imp. Acad. Japan*, **6**, 341–344 (1930).

⁵⁴ Y. Yokoyama and B. Suzuki, *Proc. Imp. Acad. Japan*, **8**, 183–185 (1932).

⁵⁵ U. Nishimoto and B. Suzuki, *Proc. Imp. Acad. Japan*, **8**, 424–427 (1932).

⁵⁶ U. Nishimoto, *Proc. Imp. Acad. Japan*, **10**, 578–581 (1934).

⁵⁷ W. R. Bloor, *J. Biol. Chem.*, **77**, 53–73 (1928).

alcohol fractions are combined and are used for the determination of the lecithin isomers.

The alcohol is evaporated from the lecithin fractions, after which the residue is dissolved in a small amount of ether. This is allowed to stand overnight in the refrigerator for the precipitation of sphingomyelin. After removal of the sphingomyelin, the lecithin is precipitated as the cadmium salt by the addition of a cold saturated solution of cadmium chloride in absolute alcohol until no more precipitation occurs. After centrifugation, the precipitate is further washed twice with 5-ml. portions of a 7:3 alcohol-ether mixture.

In order to separate the α - and β -lecithins, acetone is added and is thoroughly mixed with the precipitate by means of a stirring rod. The tube is placed in a hot-water bath which is gently heated to boiling for 2 minutes. The cadmium salt of β -lecithin dissolves in the acetone, while the salt of the α -lecithin is insoluble. After the residue is washed twice with acetone, the β -lecithin is determined on the combined acetone washings, while the α -lecithin is estimated on the residue. Welch⁵⁵ employed the Bloor oxidation method⁵⁸ for the determination of the lecithin in each of these fractions.

(5) *Synthesis of the Lecithins*

The first attempts to synthesize lecithin were made by Hundeshagen²⁸ as early as 1883 and by Gilson²⁹ 5 years later. These workers did not obtain well-defined products which gave the expected hydrolysis products. Although Grün and Kade⁵⁹ succeeded in preparing the glycolchlorohydrin ester of distearylglycerol phosphoric acid, they failed to obtain sufficient amounts of lecithin on treatment of this compound with trimethylamine to render its identification certain.

a. α -Lecithin. The first satisfactory synthesis of α -lecithin was made by Grün and Limpächer.^{60,61} These workers used α,β -distearin as a starting material. This was condensed with phosphoric acid anhydride, whereby the α,β -distearin metaphosphoric acid ester was formed. When this ester was treated with 2 equivalents of choline bicarbonate, the choline salt was obtained from which lecithin was synthesized in a 60–70% yield. It is necessary to use the bicarbonate to counteract the strongly basic hydroxyl in the nitrogen. If this is not done, the group will react with the phosphoric acid residue, resulting in the formation of a salt rather than of an ester. This series of reactions is illustrated here:

⁵⁸ W. R. Bloor, *J. Biol. Chem.*, *82*, 273–286 (1929).

⁵⁹ A. Grün and F. Kade, *Ber.*, *45*, 3367–3376 (1912).

⁶⁰ A. Grün and R. Limpächer, *Ber.*, *59*, 1350–1360 (1926).

⁶¹ A. Grün and R. Limpächer, *Ber.*, *60*, 147–150 (1927).

After drying, it can be pulverized. It is an amorphous product, but can be obtained in crystalline form when prepared at low temperatures. No melting points are ascertainable, even in the case of pure lecithin preparations, since the substance becomes brown and it is impossible to determine when melting has occurred.

Lecithin is soluble in the usual fat solvents such as chloroform, diethyl ether, petroleum ether, carbon disulfide, carbon tetrachloride, benzene, and alcohol, but it is not soluble in methyl acetate⁶⁵ or acetone. The precipitation by acetone is rendered more difficult when fat is present. In chloroform and diethyl ether solution, precipitation is only about 50% complete with acetone.⁶⁶ However, the precipitation can be made complete if a little saturated magnesium chloride solution is added to the acetone.⁶⁷ Lecithin can also be thrown out of ether solution by paraldehyde.⁶⁸

The solubility of the synthetic distearolecithin in various solvents is given in Table 2.

TABLE 2
SOLUBILITY OF SYNTHETIC DISTEAROLECITHIN IN VARIOUS SOLVENTS AT LOW TEMPERATURES^a

Solvent	Temperature, °C.	g. dissolved per 100 ml. solvent
80% alcohol	-20	0.55
Absolute alcohol	-20	0.33
Petroleum ether	-15	0.14
Benzene	± 0	0.06
Ethyl acetate	+ 5	0.03
Carbon tetrachloride	+15	1.58

^a H. Thierfelder and E. Klenk, *Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930, p. 122.

Synthetic distearolecithin was found to dissolve in aromatic solvents as well as in high-boiling hydrocarbons. It is also readily soluble at room temperature in amyl alcohol, ethyl and amyl acetates, carbon disulfide, pyridine, chloroform, carbon tetrachloride, and dichlorohydrin; on heating it goes into solution in methyl, ethyl, and propyl alcohols, ethylene chloride, glycolchlorohydrin, and acetic acid, but it is very difficultly soluble at room temperature. It also dissolves in glycerol, but, like other natural lecithins, it has been found to be practically completely insoluble in diethyl ether, petroleum ether (60-70°C.), and acetone.

⁶⁵ E. Winterstein and O. Hiestand, *Z. physiol. Chem.*, 54, 288-330 (1907-1908).

⁶⁶ J. Nerking, *Biochem. Z.*, 10, 193-203 (1908).

⁶⁷ J. Nerking, *Biochem. Z.*, 23, 262-269 (1909).

⁶⁸ E. A. Cooper, *Biochem. J.*, 18, 948-950 (1924).

Lecithin forms a colloidal solution with water from which it may be precipitated by acetone. Leathes⁶⁹ demonstrated that, when a lecithin surface comes into contact with water, peculiar finger-like growths of lecithin result. Such myelin forms are inhibited by calcium ions, except when cholesterol is present. These irregular growths on the surface presumably occur because of the very considerable surface area occupied by a film of lecithin; the extent of this film exceeds that of the component fatty acids, apparently because the other groups in the lecithin molecule separate the fatty acid chains, thus preventing any close packing.

Aqueous solutions of lecithin can be prepared in the presence of bile salts up to 80% of the weight of the salts, and only part of the solute in this case is precipitable by acetone. The presence of many inorganic salts, such as barium chloride, accelerates the solubility of lecithin under such conditions.⁷⁰ On the other hand, it has been stated that lecithin prevents the precipitation of bile salts from their alcoholic solutions by ether. Hammarsten⁷¹ believes that this may be explained by the assumption that a lecithin-bile salt combination is formed which is soluble in ether, chloroform, and benzene.

In addition to its possible combination with bile salts, lecithin combines with inorganic salts, sugars, and possibly also with protein. It forms a loose combination with sodium chloride which is soluble in ether, but which dissolves with difficulty in alcohol, and is insoluble in acetone.⁷² Strecker²² first proved that lecithin can be completely precipitated from an alcohol solution by cadmium chloride as a white flocculent precipitate. However, the proportions of lecithin and cadmium were found to be variable,^{30,73,74} and it is now believed that cephalin is also precipitated by the same salt. After removal of cephalin, however, the precipitation as the cadmium chloride salt is very useful in the preparation of pure lecithin. Lecithin precipitates as a double salt, on the addition of an alcoholic solution of platinum chloride to an alcohol solution of lecithin.²² This platinum salt dissolves readily in ether, carbon disulfide, chloroform, and benzene, and corresponds to the empirical formula $(C_{42}H_{83}NPO_8Cl)_2 \cdot PtCl_4$. Discrepancies in the composition of this salt reported by several workers can probably be traced to the fact that it may also contain cephalin in amounts which, in one preparation, were as high as 30%.⁷⁵ Bing⁷⁶ and Mayer⁷⁷ have

⁶⁹ J. B. Leathes, *Lancet*, 1925, I, 803-807, 853-856, 957-962, 1019-1022.

⁷⁰ J. H. Long and F. Gephart, *J. Am. Chem. Soc.*, 30, 1312-1319 (1908).

⁷¹ O. Hammarsten, *Ergeb. Physiol.*, 4, 1-22 (1905).

⁷² H. J. Bing, *Skand. Arch. Physiol.*, 11, 166-175 (1901).

⁷³ A. Erlandsen, *Z. physiol. Chem.*, 51, 71-155 (1907).

⁷⁴ J. E. Eppler, *Z. physiol. Chem.*, 87, 233-254 (1913).

⁷⁵ S. Fränkel and A. Käszt, *Biochem. Z.*, 124, 216-227 (1921).

⁷⁶ H. J. Bing, *Skand. Arch. Physiol.*, 9, 336-411 (1899).

⁷⁷ P. Mayer, *Biochem. Z.*, 1, 81-107 (1906).

found that the lecithin-glucose compounds, which are loosely bound products, have a quite variable composition. This fact raises the question as to whether or not they should be considered as true compounds.⁷²

The combination of lecithin with protein has been widely accepted, since such lecithoproteins were first obtained from egg-yolk by Hoppe-Seyler,⁷³ as well as by Weyl,⁷⁹ and more recently by Osborne and Campbell.⁸⁰ Lecithoproteins are soluble in salt solution, and they precipitate on dialysis or on dilution with water. The lecithin cannot be extracted with water, but it can be removed with alcohol. Synthetic lecithoproteins, soluble in chloroform, have been prepared with ovalbumin⁸¹ and casein.⁸² De Jong and Westerkamp⁸³ questioned the chemical nature of such conjugate proteins, since combination occurred only within the range of their isoelectric points when they had opposite electrical charges. Such a combination would thus appear in all probability to be an adsorption complex rather than a chemical compound.

Since lecithins almost invariably contain unsaturated fatty acids, they are capable of adding the halogens. Thus, on treatment with bromine, octobromo- and hexabromolecithins have been prepared from liver, and a tetra- and dibromide from egg lecithin.⁸⁴ On hydrolysis, octabromarachidic, hexabromostearic, and tetrabromostearic acids were prepared, indicating that the unsaturated double bonds were present in the constituent fatty acids. The iodine numbers obtained with various lecithin preparations obviously depend upon the unsaturated acids represented, and will vary from 33 to 127 for the common lecithins. However, since oxidation results so readily when lecithin is exposed to air, with the consequent obliteration of the unsaturated linkages, the iodine number is of value only when the lecithin is so prepared that oxidation is scrupulously avoided. The theoretical values for the iodine numbers of some lecithins are given in Table 3, together with results obtained by Levene and co-workers with some of their pure preparations.

Because of the unsaturated bonds, lecithin will also add hydrogen to form hydrolecithin. Hydrogenation is brought about by treating an alcoholic solution of lecithin with hydrogen in the presence of a colloidal palladium catalyst. Pure hydrolecithin was first prepared by Ritter⁸⁵; the only fatty acid obtained from it on hydrolysis was stearic acid. Cephalin-free hydrolecithin was also prepared from egg-yolk⁸⁶ and brain,⁵¹

⁷³ F. Hoppe-Seyler, *Med.-Chem. Untersuch.*, **2**, 215-220 (1867).

⁷⁹ T. Weyl, *Z. physiol. Chem.*, **1**, 72-100 (1877-1878).

⁸⁰ T. B. Osborne and G. F. Campbell, *J. Am. Chem. Soc.*, **22**, 413-422 (1900).

⁸¹ G. Galeotti and G. Giampalmo, *Arch. fisiol.*, **5**, 503-519 (1908).

⁸² T. R. Parsons, *Biochem. J.*, **22**, 800-810 (1928).

⁸³ H. G. B. De Jong and R. F. Westerkamp, *Biochem. Z.*, **234**, 367-400 (1931).

⁸⁴ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, **67**, 659-666 (1926).

⁸⁵ F. Ritter, *Ber.*, **47**, 530-532 (1914).

⁸⁶ P. A. Levene and C. J. West, *J. Biol. Chem.*, **33**, 111-117 (1918); **34**, 175-186 (1918).

which likewise contained stearic acid as the sole fatty acid. Hydrolecithin was found to retain its optical activity; the values for $[\alpha]_{26}$ in chloroform varied between $+5.5^\circ$ and $+6.0^\circ$, depending upon the concentration.

TABLE 3
IODINE NUMBERS OF VARIOUS LECITHIN PREPARATIONS

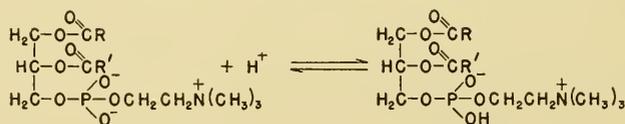
Type of lecithin	Iodine No.
Theoretical Calculations	
Palmityl-oleyl-.....	32.7
Palmityl-arachidonyl-.....	127.0
Stearyl-oleyl-.....	31.5
Stearyl-arachidonyl-.....	122.6
Actual Determinations	
Egg-yolk-.....	47 ^a
	70 ^b
Brain-.....	61 ^b
Liver-.....	72.7 ^c
	76 ^a
	90 ^b

^a P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *67*, 659-666 (1926).

^b P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *72*, 587-590 (1927).

^c P. A. Levene and T. Ingvaldsen, *J. Biol. Chem.*, *43*, 359-378 (1920).

Lecithin probably exists in the form of a zwitterion which would presumably react with a hydrogen ion as follows:



In support of this hypothesis, both Jukes⁸⁷ and Fischgold and Chain⁸⁸ have shown that lecithin cannot combine with alkali, in contrast to cephalin, which is able to do so. Cephalin can be titrated with phenolphthalein under certain conditions as a monobasic acid, while lecithin behaves as a neutral substance under such conditions.^{60,61,89,90} This is obviously related to the strong basicity of the choline residue in contrast to that of ethanolamine, which causes lecithin to act as an internally neutralized

⁸⁷ T. H. Jukes, *J. Biol. Chem.*, *107*, 783-787 (1934).

⁸⁸ H. Fischgold and E. Chain, *Proc. Roy. Soc. London*, *B117*, 239-257 (1935).

⁸⁹ A. Grün and R. Limpächer, *Ber.*, *60*, 151-156 (1927).

⁹⁰ H. Rudy and I. H. Page, *Z. physiol. Chem.*, *193*, 251-268 (1930).

these are generally precipitated in a gelatinous mass on acidification. It forms a precipitate in alcohol solution when cadmium chloride is added. The specific rotation is much less than that of lecithin, being $+0.8^\circ$ (acetic acid), $+1.2^\circ$ (pyridine), or -2.6° (chloroform).

Lysolecithin has also been prepared by the action of fer-de-lance snake venom (*Bothrops atrox*) on egg-yolk phospholipid.⁹⁹ Such preparations were found to be hemolytic. Chargaff and Cohen¹⁰⁰ were able to prepare lysolecithins in 15 to 50% yield from beef-brain and egg-yolk lecithins by the use of water-moccasin (water-viper) snake venom (*Ancistrodon piscivorus*). The analytical data on these compounds are included in Table 4.

TABLE 4
FORMATION OF LYSOLECITHIN FROM LECITHIN BY WATER-MOCCASIN SNAKE VENOM
(*Ancistrodon piscivorus*)^a

No.	Preparation ^b	P, %	N, %	NH ₂ -N		Iodine value	N:P
				%	% of total N		
1	Lec from beef brain	3.7	1.9	0.08	4.2	35.4	1:0.9
2	Lys from 1	6.2	2.9			2.3	1:1
3	Lec from beef brain	3.6	1.9	0.26	13.3	56.7	1:0.9
4	Lys from 3	6.2	3.0	0.11	3.7	1.5	1:0.9
5	Lec from egg-yolk	4.0	1.7	0.16	9.4	44.9	1:1.1
6	Lys from 5	5.9	2.4	0.08	3.3	5.9	1:1.1
X	Recovered unsplit Lec from brain 3	4.2	1.9	0.2	—	54.2	—

Calculated for palmityl lysolecithin, C₂₄H₅₂O₈NP (mol. wt. 513.4), P 6.05, N 2.73.

Calculated for stearyl lysolecithin, C₂₆H₅₆O₈NP (mol. wt. 541.4), P 5.73, N 2.59.

^a E. Chargaff and S. S. Cohen, *J. Biol. Chem.*, 129, 619-628 (1939), pp. 623, 624.

^b Lec = lecithin, Lys = lysolecithin.

Chargaff and Cohen¹⁰⁰ demonstrated that no fractionation of lecithin occurs in the course of the formation of lysolecithin, since the iodine number and other analytical figures are the same for the unsplit lecithin recovered from the reaction mixture (Preparation X, Table 4) as for the original material. Another interesting observation by the above investigators was the fact that a highly purified cadmium chloride double salt of lecithin could likewise be split by snake venom. This reaction, however, did not occur any more completely with the cadmium salt than with lecithin, since only a 21% transformation was noted. This result is in contrast to the earlier report of Contardi and Latzer,¹⁰¹ who indicated that the cadmium salt was split with greater ease than was free phospholipid.

⁹⁹ E. J. King and M. Dolan, *Biochem. J.*, 27, 403-409 (1933).

¹⁰⁰ E. Chargaff and S. S. Cohen, *J. Biol. Chem.*, 129, 619-628 (1939).

¹⁰¹ A. Contardi and P. Latzer, *Biochem. Z.*, 197, 222-236 (1928).

(8) *Enzymic Hydrolysis of the Lecithins*

Most lipases presumably act on lecithin as well as on fats. Thiele¹⁰² found an enzyme in the blood which is able to hydrolyze lecithin but not fat. Such an enzyme would be considered to be an esterase but not a lipase. Porter,¹⁰³ after examination of the enzymes in many tissues, concluded that lipases and lecithinases act independently. Moreover, four different types of enzymes are now known which are believed to produce specific effects on each of the ester linkages in the lecithin molecule.¹⁰⁴ These enzymes may be listed as follows:

a. **Lecithinase A.** This liberates only one fatty acid from the molecule, with the formation of lysolecithin.

b. **Lecithinase B.** This enzyme hydrolyzes off both fatty acids from the lecithin molecule.

c. **Lecithinase C.** This enzyme splits only the choline from lecithin, by rupturing the ester linkage between choline and phosphoric acid.

d. **Lecithinase D.** This is a true glycerophosphatase which acts on the ester linkage between phosphoric acid and glycerol, producing phosphorylcholine and a diglyceride.

Considerable work has been done on the undifferentiated lecithinases, using the production of soluble phosphate as an index of their activity. King¹⁰⁵ found that the optimum pH was 7.5, and that such enzymes were widely distributed in nature. The highest content was present in kidney, while a decreasing activity was observed in various tissues in the following order: small intestine, spleen, liver, testes, pancreas, large intestine, brain, ovary, bone, suprarenals, lung, blood vessels, cardiac muscle, and skeletal muscle. In later investigations, King¹⁰⁶ found that lecithinase had an optimum activity at body temperature, while artificial hydrolecithins were also acted upon as rapidly as was the parent lecithin. However, the synthetic lecithin of Grün, and distearyl phosphate, were acted upon much more slowly than was the natural product, and no pH optimum was noted. The behavior was so divergent that it leads one to question the identity of the synthetic and the natural lecithins. On the other hand, the phosphate is set free from lysolecithin at about twice the rate, and also from bromolecithin at a greatly accelerated speed as compared with that of lecithin. The effect of pH on hydrolysis of natural and synthetic lecithin is shown in Figure 1.

More recent work has been concerned with the activity of specific lecithinases. Lecithinase A, or "phospholipase" as termed by Fairbairn,¹⁰⁷ which is present in the venom of many poisonous snakes such as the cobra

¹⁰² F. A. Thiele, *Biochem. J.*, **7**, 275-286, 287-296 (1913).

¹⁰³ A. E. Porter, *Biochem. J.*, **10**, 523-533 (1916).

¹⁰⁴ A. Contardi and A. Ercoli, *Biochem. Z.*, **261**, 275-302 (1933).

¹⁰⁵ E. J. King, *Biochem. J.*, **25**, 799-811 (1931).

¹⁰⁶ E. J. King, *Biochem. J.*, **28**, 476-481 (1934).

¹⁰⁷ D. Fairbairn, *J. Biol. Chem.*, **157**, 633-644 (1945).

(*Naja*), the rattlesnake (*Crotalus*), and the water-moccasin (*Ancistrodon piscivorus*), causes the formation of lysolecithin, as described earlier, by the removal of the single unsaturated fatty acid residue from lecithin.⁸⁷ Fairbairn prefers the term phospholipase since this enzyme is not specific for lecithin but acts also upon cephalin; however, it is without activity on

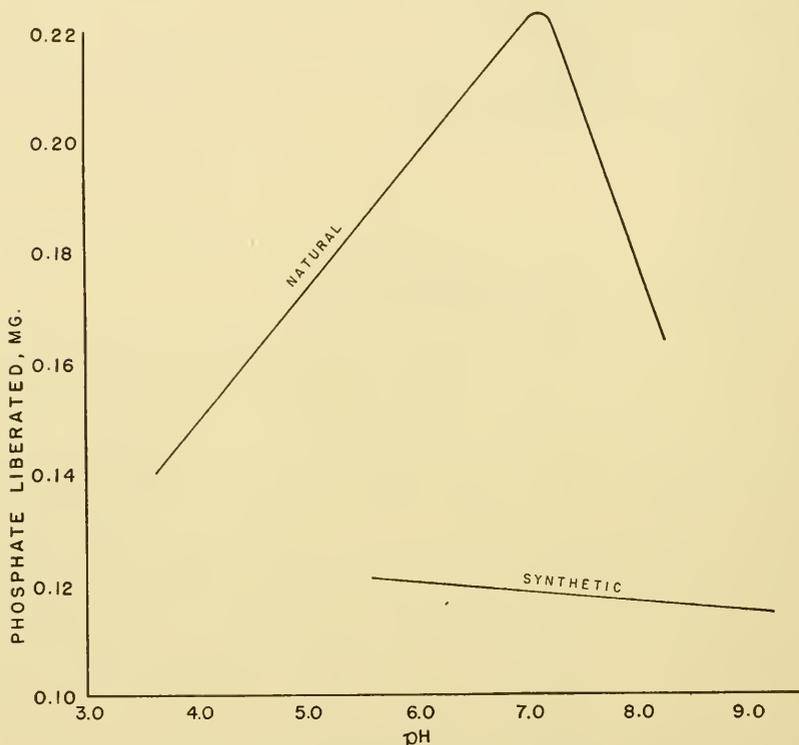


Fig. 1. The relationship between the pH and the phosphate liberated by lecithinase with natural or synthetic lecithin as the substrate.¹⁰⁶

sphingomyelins, cerebrosides, lysophospholipids, and acetal phospholipids.¹⁰⁷ This enzyme has been crystallized by Slotta and Fraenkel-Conrat¹⁰⁸ from the venom of the Mexican rattlesnake (*Crotalus terrificus*), as well as from cobra venom (*Naja tripudians*).^{109,110} Apparently lecithinase A is widely distributed in pancreas^{111,112} and in other tissues.¹¹³

¹⁰⁸ K. H. Slotta and H. L. Fraenkel-Conrat, *Ber.*, *A71*, 1076-1081 (1938).

¹⁰⁹ S. S. De, *Ann. Biochem. Exptl. Med.*, *4*, 45-56 (1944); *Chem. Abst.*, *39*, 3799 (1945).

¹¹⁰ S. S. De, *J. Indian Chem. Soc.*, *21*, 290-294, 307-310 (1944); *Chem. Abst.*, *39*, 4337 (1945).

¹¹¹ K. Ogawa, *J. Biochem. Japan*, *24*, 389-405 (1936); *Chem. Abst.*, *31*, 3078 (1937).

¹¹² V. Gronchi, *Sperimentale*, *90*, 223-239, 262-280 (1936).

¹¹³ M. Francioli, *Fermentforschung*, *14*, 241-249 (1934).

Cobra lecithinase has recently been shown to have an isoelectric point of 8.5–8.6 as determined by electrophoretic studies, and a molecular weight of 31,600; this figure was established from data on the rate of diffusion.^{109,110}

Lecithinase B, which causes the hydrolysis of both fatty acid residues from the glycerol and leaves glycerylphosphorylcholine, has been reported in extracts from rice hulls,¹¹⁴ as well as in the venom of the bee (*Apidae*), hornet (*Vespa crabro*), and wasp (*Vespidae*).¹¹⁵ Fairbairn,¹¹⁶ who has isolated this enzyme from the clay mold, *Penicillium notatum*, calls it "lysophospholipase." In addition to acting on lysolecithin, this ferment causes the breakdown of lysocephalin. Schmidt, Hershman, and Thannhauser¹¹⁷ demonstrated that this enzyme was also present in beef pancreas; these workers were able to isolate α -glycerylphosphorylcholine after beef pancreas had been incubated at 37°C. for 3 hours. A simultaneous disappearance of a large part of the preformed phosphatides was noted; this is presumptive evidence that the isolated diester originated from enzymic hydrolysis of lecithin. Kahane and Lévy¹¹⁸ have shown the presence in rat intestines of a lecithinase B which hydrolyzes lecithin to fatty acids, and to a water-soluble choline derivative which contains glycerophosphate. The properties of the crude fraction obtained by Kahane and Lévy are similar to those of the glycerylphosphorylcholine of Schmidt *et al.*¹¹⁹ The latter workers have also shown that lecithin disappears during the autolysis of minced rat intestine concomitantly with the appearance of glycerylphosphorylcholine and free choline. King and Aloisi¹²⁰ have likewise isolated a water-soluble choline ester of glycerophosphoric acid from beef pancreas. This compound had a ratio of glycerophosphate:choline of 1:2.

Although little is known of lecithinase C, which splits choline from the lecithin molecule, the role of lecithinase D has become more important in view of the recent demonstration of its presence in the gas bacillus (*Clostridium welchii*).¹²¹ This enzyme was found to decompose lecithin into phosphocholine and a diglyceride. It is believed to be the specific α -toxin which is the lethal, hemolytic, and necrotic substance present in the filtrates of type A cultures. Protective sera have an activity which is

¹¹⁴ A. Contardi and A. Ercoli, *Arch. sci. biol. Italy*, *21*, 1–44 (1935).

¹¹⁵ S. Belfanti, *Z. Immunitäts.*, *56*, 449–463 (1928).

¹¹⁶ D. Fairbairn, *J. Biol. Chem.*, *173*, 705–714 (1948).

¹¹⁷ G. Schmidt, B. Hershman, and S. J. Thannhauser, *J. Biol. Chem.*, *161*, 523–536 (1945).

¹¹⁸ E. Kahane and J. Lévy, *Compt. rend.*, *219*, 431–433 (1944).

¹¹⁹ G. Schmidt, L. Hecht, and S. J. Thannhauser. Cited from S. J. Thannhauser and G. Schmidt, *Physiol. Revs.*, *26*, 310 (1946).

¹²⁰ E. J. King and M. Aloisi, *Biochem. J.*, *39*, 470–473 (1945).

¹²¹ M. G. MacFarlane and B. C. J. G. Knight, *Biochem. J.*, *35*, 884–902 (1941).

proportional to the antilecithinase activity. Moreover, it has been demonstrated that a general relationship obtains between the production *in vitro* of α -toxin or lecithinase D and the virulence of the organism.^{122,123} Lecithinase D is activated by the calcium ion and inhibited by fluoride, citrate, or phosphate; although relatively heat-stable, it was shown to be readily inactivated by surface denaturation and by sodium dodecylsulfate.¹²¹

(9) Chemistry and Properties of Hydrolysis Products

a. Fatty Acid Components of Lecithin. The fatty acids which have been reported in various lecithin preparations include the saturated acids palmitic and stearic and the unsaturated acids oleic, linoleic, linolenic, arachidonic, and clupanodonic. Hart and Heyl^{124,125} have suggested the possibility that a triply unsaturated fatty acid with 20 carbon atoms occurs in the phospholipid present in corpora lutea. A number of lecithins have been separated from the same natural sources, so that it must be assumed that a wide range of different compounds exists in nature.

Levene and Rolf⁴⁸ isolated a lecithin preparation from egg-yolk which contained palmitic, stearic, and oleic acids. Since only two fatty acids can occur in any single lecithin molecule, it is evident that the product described above must have been a mixture of several different lecithins. Moreover, these same workers later reported¹²⁶ another preparation from egg-yolk in which they isolated the three unsaturated acids oleic, linoleic, and arachidonic acids. Still later, these investigators⁸⁴ isolated palmitic, stearic, and tetrabromostearic acids from a fraction of brominated lecithin. This must have differed from the first preparation, in which the unsaturated acid was oleic. Sueyoshi and Furukubo¹²⁷ reported the presence of "isopalmitic" and clupanodonic acids in egg lecithin, as well as of oleic and linoleic acids. Brain lecithin was shown in one case to contain palmitic, stearic, and oleic acids,⁵¹ while the same workers later reported¹²⁸ a brain lecithin devoid of saturated fatty acids and containing only oleic and arachidonic acids. The lecithin preparations isolated from samples of liver have shown even wider variations. Thus, Levene and Ingvaldsen¹²⁹ demonstrated the presence in one sample of only stearic acid and of an unsaturated acid which was probably in the linoleic series; in another study

¹²² D. G. Evans, *J. Path. Bact.*, *57*, 75-85 (1945).

¹²³ E. H. Kass, H. C. Lichstein, and B. A. Waisbren, *Proc. Soc. Exptl. Biol. Med.*, *58*, 172-175 (1945).

¹²⁴ M. C. Hart and F. W. Heyl, *J. Biol. Chem.*, *70*, 663-674 (1926).

¹²⁵ M. C. Hart and F. W. Heyl, *J. Biol. Chem.*, *72*, 395-402 (1927).

¹²⁶ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *51*, 507-513 (1922).

¹²⁷ Y. Sueyoshi and T. Furukubo, *J. Biochem. Japan*, *13*, 155-175, 177-183 (1931).

¹²⁸ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, *54*, 99-100 (1922).

¹²⁹ P. A. Levene and T. Ingvaldsen, *J. Biol. Chem.*, *43*, 355-358, 359-378 (1920)

on liver lecithin, Levene and Simms¹³⁰ found palmitic and stearic acids, and two unsaturated fatty acids, one of which could be transformed by hydrogenation to stearic acid, while the second one (probably arachidonic acid), on similar treatment, yielded arachidic acid. On another occasion, Levene and Simms¹³¹ reported the isolation of oleic and arachidonic acids from the sample. Brominated liver lecithin yielded palmitic, stearic, and octobromarachidic acid, which may have differed from the other preparation of Levene and Simms.¹³⁰ Further proof of differences in composition may be gleaned from the fact that the purified cadmium chloride compounds of liver lecithin are more highly unsaturated than are those of egg-yolk lecithin; this is indicated by the fact that the iodine number varied between 59 and 84 in the former case as contrasted with 30 to 54 in the later products.¹²⁶

There is no reason to question the authenticity of the various fatty acid combinations which have been isolated from the different lecithin preparations obtained from the same natural sources. It is possible that wide varieties of fatty acids may exist in any single preparation, while only certain lecithins would be separated by the specific methods employed in purification. According to Sinclair,¹³² it is more probable that variations in the fatty acid makeup of animal lecithins do occur from time to time, depending upon the nature of the diet. Thus, the appearance of clupanodonic acid in the egg lecithin from Japan might readily be traced to the presence of fish oils in the diet of the hens.

The general opinion seems to be prevalent that the lecithin molecule contains equal proportions of saturated and of unsaturated fatty acids.^{4,48} There has been no confirmation of the reported isolation of an egg-yolk lecithin containing only stearic acid¹³ or of another preparation from a similar source composed only of two oleic acid molecules.^{30,133} Bloor¹³⁴⁻¹³⁶ prepared the lecithin fractions of numerous phospholipids from heart and other muscles, liver, kidney, pancreas, and lung. When these were further fractionated into the liquid and solid fatty acids, it was found that both types of acids were invariably present. However, the later work of Bloor¹³⁶ and of Sinclair¹³⁷ leaves the 1:1 ratio of saturated to unsaturated acids open to question, since the proportion of saturated fatty acids was too low (25-30%), although the unsaturated (liquid) acids were found to be present in about the theoretical amount (50%). However, Thierfelder

¹³⁰ P. A. Levene and H. S. Simms, *J. Biol. Chem.*, *48*, 185-196 (1921).

¹³¹ P. A. Levene and H. S. Simms, *J. Biol. Chem.*, *51*, 285-294 (1922).

¹³² R. G. Sinclair, *J. Biol. Chem.*, *86*, 579-586 (1930).

¹³³ P. Bergell, *Ber.*, *33*, 2584-2586 (1900).

¹³⁴ W. R. Bloor, *J. Biol. Chem.*, *68*, 33-56 (1926).

¹³⁵ W. R. Bloor, *J. Biol. Chem.*, *72*, 327-343 (1927).

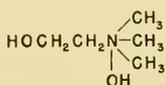
¹³⁶ W. R. Bloor, *J. Biol. Chem.*, *80*, 443-454 (1928).

¹³⁷ R. G. Sinclair, *J. Biol. Chem.*, *97*, xxxiv-xxxv (1932).

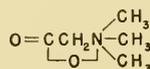
and Klenk⁴ do not feel that these experiments offer absolute proof of this hypothesis. No lecithin has been found which will not absorb iodine.¹³⁸

In the case of plant phosphatides, some instances are known in which both component fatty acids are unsaturated. This would appear to be the case with soybean lecithin, in which the principal unsaturated acids are linoleic, linolenic, and oleic. Although palmitic and stearic acids are also present, the higher proportion of unsaturated acids would seem to indicate that some diunsaturated lecithins must necessarily be present.¹³⁹⁻¹⁴¹ On the basis of the results of Daubney and Smedley-MacLean,¹⁴² it is calculated that the lecithin of yeast is composed of a mixture of 75% palmito-oleo-lecithin and 25% dioleo-lecithin. Phosphatidic acid with two unsaturated acids has also been reported in cabbage.^{143,144}

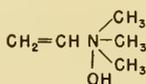
b. Choline. The essential component in the lecithin molecule is the quaternary ammonium base choline. This compound is likewise present as one of the nitrogenous bases of sphingomyelin. It may be considered to be hydroxyethyltrimethylammonium hydroxide. It is also closely related to betaine, which exerts the same qualitative physiologic effects as does choline, although the quantitative relations may differ. Neurine, with which choline was originally confused, is now known to differ from the latter because of the vinyl side chain which replaces the hydroxy-ethyl group. Muscarine, on the other hand, has an aldehyde group in place of the alcohol on the ethyl side chain of choline.



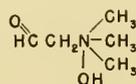
Choline



Betaine



Neurine



Muscarine

Choline has been referred to as sinkaline, amanitine, fagin, bilineurine, as well as neurine. The term *choline* first appeared in the literature in 1862, although Strecker, who named it, had reported its isolation from hog bile 13 years earlier.¹⁴⁵ Von Babo and Hirschbrunn¹⁴⁶ had also isolated the

¹³⁸ J. Cruikshank, *J. Path. Bact.*, 18, 428-431 (1913-1914).

¹³⁹ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, 62, 759-766 (1925).

¹⁴⁰ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, 65, 545-549 (1925).

¹⁴¹ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, 68, 285-293 (1926).

¹⁴² C. G. Daubney and I. Smedley-MacLean, *Biochem. J.*, 21, 373-385 (1927).

¹⁴³ A. C. Chibnall and H. J. Channon, *Biochem. J.*, 21, 233-246 (1927).

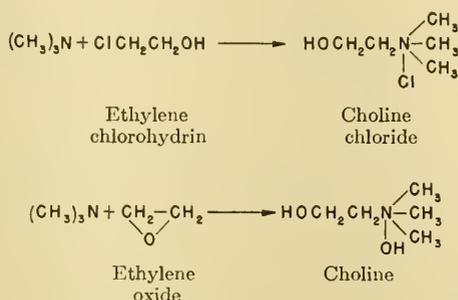
¹⁴⁴ H. J. Channon and A. C. Chibnall, *Biochem. J.*, 21, 1112-1120 (1927).

¹⁴⁵ A. Strecker, *Ann.*, 70, 149-197 (1849).

¹⁴⁶ L. von Babo and M. Hirschbrunn, *Ann.*, 84, 10-32 (1852).

compound from sinapine in 1852. This product is an alkaloid present in white mustard seeds. However, it was a number of years before this latter derivative was demonstrated to be identical with choline.¹⁴⁷ Obviously, Strecker assigned the name to choline because of his belief that it was principally found in bile.

Choline chloride was synthesized by Wurtz¹⁴⁸ in 1867 by the condensation of trimethylamine with ethylene chlorohydrin in a sealed tube for 24 hours. The same worker later synthesized the free base by treating a concentrated aqueous solution of trimethylamine, $(\text{CH}_3)_3\text{N}$, with ethylene oxide.¹⁴⁹ These reactions are illustrated here.



Since the pioneer efforts in the isolation and characterization of choline, it has been found that its distribution is widespread in both the animal and the plant kingdom. In fact, it is now believed to be a component of all living cells, although in many cases it may appear only in the combined form. The highest concentrations are in brain, egg-yolk, liver, kidney, heart, and nervous tissue, where it exists almost entirely in combination as lecithin. A recent compilation of the choline content of foods by Hawk, Oser, and Summerson¹⁵⁰ shows the following average amounts (in milligrams per 100 g. food): beef liver, 600; wheat germ, 400; beef kidney, 300; peas, 260; rice polishings, 130; beef muscle, 100; pork muscle, 100; potatoes, 100; oats, 40–100; white flour, 50; whole wheat flour, 30; corn, 21; milk, 15.

The biochemical occurrence of choline has been recently summarized in the monograph of Guggenheim,¹⁵¹ as well as in the shorter reviews of Alberts,¹⁵² and of Best and Lucas,¹⁵³ while the physiological significance

¹⁴⁷ A. Claus and C. Keesé, *J. prakt. Chem.*, 102, 24–27 (1867).

¹⁴⁸ A. Wurtz, *Compt. rend.*, 65, 1015–1018 (1867).

¹⁴⁹ A. Wurtz, *Ann.*, VI, suppl., 116–119, 197–202 (1868).

¹⁵⁰ P. B. Hawk, B. L. Oser, and W. H. Summerson, *Practical Physiological Chemistry*, 12th ed., Blakiston, Philadelphia, 1947, p. 1249.

¹⁵¹ M. Guggenheim, *Die biogenen Amine*, 3rd ed., Karger, Basel and New York, 1940, pp. 102, 110, 288–289, 554.

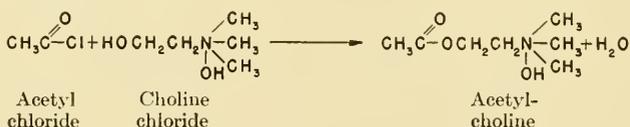
¹⁵² G. G. Alberts, *Ergeb. inn. Med. Kinderheilk.*, 43, 114–148 (1932).

¹⁵³ C. H. Best and C. C. Lucas, *Vitamins and Hormones*, 1, 1–58 (1943).

has been treated by Alles¹⁵⁴ and by Gaddum.¹⁵⁵ The importance of choline is also emphasized by its inclusion as a component of the vitamin B complex. Another important biochemical function is undoubtedly related to its ability to act as a transmethyating agent.

Another role which choline plays and which may be entirely distinct from the other functions is as an essential component of the important phospholipid lecithin.

One important compound of choline which occurs only in animal tissues is acetylcholine. Baeyer¹⁵⁶ first synthesized this compound from choline chloride ("neurine" chloride) as indicated here.



Although it was assumed at an early date that acetylcholine or similar choline esters probably exist in animal tissues, acetylcholine was not isolated from them in pure form until 1929, when Dale and Dudley¹⁵⁷ succeeded in this undertaking. The fact that acetylcholine is able to form a chloroplatinate was given as proof by Baeyer that choline also contains an alcoholic hydroxyl group, a suggestion which Wurtz had established earlier.

Loewi¹⁵⁸ had discovered earlier that this same substance is liberated in heart muscle when the vagus nerve is stimulated. It was later demonstrated that a similar reaction occurs at the nerve endings of the parasympathetic nerves when a voluntary muscle is stimulated. This liberated substance has been found to be acetylcholine. The acetylcholine so produced occurs as a result of the activity of an enzyme, *choline acetylase*, while another enzyme, *choline esterase*, controls the concentration of acetylcholine by causing the hydrolysis of any excess.¹⁵⁹

Choline was first prepared from hops as a crystalline product by Griess and Harrow,¹⁶⁰ who noted that it was a colorless, odorless compound with an extremely bitter, caustic taste. One of the most outstanding properties of choline is its great tendency to absorb water. In spite of such hygroscopicity, Meyer and Hopff¹⁶¹ were able to prepare crystals satisfactorily from saturated aqueous solutions in a high-vacuum oven over phosphorus pentoxide.

¹⁵⁴ G. A. Alles, *Physiol. Revs.*, *14*, 276-307 (1934).

¹⁵⁵ J. H. Gaddum, *Ann. Rev. Biochem.*, *4*, 311-330 (1935).

¹⁵⁶ A. Baeyer, *Ann.*, *142*, 322-326 (1867).

¹⁵⁷ H. H. Dale and H. W. Dudley, *J. Physiol.*, *68*, 97-131 (1929).

¹⁵⁸ O. Loewi, *Arch. ges. physiol. (Pflüger's)*, *189*, 239-242 (1921); *Chem. Abst.*, *16*, 1267 (1922).

¹⁵⁹ G. L. Brown, *Physiol. Revs.*, *17*, 485-513 (1937).

¹⁶⁰ P. Griess and G. Harrow, *Ber.*, *18*, 717-719 (1885).

¹⁶¹ K. H. Meyer and H. Hopff, *Ber.*, *54*, 2274-2282 (1921).

Choline is extremely soluble in water. It also dissolves to a considerable extent in methyl and ethyl alcohols and formaldehyde, but it is only slightly soluble in amyl alcohol, acetone, and chloroform. It is practically insoluble in such excellent fat solvents as dry diethyl ether, petroleum ether, benzene, carbon disulfide, carbon tetrachloride, and toluene.^{162,163} The presence of small amounts of water in diethyl ether, or in ether-containing phospholipids, will result in the solution of appreciable amounts of the base.

The free base is quite susceptible to heat. It cannot be melted without decomposition to trimethylamine¹⁶⁴ and glycol.¹⁶¹ Wurtz¹⁴⁹ found that a dilute solution of choline was quite resistant to heat, whereas concentrated solutions decomposed when heated to 190°C. Although losses occur during evaporation to dryness, even of dilute solutions, it usually amounts to less than 10% if 0.3 mg. is present, but may reach 100% if the final amount is only 7 γ .¹⁶³ Less decomposition occurs when evaporation *in vacuo* is employed. On the other hand, choline chloride is much more stable to heat, as no appreciable destruction occurs at 180°C.¹⁴⁹ Choline is stable when boiled in barium hydroxide solution for 6 hours or in sodium ethylate for 24 hours.¹⁶²

Choline itself is a strong base which liberates ammonia from its salts and precipitates the salts of heavy metals. The chloride, sulfate, nitrate, oxalate, acetate, carbonate, picrate, and picrolonate are readily soluble both in water and in alcohol; on the other hand, the monophosphate, chloroplatinate, acid tartrate, and ruffianate dissolve in water but not in ethanol. Certain other salts are practically insoluble in water, and this fact has resulted in their usage in the quantitative determination of choline. Such highly insoluble salts include the periodate (oil¹⁶⁰ or crystalline material¹⁶⁵), the phosphotungstate, the phosphomolybdate, and the reineckate ($C_5H_{14}ON \cdot C_4H_7N_6S_4Cr$), as well as the double salts with gold chloride (chloroaurate, $C_5H_{14}ON \cdot Cl \cdot AuCl_3$) and with mercuric chloride ($C_5H_{14}ON \cdot Cl \cdot 6HgCl_2$). Insoluble salts are formed with Mayer's reagent (potassium mercuric iodide), as well as with Kraut's reagent (potassium bismuth iodide). The enneaiodide ($C_5H_{14}ON \cdot I \cdot I_8$) formed by treating choline chloride with an excess of potassium triiodide is very insoluble in water, as is the hexaiodide ($C_5H_{14}ON \cdot I \cdot I_5$).¹⁵³

A large number of qualitative methods for the detection of choline, as well as quantitative procedures for its estimation, are based upon the production of such insoluble compounds as those described above. Thus, the precipitation of choline from an alcoholic solution as a double salt with platinum, gold, or iron citrate is sensitive in dilutions of 1:2,000,000, although in aqueous solutions platinum chloride is of no value.¹⁶⁶ When aqueous solu-

¹⁶² W. Gulewitch, *Z. physiol. Chem.*, *24*, 513-541 (1898).

¹⁶³ W. Roman, *Biochem. Z.*, *219*, 218-231 (1930).

¹⁶⁴ G. Klein and H. Linsler, *Biochem. Z.*, *250*, 220-253 (1932).

¹⁶⁵ V. Staněk, *Z. physiol. Chem.*, *46*, 280-285 (1905).

¹⁶⁶ I. Sakakibara and T. Yoshinaga, *J. Biochem. Japan*, *23*, 211-239 (1936).

tions are employed, choline gives a precipitate with potassium triiodide in dilutions which vary between 1:20,000 and 1:2,000,000,¹⁶⁷ depending upon the conditions^{168,169}; precipitation occurs with the reineckate at 1:50,000, while the phosphotungstate and phosphomolybdate are precipitated at dilutions of approximately 1:20,000 and 1:10,000, respectively.¹⁶²

The most satisfactory microchemical determinations of choline in biological material have been based upon the insolubility of the choline periodate and of the choline reineckate. The original procedure for the use of the periodate employed by Staněk¹⁶⁵ was later modified to prevent the simultaneous precipitation of betaine by potassium triiodide¹⁷⁰; this could be avoided by carrying out the reaction in neutral or slightly alkaline solution, under which condition choline alone was precipitated. Staněk¹⁷¹ later showed that proteins, peptones, purines, alkaloids, trimethylamine, trigonelline, stachydrine, and muscarine may also separate out under certain specific conditions on treatment with potassium triiodide. Special modifications of the Staněk method have been suggested by Sharpe¹⁷² and later by Roman.¹⁶³ Further revisions of the Roman method have recently been proposed.^{42,173}

The reineckate procedure has proved non-specific, although it is an excellent and very sensitive method in the absence of interfering substances. Other quaternary bases, tertiary and secondary amines, heterocyclic compounds as well as ω -amino acids, all of which form insoluble reineckates, may cause fallacious results. Thus, in the determination of acetylcholine in muscle, the Kapfhammer and Bischoff procedure,¹⁷⁴ which involves the use of reineckate, gave a value of 194 γ per gram,¹⁷⁵ while the values obtained by either of the biological procedures^{176,177} did not exceed 0.08 γ per gram. This discrepancy may be due in part to the failure to prevent the rapid enzymic hydrolysis of acetylcholine; the procedure employed by Bischoff *et al.*¹⁷⁵ did not prevent this change. Strack and co-workers¹⁷⁸ also, were unable to prove the presence of free choline in beef, dog, or rabbit muscle. It was finally shown that the discrepancy between the reineckate and the biological methods was to be ascribed to the fact that carnitine,

¹⁶⁷ T. Kinoshita, *Arch. ges. Physiol. (Pflüger's)*, **132**, 607-631 (1910).

¹⁶⁸ F. J. Booth, *Biochem. J.*, **29**, 2064-2066 (1935).

¹⁶⁹ E. Kahane and J. Lévy, *Bull. soc. chim. biol.*, **21**, 223-240 (1939).

¹⁷⁰ V. Staněk, *Z. physiol. Chem.*, **47**, 83-87 (1906).

¹⁷¹ V. Staněk, *Z. physiol. Chem.*, **48**, 334-346 (1906).

¹⁷² J. S. Sharpe, *Biochem. J.*, **17**, 41-42 (1923).

¹⁷³ I. Reifer, *New Zealand J. Sci. Tech.*, **B22**, 111-116 (1941).

¹⁷⁴ J. Kapfhammer and C. Bischoff, *Z. physiol. Chem.*, **191**, 179-182 (1930).

¹⁷⁵ C. Bischoff, W. Grab, and J. Kapfhammer, *Z. physiol. Chem.*, **207**, 57-77 (1932).

¹⁷⁶ F. Plattner and E. Krannich, *Arch. ges. Physiol. (Pflüger's)*, **229**, 730-737 (1932); **230**, 356-362 (1932).

¹⁷⁷ H. C. Chang and J. H. Gaddum, *J. Physiol.*, **79**, 255-285 (1933).

¹⁷⁸ E. Strack, P. Wördehoff, E. Neubaur, and H. Geissendorfer, *Z. physiol. Chem.*, **233**, 189-203 (1935).

rather than choline, was forming the insoluble reineckate. Glick¹⁷⁹ and Entenman *et al.*¹⁸⁰ have reported modifications of the reineckate procedure for determining the choline content of plasma and tissue extracts. According to Entenman and Chaikoff¹⁸¹ both of these methods give identical values for blood choline, although the Glick technic gives lower values for choline in liver extract than does the procedure of Entenman and associates.^{180,181}

In the absence of interfering substances, any of several procedures may be satisfactorily employed for the determination of choline as the reineckate. This is true of the Beattie method,¹⁸² in which the choline reineckate is dissolved in acetone and is determined by colorimetric comparisons with a standard. The procedure of Jacobi, Baumann, and Meek¹⁸³ is considerably more specific. This involves extraction of the tissues with a 1:1 alcohol-ether mixture by boiling for 3 minutes; the extract is then evaporated to dryness, and the residue is saponified for 2 hours with baryta, followed by neutralization with acetic acid and precipitation with reineckate. It is stated¹⁸³ that the results obtained by this method agree with those determined by the biological procedure using the acetylation technic of Fletcher, Best, and Solandt.¹⁸⁴ Other modifications of the reineckate procedure have been proposed by Engel,¹⁸⁵ Shaw,¹⁸⁶ and by Winzler and Meserve.¹⁸⁷

Another group of methods involves the degradation of choline to trimethylamine, which may be determined by aeration into sulfuric acid. However, such procedures as those suggested by Lintzel and associates,^{188,189} as well as by Klein and Linser,¹⁶⁴ are open to the same criticism as are the earlier ones, in that they are nonspecific.

The biological methods have long been regarded as standard. One of these is based upon the effect of acetylcholine in producing contraction of smooth muscle. The method was proposed in 1906 by Hunt and Taveau,¹⁹⁰ who found that acetylcholine was 1000 to 100,000 times as active as choline in causing such a response. Probably the most satisfactory procedure is that of Chang and Gaddum¹⁷⁷ as modified by Fletcher *et al.*¹⁸⁴

An entirely new biological procedure has been made available by the use of the ascomycete, the red bread mold *Neurospora crassa*.¹⁹¹ One of the two mutants which do not produce choline can grow only if choline itself is spe-

¹⁷⁹ D. Glick, *J. Biol. Chem.*, *156*, 643-651 (1944).

¹⁸⁰ C. Entenman, A. Tauger, and I. L. Chaikoff, *J. Biol. Chem.*, *155*, 13-18 (1944).

¹⁸¹ C. Entenman and I. L. Chaikoff, *J. Biol. Chem.*, *160*, 377-385 (1945).

¹⁸² F. J. R. Beattie, *Biochem. J.*, *30*, 1554-1559 (1936).

¹⁸³ H. P. Jacobi, C. A. Baumann, and W. J. Meek, *J. Biol. Chem.*, *138*, 571-582 (1941).

¹⁸⁴ J. P. Fletcher, C. H. Best, and O. M. Solandt, *Biochem. J.*, *29*, 2278-2284 (1935).

¹⁸⁵ R. W. Engel, *J. Biol. Chem.*, *144*, 701-710 (1942).

¹⁸⁶ F. H. Shaw, *Biochem. J.*, *32*, 1002-1007 (1938).

¹⁸⁷ R. J. Winzler and E. R. Meserve, *J. Biol. Chem.*, *159*, 395-397 (1945).

¹⁸⁸ W. Lintzel and S. Formin, *Biochem. Z.*, *238*, 438-451 (1931).

¹⁸⁹ W. Lintzel and G. Monasterio, *Biochem. Z.*, *241*, 273-279 (1931).

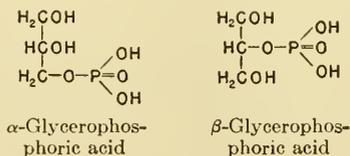
¹⁹⁰ R. Hunt and R. de M. Taveau, *Brit. Med. J.*, *1906*, *II*, 1788-1791.

¹⁹¹ N. H. Horowitz and G. W. Beadle, *J. Biol. Chem.*, *150*, 325-333 (1943).

cifically supplied. Since the growth of this fungus is proportional to the amount of choline present when a suboptimal range of choline is available, the weight of *Neurospora* produced under certain standardized conditions may be used as a method for the quantitative measurement of choline. With the so-called *cholinesless* strain, crude extracts can be analyzed, as the substances which interfere with the periodate or reineckate methods are entirely inactive. Combined choline is not determined unless hydrolysis has been carried out previously. This method has the advantage not only of being specific but also of possessing a very high sensitivity. However, it does require several days for the completion of a test. A description of methods for carrying out this procedure is given by Horowitz and Beadle,¹⁹¹ as well as by Luecke and Pearson.¹⁹²

c. Glycerophosphoric Acid. Glycerophosphoric acid is one of the end products of the hydrolysis of lecithin, especially when alkali is employed. The hydrolysis of the linkage between glycerol and phosphoric acid appears to be difficult, and the organic phosphate is the usual end product. Levene and Rolf¹⁹³ first identified glycerophosphate as a hydrolysis product of "cephalin," which they believed to be identical with the product obtained from lecithin. Glycerophosphates have also been prepared from plant phosphatides.^{34, 65, 141, 143, 144, 194-196}

Two types of glycerophosphates are known, depending upon whether the phosphoric acid is combined with the glycerol through the hydroxyl group on the α - or on the β -carbon atom. These have the structures shown here.



Both types of glycerophosphates can be separated from natural sources. They can be readily distinguished, since the α -form contains an asymmetric carbon atom and hence is optically active, while the β -compound is optically inactive. Willstätter and Lüdecke³¹ concluded in 1904 that lecithins have the α -structure, since optically-active α -glycerophosphate can be prepared from them. Although other workers were able to prepare optically active glycerophosphate from other phosphatides, it was later shown that

¹⁹² R. W. Luecke and P. B. Pearson, *J. Biol. Chem.*, **155**, 507-512 (1944).

¹⁹³ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, **40**, 1-16 (1919).

¹⁹⁴ V. Njegovan, *Z. physiol. Chem.*, **76**, 1-26 (1911-1912).

¹⁹⁵ E. Schulze and A. Ikiernik, *Z. physiol. Chem.*, **15**, 405-414 (1891).

¹⁹⁶ G. Trier, *Z. physiol. Chem.*, **86**, 1-32 (1913).

the β -form^{32,197,198} likewise occurred, as well as the α -compound.^{33,34,199-203} Bailly³³ was able to prove that a crystalline sodium salt of β -glycerophosphoric acid could be isolated from the lecithin prepared from egg and brain, as well as an amorphous sodium salt of α -glycerophosphoric acid. The two esters can be separated by virtue of the variability in solubility of their sodium soaps.¹⁹⁹ The α -form is the more soluble. On oxidation of the ester in solution, a ketone is formed which responds to Denigés' reaction. This can only be interpreted to mean that it is an α -ester.

Karrer and Salomon³⁴ have devised a quantitative method for the separation of the α - and β -glycerophosphates from water solution as the barium salts by the addition of barium nitrate. The β -ester precipitates almost quantitatively as the double salt with barium nitrate $[(C_3H_7O_6PBa)_2 \cdot Ba(NO_3)_2]$ in a practically pure state. The barium α -glycerophosphate can be removed after concentration of the filtrate. These workers were also able to separate the α -glycerophosphate from the crude mixture as the dimethyl ester of the dimethyl ether.

Glycerophosphate can readily be prepared from lecithin by the method of Willstätter and Lüdecke.³¹ This involves shaking the phosphatide with 10% barium hydroxide over a prolonged period at room temperature. The hydrolysis may also be effected in a shorter period by refluxing the mixture without influencing the end result.¹⁹³ After removal of the excess of barium hydroxide with carbon dioxide, the filtrate is concentrated and the crude barium glycerophosphate is precipitated by the addition of alcohol. After several reprecipitations from water by alcohol, it is obtained in pure form as an amorphous white powder.

Grün and Limpächer^{61,89} have suggested the possibility that β -lecithin does not occur in the natural state, or at least not in the proportions indicated by analysis, but that it is formed during the analytical procedures. However, this would seem improbable, according to the data of Karrer and Benz,²⁰⁰ since no such behavior occurred on saponification of synthetic α -glycerophosphate with barium hydroxide. Rae³⁷ has also found that no migration of phosphate from the α - to the β -position occurs during the hydrolysis of the phospholipid with baryta.

The distribution of the glycerophosphates in lecithins and other phospholipids is recorded in Table 5.

The values for optical activity observed for the various glycerophosphates, which have usually been separated as their calcium or barium salts,

¹⁹⁷ F. B. Power and F. Tutin, *J. Chem. Soc.*, 87, 249-257 (1905).

¹⁹⁸ F. Tutin and A. C. O. Hann, *J. Chem. Soc.*, 89, 1749-1758 (1906).

¹⁹⁹ L. Grimbert and O. Bailly, *Compt. rend.*, 160, 207-210 (1915).

²⁰⁰ P. Karrer and P. Benz, *Helv. Chim. Acta*, 10, 87-91 (1927).

²⁰¹ O. Bailly, *Compt. rend.*, 160, 395-398 (1915).

²⁰² O. Bailly, *Ann. chim.*, 6, 215-278 (1916).

²⁰³ O. Bailly, *Bull. soc. chim. biol.*, 1, 152-162 (1919); *Chem. Zentr.*, 1919, I, 84.

TABLE 5
PROPORTION OF β -GLYCEROPHOSPHATE OBTAINED FROM VARIOUS PHOSPHOLIPIDS^a

Phosphatide	Characterization	Minimal content obtained as Ba salt, %
Lecithin		
Egg	Crystalline	80
Egg	Seprn. by freezing from ether-purified lecithin prepd. according to Escher ^b	84
Egg	Commercial	70
Brain	—	78
Cephalin (?)		
Egg	Phospholipid difficultly sol. in alcohol	45
Sphingomyelin (?)		
Egg	Insol. phospholipid	73

^a Adapted from P. Karrer and H. Salomon, *Helv. Chim. Acta*, 9, 3-23 (1926).

^b H. H. Escher, *Helv. Chim. Acta*, 8, 686-691 (1925).

have been extremely variable. A number of the animal and plant phosphatides have yielded levo-rotatory salts,^{141,193} while other workers could not detect any optical activity in their preparations.^{197,202} In other cases, glycerophosphates having a dextro-rotation have been prepared from plant phospholipids,^{144,196} as well as from preparations of brain lecithin and cephalin.²⁰⁴ On the other hand, Karrer and Salomon³⁴ were able to separate not only an optically inactive barium salt but also a dextro-rotatory as well as a levo-rotatory glycerophosphate from the same preparation. The optical properties of some α -glycerophosphates are summarized in Table 6.

The hydrolysis of α - or β -glycerophosphoric acid to glycerol and phosphoric acid cannot be brought about by bases. It proceeds only slowly when dilute acids are employed.^{205,206} However, a much more effective hydrolysis of the ester is produced by the enzyme *glycerophosphatase*. This enzyme occurs in such plant sources as yeast,²⁰⁷ takadiastase,^{208,209} and in many plant seeds.²¹⁰⁻²¹² It has also been shown to be present in many animal tissues, including the central nervous system,²¹³ intestinal mucosa, kidneys, and, to a lesser extent, the lungs, with traces in the liver and spleen.^{214,215} It is, however, absent from the pancreas, muscle, and

²⁰⁴ S. Fränkel and L. Dimitz, *Biochem. Z.*, 21, 337-347 (1909).

²⁰⁵ F. Malengreau and G. Prigent, *Z. physiol. Chem.*, 73, 68-84 (1911).

²⁰⁶ R. H. A. Plimmer, *Biochem. J.*, 7, 72-80 (1913).

²⁰⁷ C. Neuberg and L. Karczag, *Biochem. Z.*, 36, 60-67 (1911).

²⁰⁸ S. Akamatsu, *Biochem. Z.*, 142, 184-185 (1923).

²⁰⁹ S. Akamatsu, *Biochem. Z.*, 142, 186-187 (1923).

²¹⁰ A. Nemec, *Biochem. Z.*, 93, 94-100 (1919).

²¹¹ A. Nemec, *Biochem. Z.*, 137, 570-575 (1923).

²¹² A. Nemec, *Biochem. Z.*, 202, 229-235 (1928).

²¹³ V. Vondracik, *Biochem. Z.*, 191, 88-94 (1927).

²¹⁴ P. Grosser and J. Husler, *Biochem. Z.*, 39, 1-5 (1912).

²¹⁵ R. H. A. Plimmer, *Biochem. J.*, 7, 43-71 (1913).

TABLE 6
OPTICAL ACTIVITY OF SOME α -GLYCEROPHOSPHATE DERIVATIVES

α -Glycerophosphate derivative	Formula	$[\alpha]_D^{20}$	Remarks
<i>l</i> (-)-Acid.....	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{OPO}(\text{OH})_2$	-1.45 ^a	
-dimethyl ester of dimethyl ether.....	$\text{CH}_2\text{OCH}_3\cdot\text{CHOCH}_3\cdot\text{CH}_2\text{OPO}_3(\text{CH}_3)_2$	-4.78 ^a (alc.)	B.p., 87-88°C. _{0.13}
Synthetic.....	—	-3.2 ^b	B.p., 125-126°C. _{0.3}
From phosphatides.....	—	-4.40 ^c	
From glycolysis and fermentation.....	—	-5.71 ^a (alc.)	Rotation varies with H ₂ O cont. of alc.
-diethyl ester of diethyl ether.....	$\text{CH}_2\text{OC}_2\text{H}_5\cdot\text{CHOC}_2\text{H}_5\cdot\text{CH}_2\text{OPO}_3(\text{C}_2\text{H}_5)_2$	-5.31 ^a	Homogeneous
Ag salt.....	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{O}\cdot\text{PO}_3\text{Ag}_2$	+1.0 ^a	
Ba salt.....	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{O}\cdot\text{PO}_3\text{Ba}$	-1.45 ^a	
Li salt.....	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{O}\cdot\text{PO}_3\text{Li}_2$	+3.51 ^d	
<i>d</i> (+)-Acid.....	$\text{CH}_2\text{OC}_2\text{H}_5\cdot\text{CHOC}_2\text{H}_5\cdot\text{CH}_2\text{OPO}_3(\text{C}_2\text{H}_5)_2$	+6.69 ^e (alc.)	Homogeneous
-diethyl ester of diethyl ether.....	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{O}\cdot\text{PO}_3\text{Li}_2$	+5.94 ^e	
Li salt.....		-3.02 ^d	

^a E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 128, 491-500 (1939).

^b P. Karrer and H. Salomon, *Helv. Chim. Acta*, 9, 3-23 (1926).

^c O. Meyerhof and W. Kiessling, *Biochem. Z.*, 264, 40-71 (1933); 267, 313-348 (1933).

^d E. Abderhalden and E. Eichwald, *Ber.*, 51, 1308-1312 (1918). Specific rotation determined at 18°C.

^e E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 135, 321-328 (1940).

blood.^{214,215} Forrai²¹⁶ was also able to demonstrate the enzyme in adrenal tissue, the thyroid, testis, and malignant tissue; it is found in cartilage, where it would appear to be of importance in bone formation.^{217,218} Glycerophosphatase obtained from the digestive tract, kidney, lung, liver, and bone has the optimum activity at the same pH of about 9.0.^{219,220} It is thermolabile and apparently requires a co-factor for maximum activity.²²¹⁻²²³ The enzyme acts effectively on both the α - and the β -glycerophosphates,^{224,225} although Kay²¹⁹ is of the opinion that the β -form should be more readily split than the α -isomer. Glycerophosphatase may bring about a synthesis of glycerophosphate from its components.²²⁰ It is believed to be identical with hexosediphosphatase and nucleotidase.²²⁰

Both optically active forms of α -glycerophosphate have been synthesized by Abderhalden and Eichwald.²²⁶ Karrer and Benz²²⁷ also prepared these compounds, but were only partially successful in resolving the synthetic product of Fischer and Pfähler²²⁸ through the quinine salt. Baer and Fischer²²⁹ were able to prepare $l(-)$ - α -glycerophosphoric acid from $d(+)$ -acetoneglycerol, which was found to be 100% utilizable biologically. The $d(+)$ - α -glycerophosphoric acid was later prepared by these investigators,²³⁰ starting with $l(-)$ -acetoneglycerol. It was readily hydrolyzed by various phosphatases with an even greater velocity than the $l(-)$ - α -glycerophosphoric acid. However, it could not be utilized by the muscle press juice according to the procedure of Meyerhof and Kiessling,^{231,232} in contrast to the complete utilization of the $l(-)$ -isomer. One must conclude that the $l(-)$ - rather than the $d(+)$ - α -glycerophosphate is the physiologically utilizable form.

β -Glycerophosphate has been prepared synthetically by Tutin and Hann¹⁹³ and later by King and Pyman.²³³ It is optically inactive. Bur-

²¹⁶ E. Forrai, *Biochem. Z.*, *142*, 282-290 (1923).

²¹⁷ R. Robison, *Biochem. J.*, *17*, 286-293 (1923).

²¹⁸ R. Robison and K. M. Soames, *Biochem. J.*, *18*, 740-754 (1924).

²¹⁹ H. D. Kay, *Biochem. J.*, *20*, 791-811 (1926).

²²⁰ H. D. Kay, *Biochem. J.*, *22*, 855-866 (1928).

²²¹ H. Erdtman, *Z. physiol. Chem.*, *172*, 182-198 (1927).

²²² H. Erdtman, *Z. physiol. Chem.*, *177*, 211-220 (1928).

²²³ H. Kobayashi, *J. Biochem. Japan*, *8*, 205-223 (1927).

²²⁴ P. Fleury and Z. Sutu, *Bull. soc. chim.* [4], *39*, 1716-1718 (1926).

²²⁵ P. Karrer and R. Freuler, "Die enzymatische Spaltung der α - und β -Glycerin-Phosphorsäuren," *Festschrift Alex Tschirch*, 1926, p. 42. Cited from H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930, p. 119.

²²⁶ E. Abderhalden and E. Eichwald, *Ber.*, *51*, 1308-1312 (1918).

²²⁷ P. Karrer and P. Benz, *Helv. Chim. Acta*, *9*, 23-25 (1926).

²²⁸ E. Fischer and E. Pfähler, *Ber.*, *53*, 1606-1621 (1920).

²²⁹ E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, *128*, 491-500 (1939).

²³⁰ E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, *135*, 321-328 (1940).

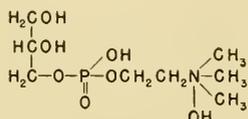
²³¹ O. Meyerhof and W. Kiessling, *Biochem. Z.*, *264*, 40-71 (1933).

²³² O. Meyerhof and W. Kiessling, *Biochem. Z.*, *267*, 313-348 (1933).

²³³ H. King and F. L. Pyman, *J. Chem. Soc.*, *105*, 1238-1259 (1914).

master²³⁴ has recently reported a microdetermination method for α - and β -glycerophosphate based on the colorimetric measurement of orthophosphate produced by the action of periodate. The recent literature on glycerophosphates has been reviewed by Fischer and Baer.²³⁵

d. Glycerolphosphorylcholine. Another lecithin intermediate which has been isolated from beef pancreas,^{117,120} from the reaction mixture when an extract of rice hulls acts upon lysolecithin,¹¹⁴ and from the action of minced intestine on lecithin,^{118,119} is glycerolphosphorylcholine. Both the α - and the β -form have now been reported as isolated from pancreas.^{120,236} This compound results from the action of lecithinase B.



α -Glycerolphosphorylcholine

Although a number of methods have been suggested for the synthesis of β -glycerolphosphorylcholine,^{236a-236c} Aloisi and Buffa²³⁶ have concluded that the product obtained in each case was the choline salt of β -glycerophosphoric acid rather than the choline ester. The latter workers²³⁶ did, however, succeed in preparing choline α - and β -glycerophosphate in beautiful crystalline form.

Pure glycerolphosphorylcholine is soluble in water and alcohol, but it does not dissolve in acetone, ether, or petroleum ether. It possesses a levo-rotation ($[\alpha]_D^{20} = -4.8^\circ$).¹¹⁷ The choline-phosphoric acid linkage in glycerolphosphorylcholine is very susceptible to rupture. Thus, when it is heated with 1 *N* HCl for 30 minutes on the water bath, a complete hydrolysis to choline and glycerophosphate takes place. This behavior is in marked contrast to that of phosphorylcholine, where the corresponding linkage is very resistant toward acids.²³⁷ Glycerolphosphorylcholine is hydrolyzed by alkaline phosphatase, but the rate of hydrolysis is as much as 150 times slower than that which occurs when glycerophosphate is the substrate. Thannhauser and Schmidt¹⁰ state that glycerolphosphorylcholine is the only intermediate of lecithin which has been identified in mammalian metabolism.

²³⁴ C. F. Burmaster, *J. Biol. Chem.*, **164**, 233-240 (1946).

²³⁵ H. O. L. Fischer and E. Baer, *Chem. Revs.*, **29**, 287-316 (1941).

²³⁶ M. Aloisi and P. Buffa, *Biochem. J.*, **43**, 157-160 (1948).

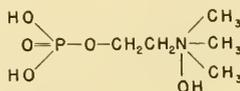
^{236a} H. Arnold, *Ber.*, **B73**, 87-90 (1940).

^{236b} C. Ravazzoni and A. Fenaroli, *Ann. chim. applicata*, **30**, 318-323 (1940); *Chem. Abst.*, **35**, 1765 (1941).

^{236c} S. Belfanti, A. Ercoli, and M. Franconioli, in E. Bamann and K. Myrbäck, *Die Methoden der Fermentforschung*, Vol. I, Thieme, Leipzig, 1941, pp. 80-110.

²³⁷ E. Baer and C. S. McArthur, *J. Biol. Chem.*, **154**, 451-460 (1944).

e. **Phosphorylcholine.** Phosphorylcholine is one of the substances formed by the action of lecithinase D on lecithin. Although there is no proof that such an enzyme occurs in animal tissues, it has been noted earlier that it is present in the gas bacillus (*Clostridium welchii*). Phosphorylcholine may play an important role in intermediary metabolism, as is indicated by Taurog's²³³ demonstration that an incorporation of P³² from phosphorylcholine into both kidney and liver phospholipid occurs *in vivo*. Riley²³⁹ has also proved that phosphorylcholine disappears rapidly from the blood stream when it is injected, and appears as an inorganic phosphate in the blood.



Phosphorylcholine

Phosphorylcholine is resistant to acid hydrolysis, in contrast to the behavior of lecithin and of other choline esters.^{240,241} This hydrolysis has been found to follow a pseudo-unimolecular reaction [$k_{\log 10} = 1.16 \times 10^{-3}$].²³⁷ However, although only an insignificant hydrolysis does occur in acid at 100°C., the fact that hydrolysis results at 125°C. leads Baer and McArthur²³⁷ to the conclusion that it should be included in the same class as α -glycerophosphoric acid, β -glycerophosphoric acid, and glyceric acid-3-phosphoric acid, insofar as stability toward acid is concerned. It has been suggested that the stability of phosphorylcholine to acid is due to the fact that it is a mono-ester of phosphoric acid.²³⁷

Phosphorylcholine is also extremely resistant to alkaline hydrolysis. It is not saponified by heating at 100°C. for 100 hours with 2 *N* alkali nor with 10 *N* alkali for 30 minutes at 118–120°C. However, on raising the saponification temperature to 125°C. and extending the period to 50 hours, Baer and McArthur were able to demonstrate a 40% hydrolysis. Many side reactions occur which result in the destruction of the free ester or of the choline. Although phosphatase hydrolyzes phosphorylcholine,²⁴⁰ it is not split by true choline esterase or by pseudocholine esterase. It is suggested that it may play a more important role in phosphate metabolism than it does in the intermediary metabolism of choline.

Phosphorylcholine can be readily synthesized by a number of procedures. These include phosphorylation of choline halide with POCl₃.^{240,242}

²³³ A. Taurog, *Unpublished experiments*, reported by I. L. Chaikoff, *Physiol. Revs.*, **22**, 310 (1942).

²³⁹ R. F. Riley, *J. Biol. Chem.*, **153**, 535–549 (1944).

²⁴⁰ R. H. A. Plimmer and W. J. N. Burch, *Biochem. J.*, **31**, 398–409 (1937).

²⁴¹ A. B. L. Beznák and E. Chain, *Quart. J. Exptl. Physiol.* **26**, 201–214 (1936–1937).

²⁴² E. Schmidt, *Ann.*, **337**, 37–121 (1904).

or with phosphorus pentoxide with or without anhydrous phosphoric acid^{60,61,240,241,243,244} or ethyl metaphosphate.²⁴⁵ Another type of procedure involves the phosphorylation of ethylene dichlorohydrin by phosphorus oxychloride^{240,243,245,246} or ethyl metaphosphate,^{245,247,248} followed by the transformation of phosphorylethylenechlorohydrin into phosphorylcholine by means of trimethylamine. Baer and McArthur²³⁷ have recently introduced an improved procedure involving the use of diphenylphosphorylchloride as the phosphorylating agent. By a slight modification of this procedure, Baer²⁴⁹ has been able to dispense with the use of the gold salts without essentially altering the yield or sacrificing the purity of the product.

(10) *Hydrolecithin*

Hydrolecithin is a completely saturated compound which was first isolated as a natural product by Lesuk and Anderson²⁵⁰ from *Cysticercus fasciolaris* (which is the larval form of the tapeworm, *Taenia crassicollis*, of cats). Dihydrosphingosine was also found. Thannhauser and co-workers²⁵¹ have more recently identified the glycerol-containing contaminant in sphingomyelin preparations of beef lung as dihydrolecithin. This new phosphatide occurs in a proportion of 20 to 40% of the total sphingomyelin. Considerable amounts likewise occur in the brain and spleen.

Hydrolecithin was tentatively identified as dipalmitolecithin.²⁵¹ In a later report,²⁵² it was shown that the product is probably identical with that obtained from the tapeworm by Lesuk and Anderson.²⁵⁰ It was found to differ markedly from the saturated lecithins prepared by hydrogenation of egg-yolk,^{85,86,253} as well as from that obtained from brain by Levene and Rof.⁵¹ It is also distinct from synthetic distearolecithins.^{60,61}

4. The Cephalins

(1) *Multiple Nature of the Cephalins*

Until the demonstration by Folch²⁵ of the multiple nature of the cephalin complex, it was assumed that only two types of cephalin existed, which were

²⁴³ E. L. Jackson, *J. Am. Chem. Soc.*, *57*, 1903-1905 (1935).

²⁴⁴ F. Inukai and W. Nakabara, *Proc. Imp. Acad. Tokyo*, *11*, 260-261 (1935).

²⁴⁵ E. Abderhalden, H. Paffrath, and H. Siekel, *Arch. ges. Physiol. (Pflüger's)*, *207*, 241-253 (1925).

²⁴⁶ R. R. Renshaw and C. Y. Hopkins, *J. Am. Chem. Soc.*, *51*, 953-954 (1929).

²⁴⁷ K. Langheld, *Ber.*, *44*, 2076-2087 (1911).

²⁴⁸ T. L. McMeeekin, *J. Am. Chem. Soc.*, *59*, 2383-2386 (1937).

²⁴⁹ E. Baer, *J. Am. Chem. Soc.*, *69*, 1253-1254 (1947).

²⁵⁰ A. Lesuk and R. J. Anderson, *J. Biol. Chem.*, *139*, 457-469 (1941).

²⁵¹ S. J. Thannhauser, J. Benotti, and N. F. Boncoddio, *J. Biol. Chem.*, *166*, 669-675 (1946).

²⁵² S. J. Thannhauser and N. F. Boncoddio, *J. Biol. Chem.*, *172*, 135-139 (1948).

²⁵³ C. Paal and H. Oehme, *Ber.*, *46*, 1297-1304 (1913).

identical except for the fact that the phosphorylethanolamine was combined with the α - or the β -carbon of glycerol. By dissolving a cephalin complex from brain in chloroform and gradually precipitating with increasing concentrations of alcohol, Folch²⁵ was able to obtain 5 fractions, 3 of which were relatively pure, while the intermediate 2 (II and IV) appeared to be mixtures. Fraction I, which contained inositol phosphatide, was least soluble in alcohol. Fraction III consisted largely of phosphatidylserine, while fraction V consisted almost entirely of phosphatidylethanolamine. Folch's data are summarized in Table 7.

TABLE 7
ANALYSIS OF FRACTIONS ISOLATED FROM BRAIN CEPHALIN BY THE CHLOROFORM-ALCOHOL METHOD^a

Components	Fr. I, %	Fr. II, %	Fr. III, %	Fr. IV, %	Fr. V, %
C	55.0	59.0	60.2	63.0	66.1
P	4.25	3.86	3.58	3.60	3.65
N	1.15	1.36	1.62	1.75	1.78
Amino N ^b	1.15	1.36	1.64	1.60	1.50
Carboxyl N ^c	0.70	0.80	1.47	0.60	<0.02
Inositol	6.8	3.4	<0.20	<0.20	<0.20
Iodine No.	65.0	—	39.8	—	78.0
Ash	16.7	—	12.8	—	2.5
Yield ^d	22.0	10.0	27.0	8.0	15.0

Fraction I, inositol phosphatide
 Fractions II and IV, mixtures
 Fraction III, phosphatidyl serine
 Fraction V, phosphatidyl ethanolamine

^a J. Folch, *J. Biol. Chem.*, *146*, 35-44 (1942).

^b By nitrous acid manometric method of D. D. Van Slyke, *J. Biol. Chem.*, *83*, 449-461 (1929).

^c By ninhydrin-CO₂ method of D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen, and P. Hamilton, *J. Biol. Chem.*, *141*, 627-669 (1941).

^d In grams per 100 g. cephalin.

It would thus appear that cephalin as formerly referred to in the literature is a mixture containing at least three types of molecules, instead of only phosphatidylethanolamine. The general facts about cephalin as gleaned from the early work in this field will be included under the section on phosphatidylethanolamine, with the full realization that much of the data may refer to preparations which are quite impure and which may, in fact, be mixtures of the several types of cephalins.

(2) Phosphatidylethanolamine and Cephalins in General

a. Structure of Phosphatidylethanolamine. The presence of a second phosphatide in the brain, in addition to lecithin, which had a N:P ratio

A preparation (cited by Renall) from cattle brain contained 87% of nitrogen in the form of amino nitrogen, while in that from sheep brain the proportion was 97.5%.

The procedure of Levene and Rolf²⁵⁶ is quite similar. Their preparations were practically free from non-amino nitrogen. The yield was 18 g. for 18 kg. of brain freed from extraneous membrane.

c. Synthesis of Phosphatidylethanolamine. α - and β -Cephalins were successfully synthesized by Grün and Limpächer⁵⁹ by methods similar to those employed for the preparation of lecithin, with the exception that colamine replaces choline. α, β -Distearocephalin and α, α' -distearocephalin have been synthesized by this procedure.

In the method of Kabashima,^{64, 257} dipalmitocephalin was synthesized by heating the monosilver salt of dipalmitoglycerophosphoric acid with bromethylamine picrate. The method was also applicable to distearo- α -cephalin.²⁵⁸ Rose²⁵⁹ has obtained cephalin synthetically by reacting either carbo-benzoxyethanolamine or β -hydroxyethylphthalimide with crude dipalmitoglycerophosphoryl chloride. The cephalin prepared by both of the latter procedures was found by Rose to be identical with that synthesized by the Kabashima method.

d. Properties of Phosphatidylethanolamine. When carefully prepared, cephalin is a colorless solid but, like lecithin, it readily darkens to a reddish brown color when exposed to light or air. It is very hygroscopic, but maintains a constant weight in a vacuum desiccator. Brain cephalin melts at 174° to 175°C.^{260, 261}

Cephalin is insoluble in dry diethyl ether, but it dissolves in all proportions in ether containing 1% of water. It is soluble in such fat solvents as chloroform, petroleum ether, carbon disulfide, benzene, and hot acetic acid. However, it does not dissolve in alcohol or acetone. It differs from lecithin by virtue of its insolubility in alcohol. Some cephalin is dissolved in alcohol in the presence of lecithin. Cephalin forms an emulsion with water, from which it may be precipitated by hydrochloric acid, other mineral acids, or concentrated solutions of oxalic acid. Cephalin produces myelin forms in the presence of water, in much the same way as does lecithin. Cephalin is also precipitated from aqueous suspension by barium hydroxide, calcium hydroxide, platinum chloride, cadmium chloride, lead acetate, ammonia, and by other metallic salts.²⁶² The cadmium chloride compound has less than an equivalent amount of the salt. The same is true in the case of the

²⁵⁷ I. Kabashima and B. Suzuki, *Proc. Imp. Acad. Japan*, 8, 492-495 (1932).

²⁵⁸ I. Kabashima, *Ber.*, 71, 1071-1073 (1938).

²⁵⁹ W. G. Rose, *J. Am. Chem. Soc.*, 69, 1384-1387 (1947).

²⁶⁰ J. Parnas, *Biochem. Z.*, 22, 411-432 (1909).

²⁶¹ S. Fränkel and E. Neubauer, *Biochem. Z.*, 21, 321-336 (1909).

²⁶² J. L. W. Thudichum, *Die chemische Konstitution des Gehirns des Menschen und der Tiere*, Pietzcker, Tübingen, 1901.

platinum chloride compound. Whereas the lecithin-cadmium chloride is insoluble in ether, the opposite is true for the cephalin-cadmium chloride salt.

According to Thudichum,²⁶² the lead salt of cephalin is soluble in ether and insoluble in alcohol. However, Levene and West^{263,264} found that a purified lead salt, in the form of an amorphous yellow powder, is insoluble in ether, alcohol, or acetone, but that it is dissolved more or less in benzene, toluene, and pyridine. Its empirical formula was calculated as $C_{41}H_{74}NPO_{13}Pb_2$.²⁶³ Cephalin reacts with nitrous acid by virtue of its amino group.^{255,265,266}

Because of its unsaturated component fatty acids, cephalin will readily add bromine. The iodine number of the preparation of Fränkel and Neubauer²⁶¹ was 80, while that of Wagner²⁶⁷ was 40.7. The addition of hydrogen with a palladium catalyst proceeds somewhat unsatisfactorily.^{263,268,269} Hydrocephalin has a formula of $C_{41}H_{82}NPO_8$ and a specific rotation in chloroform of $+6.0^\circ$. The phosphatide itself exhibits a slight levo-rotation in chloroform, although a somewhat less pure preparation has been reported to show a strong dextro-rotatory activity in petroleum ether ($[\alpha]_D = +13.6^\circ$). Cephalin acts as a fairly strong acid, in contrast to the behavior of lecithin, which acts as a base. Cephalin is susceptible to autoxidation, especially in the presence of ferric chloride.

Cephalin is able to form stable compounds with proteins under certain conditions where neither lecithin nor sphingomyelin will react. Chargaff²⁷⁰ prepared a stable salmine-cephalin compound at pH ranges from 2 to 11 which was believed to be a water-insoluble salt between the strongly basic protamine and the acidic phosphatide. The salt had a composition of 80% of cephalin and 20% of salmine. Although with strongly alkaline buffers an insoluble compound was formed with lecithin, no such combination obtains within the physiological range. Chargaff²⁷⁰ was also able to synthesize a similar insoluble salt with egg albumin at a pH of 2, 3, or 4. It would appear that this ability of cephalin to react is related to the position of the isoelectric point of the protein, which is 12 in the case of salmine²⁷¹ and 4.8 for egg albumin.²⁷²

On heating with water, weakly basic or acidic solutions, phosphatidyleth-

²⁶³ P. A. Levene and C. J. West, *J. Biol. Chem.*, *24*, 41-53 (1916).

²⁶⁴ P. A. Levene and C. J. West, *J. Biol. Chem.*, *24*, 111-116 (1916).

²⁶⁵ A. Baumann, *Biochem. Z.*, *54*, 30-39 (1913).

²⁶⁶ G. Trier, *Z. physiol. Chem.*, *86*, 141-152 (1913).

²⁶⁷ R. Wagner, *Biochem. Z.*, *64*, 72-81 (1914).

²⁶⁸ P. A. Levene and C. J. West, *J. Biol. Chem.*, *25*, 517-519 (1916).

²⁶⁹ P. A. Levene and S. Komatsu, *J. Biol. Chem.*, *39*, 91-104 (1919).

²⁷⁰ E. Chargaff, *J. Biol. Chem.*, *125*, 661-670 (1938).

²⁷¹ S. Miyake, *Z. physiol. Chem.*, *172*, 225-229 (1927).

²⁷² C. L. A. Schmidt, in M. Sahyun, *Outline of the Amino Acids and Proteins*, Reinhold, New York, 1944, pp. 41-72.

anolamine can be hydrolyzed to fatty acids, glycerophosphoric acid, and colamine. Hydrolysis with barium hydroxide proceeds very slowly, in contradistinction to the reaction between alkali and lecithin. While lecithin is hydrolyzed to the extent of 90% merely by shaking with a saturated barium hydroxide solution at room temperature for 16 hours, phosphatidylethanolamine under similar conditions is split only to the extent of 19%. Levene and Rolf¹⁹³ have likewise shown the difficulty of preparing nitrogen-free glycerophosphoric acid from cephalin. Complete hydrolysis of cephalin occurs when the solution is boiled for 2 to 3 hours with 6% hydrochloric acid,²⁷³ or on continuous agitation of a 50-g. sample of cephalin at 60°C. with a mixture of 300 ml. of a 20% sulfuric acid solution or a mixture of 75 g. of cephalin in 250 ml. of water and 550 ml. of concentrated hydrochloric acid.²⁶⁵ In spite of the difficult hydrolysis with barium hydroxide, there seems to be little evidence that phosphatidylethanolamine is more difficultly hydrolyzable with other hydrolyzing agents than is lecithin. There is no apparent mechanism of enzymatic destruction or hydrolysis of cephalin. A breakdown may possibly be caused by a non-specific esterase, but such a reaction has not as yet been demonstrated.

e. Lysocephalin. Although the snake venoms bring about profound changes in the cephalin molecule, the proof of the nature of the lysocephalin molecule so produced is not so clear-cut as is the case with lysolecithin. Lysocephalin produced by cobra venom has too low a carbon value to correspond to the theory for monostearocephalin, and the ash content is especially high.

Lysocephalin has been shown to form a definite part of the lysophosphatides produced by snake venoms. After cephalin had been acted on by cobra venom (*Naja flava*), lysocephalin was found to comprise 22%⁶³ and 20%¹⁰⁰ of the total lysophosphatides; after treatment with fer-de-lance venom (*Bothrops atrox*), the corresponding amounts of lysocephalin found were 23⁹⁹ and 24.5%¹⁰⁰, while after the action of water-moccasin venom (*Ancistrodon piscivorus*) the values varied between 30 and 32%¹⁰⁰. Chargaff and Cohen¹⁰⁰ have shown that purified preparations of lysocephalins have no effect upon blood clotting, in marked contrast to the great activity exhibited by untreated cephalin. This is in line with the earlier report of Billing,²⁷⁴ who demonstrated that the thromboplastic effect of cephalin emulsions was destroyed by *Crotalus adamanteus* venom (diamond-back rattlesnake), and also with the observation^{275,276} that "cytozyme" is similarly inactivated by cobra venom (*Naja tripudians*, *N. flava*). Since it is known

²⁷³ H. Cousin, *J. pharm. chim.* [6], 24, 101-108 (1906).

²⁷⁴ W. M. Billing, *J. Pharmacol.*, 38, 173-196 (1930).

²⁷⁵ L. Hirschfeld and R. Klinger, *Biochem. Z.*, 70, 398-415 (1915).

²⁷⁶ T. Link, *Z. Immunitäts.*, 85, 504-512 (1935); *Zentr. Bakt. Parasitenk.*, I, 120, 396 (1936); *Chem. Abst.*, 30, 1435 (1936).

that an unsaturated acid component must be present in such a thromboplastic agent, the fact that only stearic acid is present in the lysocephalin would explain the ineffectiveness of the latter.

Lysocephalin also has a strong hemolytic activity, although Magistris²⁷⁷ attributes this effect to a contamination of the preparations with lysolecithin. It is evident that this field of endeavor requires more experimental work before a clear picture will be available, especially in view of the recent demonstration that the cephalin fraction is made up of a mixture which contains at least three independent molecular complexes.

f. Distribution of α - and β -Cephalins. The method for the fractionation of α - and β -lecithin employed by Welch³⁵ has also been used for the determination of the distribution of the α - and β -forms of cephalin. The distribution of the isomers in the liver, heart, and brain of several species is given in Table 1. The β -form of cephalin is found to be predominant in the liver and heart cephalins of the several species studied (liver: beef, rat, guinea pig; heart: beef, cat), while the α -form is present in a larger proportion in the case of brain (beef, rat). This is in contradistinction to the distribution of lecithin, the α -form of which is always present in a larger proportion. The conclusions as to the distribution in the liver and heart as determined by Welch³⁵ are in agreement with those of Yoshinaga.³⁶

g. Chemistry and Hydrolysis Products of Phosphatidylethanolamine. Glycerophosphate is also a hydrolysis product of phosphatidylethanolamine, as well as of lecithin. Phosphorylethanolamine and glycerylphosphorylethanolamine, which would be comparable to the compounds produced in the hydrolysis of lecithin, have not been characterized.

(a) *Fatty Acids.* Thudichum²⁶² demonstrated the presence of stearic, oleic, linoleic, and arachidonic acids in the cephalin molecule. Klenk,⁴ however, could find no trace of a C₂₀ unsaturated acid in a crude cephalin obtained from brain, but only one which on hydrogenation yielded behenic acid, C₂₂H₄₄O₂.

Cephalin differs from lecithin in having stearic acid as the sole saturated fatty acid. This was first proved by Parnas,^{260,278} who used a lecithin-free cephalin preparation. Similar results were obtained by MacArthur and Burton²⁷⁹ and by Levene and Rolf,²⁸⁰ as well as by several others.^{262,273,281} Oleic acid is the principal unsaturated acid. Parnas²⁶⁰ reported 18% of this acid, while MacArthur and Burton²⁷⁹ noted the presence of 50% in their preparation. Levene and Rolf²⁸⁰ believed that arachidonic acid also occurred as a second unsaturated acid in brain cephalin, but the assumption is in doubt, in view of the subsequent negative results of Klenk.

²⁷⁷ H. Magistris, *Biochem. Z.*, 210, 85-119 (1929).

²⁷⁸ J. Parnas, *Biochem. Z.*, 56, 17-20 (1913).

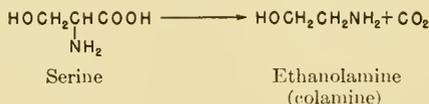
²⁷⁹ C. G. MacArthur and L. V. Burton, *J. Am. Chem. Soc.*, 38, 1375-1382 (1916).

²⁸⁰ P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, 54, 91-100 (1922).

²⁸¹ P. A. Levene and C. J. West, *J. Biol. Chem.*, 16, 419-422 (1913).

(b) *Colamine*. Although choline was at first thought to be the nitrogenous component of cephalin,^{23,282} it was later shown to be absent from purer preparations of this phosphatide. According to Thierfelder and Klenk,⁴ Baumann,²⁶⁵ and Renall,²⁵⁵ the identity of the nitrogen-containing compound with ethanolamine (colamine) was proved by the experiments of Parnas. The view was then generally accepted that colamine was the sole nitrogenous component of cephalin, and the conflicting evidence of MacArthur²⁸³ as to the possible acid nature of the compound was largely forgotten. Colamine was first separated as an hydrolysis product of bean phosphatides by Trier²⁸⁴ in 1911, although as early as 1884,^{4,23} and later in 1901,²⁶² Thudichum had predicted that this compound would be present in the phosphatides.

Serine is believed to be the mother substance of colamine. Nord²⁸⁵ has suggested that it originates in putrefactive anaerobes, by decarboxylation of serine.



After the discovery by Folch and Schneider²⁸⁶ of the presence of phosphatidylserine in cephalin preparations, it was suggested that the classical type of cephalin in which colamine is pictured as the nitrogenous base may not actually exist in nature, but that colamine may be an artifact. Such a possibility cannot be overlooked, since phosphatidylethanolamine may conceivably originate from phosphatidylserine merely by means of decarboxylation of serine due to postmortem changes in the tissues, or as a result of the analytical procedures employed in its isolation. However, Folch²⁵ has since shown that such an assumption is erroneous. In the first place, the fatty acid makeup of phosphatidylethanolamine and that of phosphatidylserine prepared from the same cephalin are quite different. Whereas the iodine number of the serine-containing fraction was found to be 33, that for the ethanolamine-containing portion was 78. Phosphatidylserine was isolated mostly as the potassium salt, and it contained a large portion of ash, while the phosphatidylethanolamine prepared without acid treatment was largely free from ash. Finally, Folch²⁵ proved that the postmortem changes were not the cause of the presence of phosphatidylethanolamine, since cephalin, prepared from the brain of an anesthetized animal by immediate maceration after removal from the animal, and by mixing with acetone

²⁸² H. Cousin, *Compt. rend. soc. biol.*, **62**, 238-240 (1907).

²⁸³ C. G. MacArthur, *J. Am. Chem. Soc.*, **36**, 2397-2401 (1914).

²⁸⁴ G. Trier, *Z. physiol. Chem.*, **73**, 383-388 (1911).

²⁸⁵ F. F. Nord, *Biochem. Z.*, **95**, 281-285 (1919).

²⁸⁶ J. Folch and H. A. Schneider, *J. Biol. Chem.*, **137**, 51-62 (1940).

at -72°C ., contained the same amount of this fraction as did cephalin prepared by the usual manner. This does not preclude some enzyme action.

In contradistinction to choline, which can be shown to be an intermediate of lecithin, the presence of free colamine cannot be demonstrated in animal or in plant tissues. It is possible that the activity of lecithinase C, by which the nitrogenous base is set free, may be limited to lecithin. Under such conditions, the phosphoric acid would not be split from ethanolamine, and it could be expected that the ester of the nitrogenous base would appear as a decomposition product. That such is the case is suggested by the demonstration that colamine phosphate, $\text{H}_2\text{N}\cdot\text{CH}_2\text{CH}_2\text{O}\cdot\text{PO}(\text{OH})_2$, is present to the extent of 36 milligram per cent of the fresh weight of human malignant tumors, as well as to a maximum of 1 milligram per cent in pancreas, liver, cattle embryo, and human benign tumors.²⁸⁷ However, colamine is quickly destroyed by liver perfusion so that it can no longer be detected after 2 hours,^{151,288} and this fact may account for the failure to detect the free base in animal tissues. Another possibility arises that colamine may be transformed to monomethylaminoethanol, $\text{CH}_3\text{NH}\cdot\text{CH}_2\text{CH}_2\text{OH}$, or even to dimethylaminoethanol, $(\text{CH}_3)_2\text{N}\cdot\text{CH}_2\text{CH}_2\text{OH}$. Such compounds have been detected in tissues, and are believed to represent intermediate stages between ethanolamine and choline (or, in fact, between phosphatidylethanolamine and lecithin).^{255,265}

One of the difficulties in the determination of the proportion of phosphatidylethanolamine has been the lack of a satisfactory method for the quantitative determination of colamine. Although this base gives a characteristic gold salt which has a composition represented by the formula $\text{C}_2\text{H}_8\text{NOAuCl}_4$, the formation is not a quantitative one. The determination of free amino nitrogen furnishes a method for the estimation of colamine in the absence of other primary amines, amino acids, or phosphatidylserine; however, even if only phosphatidylethanolamine is present, it cannot be used to determine the extent of the hydrolysis, as the reaction takes place in the unhydrolyzed phosphatide as well as in the hydrolysates. The gold salt precipitation procedure has been used in the Thierfelder-Schulze method,²⁸⁹ which was later modified by Levene and Ingvaldsen.¹²⁹

Artom²⁹⁰ recently proposed an analytical procedure which gives quantitative results for ethanolamine and for serine, separately or when they are mixed. Ethanolamine is quantitatively separated from serine by adsorption on Permutit, while the amino acid is not adsorbed. Ethanolamine is subsequently eluted from the Permutit column with concentrated sodium chloride solution. The ethanolamine can be determined in the eluate by

²⁸⁷ E. L. Outhouse, *Biochem. J.*, **31**, 1459-1463 (1937).

²⁸⁸ M. Guggenheim and W. Löffler, *Biochem. Z.*, **74**, 208-218 (1916).

²⁸⁹ H. Thierfelder and O. Schulze, *Z. physiol. Chem.*, **96**, 296-308 (1915-1916.)

²⁹⁰ C. Artom, *J. Biol. Chem.*, **157**, 585-594 (1945).

determination of the ammonia set free or by the formaldehyde produced when it reacts with sodium periodate. The ammonia produced is separated by steam distillation by means of a Parnas-Wagner apparatus.²⁹¹

When this procedure is applied to the lipids extracted from tissues, a preliminary hydrolysis is carried out with methanolic hydrochloric acid. This is later removed, and the liquid hydrolysate is dissolved in water, extracted with diethyl (or petroleum) ether, filtered, and the ethanolamine and serine are determined on the filtrate. When serine is present, total ammonia is determined on treatment with alkaline periodate before and after adsorption on Permutit. According to Nicolet and Shinn,²⁹² serine likewise reacts quantitatively with periodate. This reaction has been confirmed by Artom.²⁹⁰ Data obtained from this reaction are in agreement with the results obtained by Van Slyke *et al.*²⁹³ with another hydroxyamino acid (hydroxylysine). Jones²⁹⁴ has proposed still another procedure for the determination of ethanolamine. Burmaster²⁹⁵ suggested a micromethod for the determination of ethanolamine in the presence of serine which involves the microdiffusion of ammonia produced by periodate in a solution nearly saturated with potassium metaborate. Special microdiffusion cells made from Petri dishes are required for the latter determination.

(3) *Phosphatidylserine*

The fact that the phosphatides might contain an amino acid residue was first suggested by MacArthur.²⁸³ He based this assumption upon the demonstration of the solubilizing effect of cephalin upon copper obtained from cupric hydroxide. This is the same reaction upon which the Kober-Sugiura method²⁹⁶ for the determination of amino acids is based. However, because of the non-specificity of the latter method, and because of the proof by other workers of the presence of ethanolamine in cephalin, little attention was paid to MacArthur's results until recently.

The proof that cephalin may contain an amino acid fraction has recently been brought forward by Folch and Schneider.²⁸⁵ These investigators found that four different presumably pure samples of brain cephalin, which possessed the constants usually accepted for this phosphatide, all gave values for amino nitrogen when analyzed by the ninhydrin-carbon dioxide method slightly modified from that of Van Slyke and Dillon.²⁹⁷ Only α -

²⁹¹ J. K. Parnas and R. Wagner, *Biochem. Z.*, *125*, 253-256 (1921).

²⁹² B. H. Nicolet and L. A. Shinn, *J. Biol. Chem.*, *139*, 687-692 (1941).

²⁹³ D. D. Van Slyke, A. Hiller, and D. A. MacFadyen, *J. Biol. Chem.*, *141*, 681-705 (1941).

²⁹⁴ J. H. Jones, *J. Assoc. Official Agr. Chem.*, *27*, 462-467 (1944).

²⁹⁵ C. F. Burmaster, *J. Biol. Chem.*, *165*, 1-6 (1946).

²⁹⁶ P. A. Kober and K. Sugiura, *J. Am. Chem. Soc.*, *35*, 1546-1584 (1913).

²⁹⁷ D. D. Van Slyke and R. T. Dillon, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, *22*, 480-486 (1938).

amino acids having a free amino and a free carboxyl group will respond to the above reaction; ethanolamine, either alone or in the presence of oleic acid, gave entirely negative results. Since Folch and Schneider²⁸⁶ carried out these tests on unhydrolyzed cephalin, it is evident that the amino acid must be combined in the cephalin molecule in such a way that both the amino and the carboxyl groups are unattached. The amino acid could still be quantitatively demonstrated in the water-soluble fraction after hydrolysis by the use of the ninhydrin test. Further proof of the amino acid nature of the product is afforded by the fact that, accompanying the liberation of carbon dioxide, an equimolecular amount of ammonia is also released. The liberation of both carbon dioxide and ammonia with ninhydrin is a reaction characteristic only of the α -amino acids.

The amino acid component was believed to be serine, on the basis of its reaction with periodate.²⁹⁸ The finding of glycolic aldehyde as an end product of the ninhydrin reaction also adds weight to this hypothesis. The hydroxyl group on serine would afford a means for esterification with phosphoric acid while leaving both the amino and the carboxyl group free on the unhydrolyzed molecule. The amino acid is not an adsorbed impurity, but is actually an integral part of the cephalin molecule; this is indicated by the large quantity which is present. Moreover, in the preparation of cephalin, it flocculated from solution in such a way as to preclude the adsorption of water-soluble impurities. The final proof of the identity of the acid was obtained by the preparation of pure phosphatidylserine²⁹⁸ and the identification of the amino acid component as L-serine. The purified phosphatidylserine contained 97% of its nitrogen in the form of amino nitrogen; on hydrolysis, 38% of the theoretical amount of serine was prepared from it in crystalline form. Schuwirth^{299,300} demonstrated the presence of serine in human brain phosphatides following its extraction with butyl alcohol from the barium hydroxide hydrolysate, as well as by the preparation of the β -naphthalene sulfonic acid derivative. The methods for the determination of phosphatidylserine which are based upon the determination of serine are discussed in the section on phosphatidylethanolamine.

Folch³⁰¹ was able to prepare phosphatidylserine in 92 to 97% purity from human brain. Stearic and oleic acids were the chief fatty acids present. On hydrolysis, glycerophosphoric acid, L-serine, and fatty acids were separated in molecular ratios of 1:1:2. Phosphatidylserine was shown to comprise 60% of the nitrogenous lipid carboxyl in the brain.

There are, in all probability, two types of phosphatidylserine, namely, α and β , depending upon the carbon atom of glycerol with which phosphoric

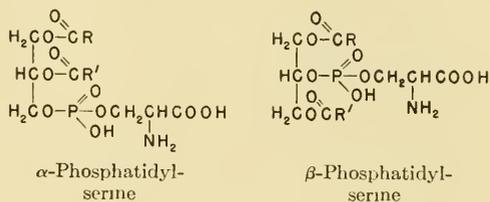
²⁹⁸ J. Folch, *J. Biol. Chem.*, 139, 973-974 (1941).

²⁹⁹ K. Schuwirth, *Z. physiol. Chem.*, 270, I-III (1941).

³⁰⁰ K. Schuwirth, *Z. physiol. Chem.*, 277, 87-96 (1942).

³⁰¹ J. Folch, *J. Biol. Chem.*, 174, 439-450 (1948).

acid is combined. The structures of the α - and β -phosphatidylserine are represented in the accompanying formulas.



(4) Inositol Phosphatides

The occurrence of inositol as a component of soybean phosphatides was discovered by Klenk and Sakai,³⁰² although Anderson³⁰³ had previously proved that this lipid was present in tubercle bacilli. Folch and Woolley²⁶ were the first to demonstrate inositol as a constituent of an animal phosphatide which was isolated both from the brain and from the spinal cord. It was noted that the so-called brain cephalin was especially rich in this substance. The inositol-containing phosphatide was prepared as a friable white powder which contained 4.5% of phosphorus and about 1% of nitrogen, all of which was in the form of amino nitrogen. The inositol content varied between 6.8 and 8.6%, although one preparation contained as much as 10%. About one-fourth of the brain cephalin, or 0.4% of the net weight of the brain, was found to be made up of the inositol-containing phospholipid.

By the use of fractional precipitation of a chloroform solution of brain cephalin with increasing proportions of alcohol, Folch²⁵ was able to separate practically pure inositol phosphatide in the portion which was least soluble in alcohol. Glycerophosphoric acid and serine were prepared from this fraction. It was believed that the presence of serine indicated that the product was contaminated with phosphatidylserine.

Woolley,³⁰⁴ using the Folch method²⁵ for the fractionation of the phosphatides, prepared an inositol-containing lipid from soybean oil which contained as much as 16% of inositol. It was found to contain carbohydrate, oleic acid, phosphorus, and inositol in approximately molecular ratios; therefore, this author believed the preparation to be essentially pure. Because this substance is a lipid which contains inositol, Woolley proposed calling the product soybean *lipositol*.

Although Woolley has not suggested a structural formula for lipositol, the products of complete acid hydrolysis have been identified as inositol, phosphoric acid, oleic acid, saturated fatty acids, a black, humin-like material,

³⁰² E. Klenk and R. Sakai, *Z. physiol. Chem.*, **253**, 33-38 (1939).

³⁰³ R. J. Anderson, *J. Am. Chem. Soc.*, **52**, 1607-1608 (1930).

³⁰⁴ D. W. Woolley, *J. Biol. Chem.*, **147**, 581-591 (1943).

as well as traces of ethanolamine and tartaric acids. The saturated fatty acids were composed of 5% cerebronic acid, and 95% of a mixture made up of 70% palmitic acid and 30% of stearic acid. On the other hand, when alkaline hydrolysis was employed, a non-reducing carbohydrate was prepared which was shown to yield galactose after mild hydrolysis. The tartaric acid was found to be a *d*-isomer, while the molecular ratios of galactose, inositol, and tartaric acid were in the proportions of 1:1:1. A summary of the constituents of lipositol as determined by analysis and by isolation is given in Table 8. The fact that the components identified represent the major portion of all constituents present in the molecule is indicated by the finding that they account for 102% of the lipositol, whereas a theoretical recovery would amount to 109%.

TABLE 8
CONSTITUENTS OF SOYBEAN LIPOSITOL^a

Constituent	Per cent found by		Constituent	Per cent found by	
	Isolation	Anal.		Isolation	Anal.
Inositol	15	16	Oleic acid	23.6	—
Galactose	—	15.5	Phosphoric acid	—	9.8
Tartaric acid	8.3	—	Saturated acids	21.2	—
Ethanolamine	0.44	—	Potassium	—	3.4

^a D. W. Woolley, *J. Biol. Chem.*, 147, 581-591 (1943).

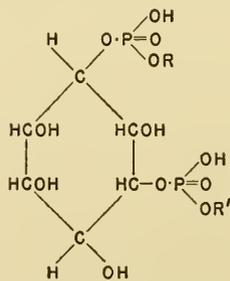
The structural relationships of the inositol phosphatide may, to a considerable extent, be deduced from its partial hydrolysis products. The fact that inositol monophosphate results from partial hydrolysis with acid, as also proved earlier by Klenk and Sakai,³⁰² is convincing proof that the phosphate is bound to the inositol in such a molecule. Since a non-reducing carbohydrate is formed on alkaline hydrolysis while a reducing sugar is set free on subsequent acid hydrolysis, there is cogent evidence that the sugar, which is galactose, is present in galactoside linkage. After assessing the experimental evidence as to whether tartaric acid or inositol is involved in this galactoside combination, Woolley³⁰⁴ came to the conclusion that it must be the latter. Since free ethanolamine and free tartaric acid were liberated only after alkaline hydrolysis, it was concluded that ethanolamine must be present in the form of a tartrate. It is believed that, in lipositol, the second carboxyl group of tartaric acid is esterified with inositol, although it might also be joined with an alcohol group of galactose. In any event, if tartaric acid is not combined with inositol, an oleic acid residue must be united with this hexahydric alcohol, since at least three of the groups are combined. These must be joined on alternate hydroxyls, as no two adjacent hydroxyl groups, of the six present, are free.

There would thus seem to be considerable evidence for the supposition

that a similarity in structure obtains between brain inositol phosphatide and soybean lipositol. The rate of hydrolysis of the two products is identical; the carbohydrate from both phosphatides is galactose. Finally, tartaric acid is liberated, in either case, only if an alkaline hydrolysis is employed.

Lipositol is a fine, creamy, white powder which is quite friable when dry. On exposure to air, it darkens slowly. When no precautions are observed to prevent oxidation during its preparation, one obtains a light brown rather than a white powder. Lipositol dissolves in petroleum ether, benzene, diethyl ether, and chloroform when they are moist, but it is insoluble in the anhydrous solvents. Moreover, it is insoluble in ethanol, methanol, acetone, and dioxane; it does, however, form an emulsion with water.

a. Brain Diphosphoinositide. Considerable doubt is cast on the validity of the findings as to the purity of the original brain inositol phosphatides by the recent reports by Folch^{305,306} of the preparation of a relatively pure brain diphosphoinositide which contains small amounts of carbohydrate and only traces of carboxyl nitrogen. This product has been separated from brain cephalin by fractionation with methyl alcohol³⁰⁷; the separation is based upon the fact that brain diphosphoinositide is less soluble in this solvent than are phosphatidylethanolamine or phosphatidylserine. This sample contained 16% of inositol, which is the figure also obtained for soybean lipositol. This fraction accounts for all the inositol in the brain cephalin. The proportions of inositol, phosphoric acid, glycerol, and fatty acid were 1:2:1:1. It is not certain as yet whether a nitrogen-containing compound is present as a portion of the molecule or whether it is a contaminant. Folch³⁰⁶ was able to separate inositol metadiphosphate from the purified diphosphoinositide, following a short-time acid hydrolysis. This compound has been identified on the basis of its elementary composition, by titration with alkali, by the isolation of inositol from it, and by a study of its reaction products with HIO_4 . The last reaction proves that



Inositol metadiphosphate

³⁰⁵ J. Folch, *Federation Proc.*, 5, 134 (1946).

³⁰⁶ J. Folch, *J. Biol. Chem.*, 177, 505-519 (1949).

³⁰⁷ J. Folch, *J. Biol. Chem.*, 177, 497-504 (1949).

the phosphate radicals are in *meta* position to each other. The structure assigned is given herewith, R and R' referring to unknown groups.

Diphosphoinositide is a white gritty powder, not emulsified in water and insoluble in most organic solvents with the exception of wet chloroform. It contains 7.3% phosphorus. Fatty acids, glycerol, and inositol metaphosphate are present in approximately equimolecular amounts. The inositol metaphosphate accounts for all the phosphorus in the molecule. Diphosphoinositide is an acidic phosphatide and, when prepared from brain tissue with neutral solvents, it is obtained as the calcium and magnesium salt in a base-to-phosphorus ratio indicative of a monophosphate.

(5) *Distribution of Phosphatidylethanolamine and Phosphatidylserine*

Although the methods for separation of the cephalin fractions employed by Folch²⁵ are not quantitative, the approximate quantities of the several fractions in ox brain, correcting for 100% recovery and for the mixed fractions, are as follows: phosphatidylethanolamine, 23%; phosphatidylserine, 48%; and inositol phosphatide, 29%.

Artom,³⁰⁸ making use of his new method for the estimation of ethanolamine and serine,²⁹⁰ has reported the proportion of phospholipids containing these nitrogenous compounds in rat tissues and in human plasma. These results are recorded in Table 9.

TABLE 9
DISTRIBUTION OF INDIVIDUAL PHOSPHOLIPIDS IN RAT TISSUES^a

Tissue examined	No. of groups	Total P.L.	Choline-contg. P.L.	Ethanolamine-contg. P.L.	Serine-contg. P.L.	Ethanolamine + serine-contg. P.L.	
						by anal.	by diff.
Liver	3	39.0	23.4	11.3	3.8	15.1	15.7
Skeletal muscle	3	12.6	6.0	4.9	1.4	6.3	6.6
Heart	2	20.6	5.9	10.2	1.9	12.1	14.7
Testis	1	16.1	8.4	5.5	2.1	7.6	7.7
Spleen	2	16.2	6.2	7.1	3.3	10.4	10.0
Lung	2	22.5	13.6	10.6	2.2	12.8	8.9
Kidney ^b	2	32.7	17.4	15.2	8.0	23.2	15.3
Kidney	1	29.8	12.3	16.4	2.1	18.5	17.5
Brain ^b	2	65.0	23.6	33.4	19.0	52.4	41.5
Brain	1	57.7	22.5	23.2	12.4	35.6	35.2

^a Adapted from C. Artom, *J. Biol. Chem.*, 157, 595-599 (1945).

^b Ether-soluble fraction of lipid extract.

According to Artom³⁰⁸ the values obtained compare well with those for phosphatidylethanolamine found by Chargaff *et al.*³⁰⁹ by the isotope dilu-

³⁰⁸ C. Artom, *J. Biol. Chem.*, 157, 595-599 (1945).

³⁰⁹ E. Chargaff, M. Ziff, and D. Rittenberg, *J. Biol. Chem.*, 144, 343-352 (1942).

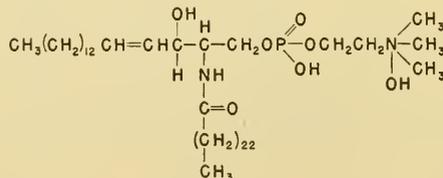
tion method. The per cent of total lipid in this form reported by Artom and calculated on the figures of Chargaff *et al.* are, respectively: liver, 27.2–32.2 and 27.2; heart, 48.6–51.2 and 38.6; brain, 40.2 and 44.4. The phosphatidylserine (in per cent of total lipid) compares with lipid amino nitrogen determined by Chargaff *et al.* as follows: liver, 7.4–11.3 and 6.7; heart, 7.2–11.3 and 7.0; lung, 7.8–12.0 and 15.7; brain, 21.5 and 21.6.

5. The Sphingomyelins

Sphingomyelin was first discovered by Thudichum²³ in 1884. It is readily distinguished from other phospholipids because it contains two nitrogen atoms to each phosphorus atom present in the molecule. Moreover, it is insoluble in diethyl ether. Sphingomyelins are also unique in containing no glycerol in the molecule, although the recent elucidation of the composition of lipositol has shown that sphingomyelin is not the only phosphatide from which glycerol is absent. Not only has sphingomyelin been found in the free state in the tissues, but it may also occur as a fatty acid ester.³¹⁰

(1) Structure of the Sphingomyelins

The best insight as to the structure of sphingomyelin is gained from an examination of its hydrolysis products, and especially of the intermediates which can be isolated when only a partial hydrolysis obtains. Thus, a fatty acid, two nitrogenous bases—choline and sphingosine—and phosphoric acid have long been recognized as the ultimate hydrolysis products. The fatty acid has most frequently been identified as lignoceric acid. The identification of lignoceryl sphingosine as a product of partial hydrolysis offers considerable confirmation of the fact that the fatty acid and sphingosine are joined by a NH—CO linkage through the carboxyl and amino groups, respectively. It is believed that choline phosphate is joined to sphingosine through a hydroxyl group in ester linkage. The original structure for sphingomyelin was suggested by Levene,³¹¹ but this must now be modified to agree with the present conception of the structure of sphingosine which has recently been demonstrated by Carter *et al.*³¹²



Sphingomyelin

³¹⁰ S. J. Thannhauser and M. Reichel, *J. Biol. Chem.*, **135**, 1–13 (1940).

³¹¹ P. A. Levene, *J. Biol. Chem.*, **24**, 69–89 (1916).

³¹² H. E. Carter, F. J. Glick, W. P. Norris, and G. E. Phillips, *J. Biol. Chem.*, **142**, 449–450 (1942).

Although Thudichum²⁶² separated a fatty acid which he called sphingostearic acid from sphingomyelin, it is not probable that this product, which had an empirical formula of $C_{18}H_{36}O_2$ and a melting point of $57^\circ C.$, was a homogeneous substance. Sphingomyelin prepared by the older methods from brain and spleen was shown to yield palmitic, stearic, and lignoceric acids on hydrolysis.^{311,313-314} Pyridine extraction has also been used.³¹⁵ Merz,³¹⁶ using an acetone fractionation method with brain phospholipids, found that, in addition to these saturated acids, the unsaturated acid nervonic acid was also present in sphingomyelin. The presence of palmitic acid in brain sphingomyelin appears questionable, since it has been shown that sphingomyelin prepared by the earlier methods is a mixture which contains hydrolecithin. Thannhauser and Boncoddio³¹⁷ have recently proved that brain sphingomyelin has a different fatty acid composition from that prepared from other organs. Stearic, lignoceric, and nervonic acids were shown to comprise the sole fatty acids in brain sphingomyelin, while palmitic and lignoceric acids are the fatty acids present in lung and spleen sphingomyelins.³¹⁸ Hydrosphingosine occurred along with sphingosine in the case of both brain and spleen sphingomyelins. Fränkel *et al.*³¹⁹ prepared a sphingomyelin which contained sphingosine, choline, phosphoric acid, and fatty acids in the expected proportions. There were three fatty acids present—palmitic, stearic, and lignoceric—which were found in equivalent proportions. Fränkel *et al.* believe that sphingomyelin consists of salt-like complexes which are formed by the condensation of several single sphingomyelin molecules by elimination of water through interaction of adjacent molecules of choline and phosphoric acid. It is believed that such long-chain polyaminophospholipids may be of great importance in producing the properties of irritability and conductivity characteristic of nerve tissue.

a. Sphingomyelin Fatty Acid Ester. The possibility that sphingomyelin may exist in nature, combined with a second fatty acid in ester linkage on the hydroxyl group of the sphingosine which has usually been considered to be free, has recently been revived by Thannhauser and Reichel.³¹⁰ These workers were able to demonstrate that, when spleen sphingomyelin was hydrolyzed with a liver enzyme or with a purified pancreatic lipase, palmitic acid could be isolated, in addition to choline, phosphoric acid, and lignoceryl sphingosine. When the Henriques method of alkaline

³¹³ P. A. Levene, *J. Biol. Chem.*, **15**, 153-154 (1913).

³¹⁴ P. A. Levene, *J. Biol. Chem.*, **18**, 453-462 (1914).

³¹⁵ O. Rosenheim and M. C. Tebb, *J. Physiol.*, **41**, *Proc.*, i-ii (1910-1911).

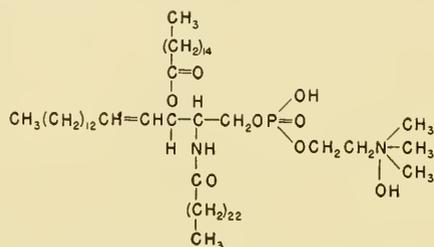
³¹⁶ W. Merz, *Z. physiol. Chem.*, **193**, 59-87 (1930).

³¹⁷ S. J. Thannhauser and N. F. Boncoddio, *J. Biol. Chem.*, **172**, 141-147 (1948).

³¹⁸ S. J. Thannhauser, J. Benotti, and N. F. Boncoddio, *J. Biol. Chem.*, **166**, 677-681 (1946).

³¹⁹ E. Fränkel, F. Bielschowsky, and S. J. Thannhauser, *Z. physiol. Chem.*, **218**, 1-11 (1933).

saponification was employed, which will not split the NH—CO linkage, palmitic acid and unesterified lignocerylsphingomyelin were obtained. The authors believe that sphingomyelin exists in both the esterified and the free form in nature. However, the suggestion is made that it may occur only in the form of fatty acid esters; the appearance of free sphingomyelin would then be the result of autolysis of the ester during the process of isolation. A method is given by Thannhauser and Reichel³¹⁰ to determine the proportion in the free and in the ester form by the ketene acetylation of the ceramide. A value of 67.5% was reported for one sample. The formula for the palmitic acid ester of lignocerylsphingomyelin would show the accompanying structure. Reichel and Thannhauser³²⁰ have prepared a number of lignocerylsphingosine fatty acid esters, which they call sphingosine



Palmitic acid ester of lignocerylsphingomyelin

fats. This demonstrates that the esterification of both hydroxyl groups in sphingosine is possible.

(2) Preparation of the Sphingomyelins

a. Rosenheim and Tebb Method.³¹⁵ Brain is used as the source of sphingomyelin in this procedure. The cerebroside is first removed from the brain tissue by extraction with cold pyridine. The sphingomyelin is then extracted by allowing the brain to stand with 3 volumes of pyridine at 40–45°C. for one-half hour, after which it is filtered. The filtrate is cooled to 15°C., and the precipitated sphingomyelin is filtered off and washed with acetone. It is a white, non-hygroscopic powder, which is further purified through repeated fractional precipitation from an alcohol-chloroform solution with acetone. It is finally crystallized from pyridine.

b. Levene Method.³¹⁴ The dried brain sample is exhaustively extracted with boiling alcohol. The precipitate which forms on cooling the alcohol extract is extracted with ether and acetone. The precipitate is then taken up in hot pyridine, the crude sphingomyelin which separates on cooling is filtered off, and the sphingomyelin is precipitated by acetone after concentration of the acetic acid filtrate *in vacuo* to a small volume. The crude preparation can be further purified by dissolving in a mixture of 5

³²⁰ M. Reichel and S. J. Thannhauser, *J. Biol. Chem.*, 135, 15–21 (1940).

parts of ligroin and 1 part of alcohol,³¹¹ after which alcohol is added as long as a precipitate forms. This is filtered, and the filtrate is allowed to stand overnight at 0°C. The solution is filtered, concentrated under reduced pressure, and the sphingomyelin is precipitated by the addition of acetone. Final purifications are made by crystallization from a mixture of equal parts of pyridine and chloroform, first at room temperature, then at 30°C., and finally at 37°C. Such a preparation has been shown to be free from cerebrosides.

c. Klenk and Rennkamp Method.³²¹ A somewhat different procedure has been proposed by Klenk and Rennkamp which yields a glycerol-free sphingomyelin preparation. "Crude dry carbohydrate-free sphingomyelin is dissolved in 10 volumes of benzene (b.p., 70–80°C.) and treated with 1 volume of sodium ethylate in ethyl alcohol prepared by dissolving 1 g. of sodium in 100 cc. of ethyl alcohol. Transesterification of glycerides occurs during 1 hour of refluxing. The solvents are evaporated and the esters are removed with acetone; the sphingomyelin is freed from glycerophosphoric acid by precipitation by cadmium acetate from methanol and adsorption of the last trace by Al₂O₃. The final crystallization materials had a $[\alpha]_D^{28} = +5.30-5.35$ (0.54 g. in 10 cc. methanol-CHCl₃, 1:1)."

d. Thannhauser, Benotti, and Boncoddio Method.³¹³ Since all the methods used for the preparation of sphingomyelin result in a product which is a mixture of sphingomyelin and hydrolecithin in various proportions, the present procedures are designed to prepare lung sphingomyelin free from hydrolecithin. The difficulty in separating the two products results from the similarity of their physical properties, especially since both substances are insoluble in ether.

(a) *Method 1.* The ether-soluble lipids are first removed from macerated lung tissue which has previously been washed twice with acetone and dried *in vacuo* at 60°C., by exhaustive extraction of the powdered material for 3 days in a continuous extractor with ether.²⁵¹ The crude sphingomyelin is extracted with glacial acetic acid, precipitated with acetone, filtered and dried after washing with acetone. This material is free from unsaturated monophosphatides, but contains hydrolecithin to the extent of approximately 33%.

To remove the hydrolecithin, 10 g. of the crude phosphatide is suspended in a small amount of water and ground to a paste; 200 ml. of 0.25 *N* NaOH is added.³¹³ This suspension is then shaken for 5 days at 37°C. After acidification with glacial acetic acid, it is cooled in the refrigerator overnight and filtered with the aid of a Hyflo filter. The precipitate is washed with acetone and then extracted for 2 or 3 days in a Soxhlet apparatus, to remove any free fatty acids. The precipitate is dialyzed for 24 hours against running water, to remove inorganic contaminants. The dialyzed suspension is

³²¹ E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, 267, 145–153 (1940–1941).

again filtered, and is washed with acetone. Sphingomyelin is extracted from it by the use of a 9:1 petroleum ether-methanol mixture. The extract is filtered, concentrated to a small amount, of thin syrupy consistency, and is precipitated with a large excess of acetone (1000–1500 ml.). Since this precipitate still gives a slightly positive Molisch reaction, it is again dissolved in a 9:1 petroleum ether-methanol mixture and is put through an Al_2O_3 chromatographic column for selective adsorption of cerebrosides.³²² Hydrolecithin-free sphingomyelin is then recovered from the concentrated solution by precipitation with acetone and recrystallization from hot ethyl acetate.

(b) *Method 2.* The hydrolecithin may be effectively removed from the crude sphingomyelin by extraction with 97% acetone (97 volumes of acetone to 3 volumes of water). The crude powder obtained as described in Method 1 is mixed with sand and is extracted for 3 weeks with 97% acetone in a Soxhlet apparatus. The sphingomyelin, which remains in the residue, is dissolved in 1000 ml. of a 9:1 petroleum ether-methanol mixture; this is filtered and concentrated to a small volume. Sphingomyelin is precipitated with an excess of acetone and is recrystallized from ethyl acetate.

This method has the disadvantage of giving only a small yield of sphingomyelin, since most of the desired phospholipid is extracted by the 97% acetone, along with the hydrolecithin.

(3) *Tests for Purity of Sphingomyelin*

Because of the similarity of the properties of sphingomyelins to those of the lecithins on the one hand, and of the cerebrosides on the other hand, the preparation of reasonably pure samples presents extreme difficulties. The melting point or the optical rotation is not a sufficiently precise physical constant to be of much value in identification. The absence of cerebrosides from the samples can be proved in a reliable manner by the application of the Molisch test for carbohydrates, since this is an extremely sensitive test. The other test which has been employed is the N:P ratio, which should give a value of 2:1. However, a considerable quantity of monophosphatides might be present without significantly altering this proportion. Of considerably greater importance in establishing the purity of a sphingomyelin preparation is proof of the absence of glycerol. This is possible by the method of microdetermination for this compound which has recently been developed by Blix.³²³ When this test was applied to presumably pure sphingomyelin preparations, it was found that it was positive,^{10,321} which indicated that considerable amounts of glycerol-containing phosphatides (probably saturated lecithins) were present.

³²² S. J. Thannhauser and P. Setz, *J. Biol. Chem.*, 116, 527–531 (1936).

³²³ G. Blix, *Mikrochim. Acta*, 1, 75–77 (1937).

Thannhauser and Benotti³²⁴ have recently prepared glycerol-free sphingomyelin from lung tissue, but this has not been achieved in the case of brain sphingomyelin, where the large proportion of cerebrosides and of saturated monophosphatides causes considerable interference. Klenk and Rennkamp³²¹ succeeded in accomplishing this purification by subjection of the crude sphingomyelin to saponification with sodium alcoholate, which had a more or less selective action in producing hydrolysis of the monophosphatides. Thannhauser and Benotti³²⁴ found that saponification at a boiling temperature was too drastic a procedure; they accomplished the purification by shaking the impure sphingomyelin preparation with aqueous sodium hydroxide at 37°C. for 24 hours.

(4) *Properties of the Sphingomyelins*

Sphingomyelins are white crystalline substances which are not hydroscopic, in contradistinction to the behavior of their most important component, sphingosine. These phosphatides are resistant to air and light. They are only slightly soluble in cold alcohol or pyridine, and can be crystallized from these solvents when hot solutions are cooled. Thus, 100 ml. of a saturated pyridine solution of sphingomyelin contains 0.22 g. of the solute at 40°C. and 0.18 g. at room temperature. Sphingomyelins are almost completely insoluble in acetone and diethyl ether, but they form emulsions with water which exhibit myelin formation. In an aqueous solution of glycerol, they exhibit birefringence.³²⁵ Sphingomyelin melts at 196–198°C.³²⁶

Like lecithin, sphingomyelin forms a cadmium chloride compound which is difficultly soluble in alcohol. It also combines with platinum to give a salt sparingly soluble in alcohol, as well as in diethyl ether.³²² The phospholipid also exhibits optical activity. In a methyl alcohol-chloroform solution, a dextro-rotation is observed. Levene³¹¹ reported specific rotations for different preparations varying from 7.53° to 8.73°, while Walz³²⁷ gave a value for $[\alpha]_D$ of +5.5°. In pyridine, the optical rotation is strongly dextro-rotatory above 40° ($[\alpha]_D = +13.82^\circ$). However, when the solution is cooled, the optical activity changes from a dextro- to a levo-rotation.³²⁶

It is believed that sphingomyelin may occur in the zwitterion form. Although Chain and Kemp³²⁸ found that the isoelectric point of this phosphatide as determined electrophoretically was approximately at a pH of 6.0, Fischgold and Chain⁸³ observed that, in a benzene alcohol solution, sphingomyelin bound one equivalent of hydrogen ions, but did not yield hydrogen

³²⁴ S. J. Thannhauser and J. Benotti, *Unpublished experiments*, reported by S. J. Thannhauser and G. Schmidt, *Physiol. Revs.*, **26**, 286, 287 (1946).

³²⁵ R. Kawamura, *Die Cholesterinesterverfettung*, Fischer, Jena, 1911, p. 9.

³²⁶ M. Sano, *J. Biochem. Japan*, **1**, 1–16, 17–20 (1922).

³²⁷ E. Walz, *Z. physiol. Chem.*, **166**, 223–226 (1927).

³²⁸ E. Chain and I. Kemp, *Biochem. J.*, **28**, 2052–2055 (1934).

ions in basic solution. No buffering action of sphingomyelin could be demonstrated over a wide range of pH by Christensen and Hastings³²⁹; these authors also failed to demonstrate any appreciable combination of sphingomyelin with chloride or sodium ions.

(5) *Hydrolysis of Sphingomyelin*

There is general agreement that glycerol is not present as a hydrolysis product of sphingomyelin.^{262,330} Choline has been proved to be a component, irrespective of whether hydrolysis was brought about by barium hydroxide or by dilute sulfuric acid.^{262,311,314} Sphingosine is always present,^{262,311,314} while Thudichum²⁶² also reported that an alcohol—sphingol, $C_{18}H_{36}O_2$ —is found. This has been confirmed by Rosenheim and Tebb,³³⁰ although Levene failed to find it in the hydrolysis products.³¹¹ According to Levene,³¹¹ about 40–50% of the hydrolysis products consist of fatty acids. The nature of these has already been discussed (see Section 5(1)). When a less complete hydrolysis was carried out, lignocerylsphingosine was found.³¹¹ This compound possesses no basic character, and the nitrogen is not present as the free amino group.

Magistris²⁷⁷ reported that lignoceric acid is split from sphingomyelin by bee venom, yielding a so-called lysosphingomyelin. Thierfelder and Klenk⁴ consider that the experiments are not convincing, and they suggest that the isolated lignoceric acid might well have originated through acid hydrolysis from sphingomyelin during the analytical procedures.

Little is known about the pathways of enzymic breakdown of sphingomyelin. Lignocerylsphingosine has been identified as a product arising during the incubation of a preparation of liver extract.³¹⁰

(6) *Chemistry of Hydrolysis Products of the Sphingomyelins and Their Derivatives*

a. Lignocerylsphingosine. Levene³¹¹ first isolated lignocerylsphingosine from a partially hydrolyzed preparation of hydrogenated sphingomyelin. Thannhauser and Fränkel³³¹ prepared this compound from pig liver by the use of a mild hydrolyzing agent such as methyl alcoholic sodium hydroxide. The term *ceramides* has been coined to identify this class of compounds.³³² The presence of free lignocerylsphingosine in pig liver and beef spleen is indicated by the fact that the ceramide has been prepared from these organs without preliminary saponification.^{332,333} Klenk and von Schoenebeck³³⁴ also separated lignocerylsphingosine from unhydro-

³²⁹ H. N. Christensen and A. B. Hastings, *J. Biol. Chem.*, **136**, 387–398 (1940).

³³⁰ O. Rosenheim and M. C. Tebb, *J. Physiol.*, **38**, *Proc.*, li–liii, liv–lvi (1909).

³³¹ S. J. Thannhauser and E. Fränkel, *Z. physiol. Chem.*, **203**, 183–188 (1931).

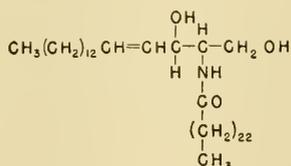
³³² E. Fränkel and F. Bielschowsky, *Z. physiol. Chem.*, **213**, 58–62 (1932).

³³³ C. Tropp and V. Wiedersheim, *Z. physiol. Chem.*, **222**, 39–43 (1933).

³³⁴ E. Klenk and O. von Schoenebeck, *Z. physiol. Chem.*, **209**, 112–133 (1932).

lyzed ox liver. The product had a melting point of 156–157°C. and yielded only sphingosine and lignoceric acid on hydrolysis. Moreover, it was shown that the conditions prevailing throughout the saponification of sphingomyelin and the subsequent isolation of the ceramide were so mild as to preclude the adventitious formation of lignocerylsphingosine.

This ceramide has no free carboxyl group and is inactive toward nitrous acid. This is taken to indicate that a NH—CO union exists between the acid and the sphingosine.



Lignocerylsphingosine

Although palmitic acid was at first thought to be present in the molecule, it was later shown to be an impurity. Lignocerylsphingosine is the only ceramide which has as yet been identified as a hydrolysis product of sphingomyelin. In contradistinction to the sphingomyelins, the ceramides are soluble in ether; they can also be crystallized from acetone.³³¹ However, in the case of the cerebrosides, not only lignocerylsphingosine has been prepared, but also cerebronyl-*N*-sphingosine.

b. Sphingosine. Sphingosine was first prepared as the sulfate from the cerebroside phrenosin by Thudichum.³³⁵ The pioneer work in establishing its structure was done by Lapworth³³⁶ and by Levene and West.^{337,338} These investigators believed that sphingosine has an empirical formula of $\text{C}_{17}\text{H}_{35}\text{NO}_2$ and a structural one of $\text{CH}_3(\text{CH}_2)_{11}\text{CH}:\text{CH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CH}_2\text{NH}_2$. Klenk³³⁹ was unable to confirm these earlier observations, since he consistently obtained myristic and palmitic acids, respectively, on oxidation of sphingosine and of dihydrosphingosine with chromic acid. Levene and West had earlier identified these acids with the C_{13} (tridecylic) and C_{15} (pentadecylic) acids. Klenk further showed that the analyses of many sphingosine derivatives were much more in harmony with an assumed composition of $\text{C}_{18}\text{H}_{37}\text{NO}_2$ than with one of $\text{C}_{17}\text{H}_{35}\text{NO}_2$. It was also pointed out by Klenk that the earlier workers had based their opinions almost exclusively upon carbon and hydrogen analyses, while a better criterion to es-

³³⁵ J. L. W. Thudichum, *Further Researches on the Chemical Constitution of the Brain and of the Organoplastic Substances*, Ninth Annual Report of the Local Government Board, 1879–1880. Suppl. B, No. 3, Report of the Medical Officer for 1879, London, pp. 143–206.

³³⁶ A. Lapworth, *J. Chem. Soc.*, 103, 1029–1034 (1913).

³³⁷ P. A. Levene and C. J. West, *J. Biol. Chem.*, 16, 549–553 (1913–1914).

³³⁸ P. A. Levene and C. J. West, *J. Biol. Chem.*, 18, 481–484 (1914).

³³⁹ E. Klenk, *Unpublished observations*, cited from H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930, p. 42.

TABLE 10
CALCULATED PERCENTAGE COMPOSITION OF SPHINGOSINE DERIVATIVES (CALCULATED AS SPHINGOSINE) BASED UPON ASSUMED CARBON
CONTENT OF 17 AND 18 ATOMS COMPARED WITH VALUES FOUND BY ANALYSIS

Derivative	C			H			N			Br		
	Based on		Found	Based on		Found	Based on		Found	Based on		Found
	C ₁₇	C ₁₈		C ₁₇	C ₁₈		C ₁₇	C ₁₈		C ₁₇	C ₁₈	
Dihydrosphingosine ^a	53.45	54.30	54.4	7.81	7.98	8.2	10.85	10.56	10.2			
Dihydrosphingosinebromide ^c	55.40	56.51	56.7	10.40	10.55	10.7						
Triacetylsphingosine	67.15	67.71	67.38 ^b	9.98	10.19	9.98 ^b						
			67.40 ^c			10.23 ^c						
			67.4 ^c			9.9 ^c						
			67.7 ^a			10.2 ^a						
Chloroplatinate of methylated am- monium base of monomethyl- dihydrosphingosine				8.46	8.60	8.7						
Preparation 1 (amorphous) ^{a,d}	45.95	46.94	47.8	8.46	8.60	8.7	17.80	17.35	17.0	5.66	5.51	6.2
									17.6			5.5
Preparation 2 (crystalline) ^{a,d}	45.95	46.94	46.8	8.46	8.60	8.2	17.80	17.30	17.4	5.66	5.51	5.5

^a Unpublished results of E. Klenk cited by H. Thierfelder and E. Klenk, *Chemie der Cerebroside und Phosphatide*, Springer, Berlin, 1930, pp. 42-44.

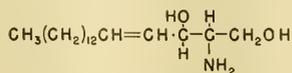
^b P. A. Levene and W. A. Jacobs, *J. Biol. Chem.*, **11**, 547-554 (1912).

^c K. Thomas and H. Thierfelder, *Z. physiol. Chem.*, **77**, 511-515 (1912).

^d E. Klenk and R. Hürle, *Z. physiol. Chem.*, **178**, 221-238 (1928).

tablish their composition would have been to include nitrogen, as well as Cl, Br, or OCH_3 , when applicable. The data on the composition of various sphingosine preparations upon which Klenk has based his conclusions are summarized in Table 10.

Carter *et al.*³¹² have demonstrated that the revised formula for sphingosine based on 18 carbon atoms should be modified by changing the assigned positions of the hydroxyl groups to carbons 1 and 3, rather than to 2 and 3 as proposed by Levene and West. This revision would seem imperative to explain the fact that benzoylsphingosine will not react with periodate, but rather forms a cyclic acetal with benzaldehyde in the presence of zinc chloride. In view of the fact that periodate will react only with vicinal glycols, while acetal formation will occur with both 1,2 and 1,3 glycols, the above results would seem to justify the assignment to sphingosine of the Carter *et al.*³¹² formula given here.



Sphingosine

Sphingosine is a component not only of sphingomyelin but also of the cerebrosides (see Section B,2). Shimizu³⁴⁰ reported the presence of sphingosine in the urine after the administration of the cerebroside phrenosine to a dog. Klenk is of the opinion that this finding has not been sufficiently established to render it certain.

Sphingosine can be crystallized from diethyl ether. It readily separates from petroleum ether in needle-like crystals. Sphingosine is easily soluble in acetone, ethyl alcohol, and methyl alcohol, but is insoluble in water. On oxidation it gives an odor of burning fat. Several well-defined derivatives of sphingosine are known. Sphingosine sulfate, $\text{C}_{18}\text{H}_{37}\text{NO}_2 \cdot \text{H}_2\text{SO}_4$, can be crystallized from hot alcohol in rosettes which consist of needles arranged in a radial formation. It melts at 240–250°C. and is very hydroscopic. It dissolves in alcohol having a slight excess of sulfuric acid. Sphingosine sulfate exhibits optical activity, its solutions being levo-rotatory, $[\alpha]_D^{20} = -13.12^\circ$ (chloroform)³⁴¹; $[\alpha]_D^{18} = -9.47^\circ$ (methyl alcohol containing H_2SO_4).³⁴² Other derivatives include the diacetate ($\text{C}_{18}\text{H}_{37}\text{NO}_2 \cdot (\text{C}_2\text{H}_4\text{O}_2)_2$), the picrolonate ($\text{C}_{18}\text{H}_{37}\text{NO}_2 \cdot \text{C}_{10}\text{H}_8\text{N}_4\text{O}_5$), and the triacetyl derivative ($\text{C}_{18}\text{H}_{34}\text{NO}_2 \cdot (\text{CH}_3\text{CO})_3$). The last compound melts sharply at 100°C. and it does not react with nitrous acid.^{341,343} A diacetyl derivative has also been reported by Levene and Jacobs.³⁴¹

³⁴⁰ T. Shimizu, *Biochem. Z.*, 117, 263–265 (1921).

³⁴¹ P. A. Levene and W. A. Jacobs, *J. Biol. Chem.*, 11, 547–554 (1912).

³⁴² O. Rosenheim, *Biochem. J.*, 10, 142–159 (1916).

³⁴³ K. Thomas and H. Thierfelder, *Z. physiol. Chem.*, 77, 511–515 (1912).

c. Dihydrosphingosine. Dihydrosphingosine, $\text{CH}_3(\text{CH}_2)_{14}\text{CHOH}\cdot\text{CH}(\text{NH}_2)\cdot\text{CH}_2\text{OH}$, is formed by the hydrogenation of sphingosine in the presence of colloidal palladium according to the method of Carter and Norris.³⁴⁴ It forms derivatives similar to those of sphingosine, *i.e.*, sulfate, triacetate,³⁴⁴ picrolonate, and picrate. Earlier analyses indicated that this molecule had 17 carbons, but the more recent work of Klenk³³⁹ with the picrate and bromide yields results which are in harmony with a C_{18} structure. Klenk³³⁹ found that, on oxidation with chromic acid, palmitic acid was produced, instead of pentadecyclic (*n*-pentadecanoic) as found by Levene and West.^{337,338} Klenk based his identification on the melting point ($60\text{--}61^\circ\text{C}$.) and the titration value of the isolated acid. The presence of dihydrosphingosine in the larval form (*Cysticercus fasciolaris*) of the cat tapeworm has been demonstrated by Lesuk and Anderson.²⁵⁰ This would indicate that it is a normal product. Although earlier workers failed to find any of the dihydro-compound associated with sphingosine in nerve tissue or brain, Carter and Norris³⁴⁴ have been able to prove that it is a normal constituent in such tissues. Carter and his associates³⁴⁵ found that the concentration of the dihydrosphingosine is considerably higher in the spinal cord than in the brain. Although it would seem probable that it may be a component of the sphingomyelin molecule, there appears to be definite evidence that it occurs in the cerebroside fraction, as it was shown to be present in the phosphatide-free portion.

A number of other related compounds can be prepared from sphingosine. These include sphingamine, an unsaturated base with the formula $\text{C}_{18}\text{H}_{37}\text{N}$, which is produced on reduction of sphingosine with hydrogen iodide. It forms a sulfate with the composition $(\text{C}_{18}\text{H}_{37}\text{N})_2\cdot\text{H}_2\text{SO}_4$.³⁴¹ *Sphingine* is an amino alcohol with the suggested formula $\text{C}_{17}\text{H}_{34}(\text{OH})(\text{NH}_2)$,^{311,346} which is formed when the reduction with hydrogen iodide occurs in acetic acid. When sphingosine is deaminized on treatment with nitric acid, dihydro-sphingosol, $\text{C}_{17}\text{H}_{35}(\text{OH})_3$, is formed.³⁴⁶ It melts at $54\text{--}55^\circ\text{C}$. On treatment with methyl iodide in the presence of silver oxide, only one hydroxyl group is methylated; the compound corresponds to the methylated ammonium base of monomethyldihydrosphingosine.³⁴⁷

d. Lignoceryl Fatty Acid Esters and Synthetic Ceramides. Reichel and Thannhauser³²⁰ succeeded in preparing a number of esters of lignocerylsphingosine, as well as some new ceramides. When sphingosine was treated with the acyl chloride of the fatty acid, it was possible to introduce three fatty acid residues into sphingosine—two in ester linkages on the hydroxyl groups and the third into the amino group. Since the esters are

³⁴⁴ H. E. Carter and W. P. Norris, *J. Biol. Chem.*, *145*, 709 (1942).

³⁴⁵ H. E. Carter, W. P. Norris, F. J. Glick, G. E. Phillips, and R. Harris, *J. Biol. Chem.*, *170*, 269–283 (1947).

³⁴⁶ P. A. Levene and C. J. West, *J. Biol. Chem.*, *24*, 63–68 (1916).

³⁴⁷ E. Klenk and R. Hårle, *Z. physiol. Chem.*, *178*, 221–238 (1928).

readily hydrolyzable, while the NH—CO linkage is quite resistant to hydrolysis, it is possible to prepare such ceramides as palmityl and stearyl-sphingosine; these had not previously been known. The properties of some of these compounds are given in Table 11.

TABLE 11

MELTING POINTS AND SOLUBILITIES OF SOME SPHINGOSINE FATS AND SOME CERAMIDES^{a,b}

Sphingosine	Melting point, °C.	Solubility				
		Ethanol	Ether	Chloroform	Acetone	Methanol
<i>O</i> -Diacetyl						
lignoceryl-.....	70-71	+++	+++	+++	++	
<i>O</i> -Dipalmityl						
lignoceryl-.....	39-40	+(H)	+++	+++	+++	
<i>O</i> -Distearyl						
lignoceryl-.....	45-47	+(H)	+++	+++	+++	
<i>O</i> -Dibenzoyl						
lignoceryl-.....	57-58	++(H)	++++	++++	++++	
Tripalmityl-.....	67-69	+(H)	++++	++++	++++	
Tristearyl-.....	72-74	+(H)	++++	++++	++++	
Tribenzoyl-.....	118-120	++	++++	++++	++++	
Palmityl-.....	86-87		++	++	++(H)	++(H)
Stearyl-.....	88-89		++	++	++(H)	++(H)

^a +, slightly soluble; ++, fairly soluble; +++, readily soluble; +++++, very soluble. H, soluble only in hot solvent.

^b Adapted from M. Reichel and S. J. Thannhauser, *J. Biol. Chem.*, 135, 15-21 (1940).

(7) Distribution of the Sphingomyelins

Sphingomyelins are found with other phospholipids and cerebrosides in highest concentration in the brain and nervous tissue. They also occur in a relatively high proportion in kidneys and liver,³¹¹ spleen,³²⁷ and to some extent in blood.³⁴⁸ Sphingomyelin-like compounds have been prepared from many other sources such as horse pancreas,³⁴⁹ kidneys and heart,^{350,351} and the slimy skin secretion from the lamprey (*Petromyzon fluviatilis*³⁵²), but not from the gastric mucosa of pigs.³⁵³ This substance is also found in erythrocytes,³⁵⁴ suprarenals,^{330,355,356} sex organs,³⁵⁵ placenta,³⁵⁷ and in traces in skeletal muscle.¹³⁵ The presence of sphingomyelin in erythrocytes is denied by Haurvitz and Sládek,³⁵⁸ who believed that the supposed compound was a mixture of lecithin and a cerebroside. Sphingomyelin or a

³⁴⁸ H. J. Channon and G. A. Collison, *Biochem. J.*, 23, 663-675 (1929).

³⁴⁹ S. Fränkel and T. R. Offer, *Biochem. Z.*, 26, 53-54 (1910).

³⁵⁰ H. MacLean, *Biochem. J.*, 6, 333-354 (1912).

³⁵¹ H. MacLean, *Biochem. Z.*, 57, 132-142 (1913).

³⁵² J. Muller and H. Reinbach, *Z. physiol. Chem.*, 92, 56-74 (1914).

³⁵³ B. Uhnoo, *Z. physiol. Chem.*, 256, 104-110 (1938).

³⁵⁴ M. Bürger and H. Beumer, *Biochem. Z.*, 56, 446-456 (1913).

³⁵⁵ D. L. Woodhouse, *Biochem. J.*, 22, 1087-1096 (1928).

³⁵⁶ H. Beumer, *Arch. expl. Path. Pharmacol.*, 77, 304-316 (1914).

³⁵⁷ C. Sakali, *Biochem. Z.*, 49, 317-325 (1913).

³⁵⁸ F. Haurvitz and J. Sládek, *Z. physiol. Chem.*, 173, 268-277 (1928).

similar product has been found in such animal products as egg-yolk,^{311,359} milk,³⁶⁰ and fish sperm,³²⁶ as well as in plant sources such as the ectoplasm of the yeast cell.³⁶¹ The principal fatty acid in the sphingomyelin of milk lipids is lignoceric.³⁶²

The quantitative relationships of sphingomyelin in a number of human tissues have been reported by Thannhauser *et al.*,³⁶³ who made use of a new method for the determination of lecithin, cephalin, and sphingomyelin.⁴¹ The following average values were found in per cent of dried tissue for total phospholipid and sphingomyelin, respectively: brain, 30.90, 5.66; lung, 6.65, 1.45; spleen, 8.56, 0.86; kidney, 8.00, 0.72; liver, 9.80, 0.38; and heart, 6.87, 0.34. It is thus apparent that, with the exception of the brain and lungs, in which the sphingomyelin accounts for 20% of the total phospholipid, in most organs sphingomyelin usually comprises only from 5 to 10% of the total phospholipid.

Schmidt and collaborators³⁶⁴ have noted differences in the distribution of sphingomyelin in man and in rats. In the rat brain, only about 5% of the total lipid phosphorus was sphingomyelin phosphorus; on the other hand, the sciatic nerve contained as high as 32% of this fraction. Sphingomyelin comprised the following percentages of total phospholipids in other rat tissues: liver, 2.6; kidney, 14.0; lung, 14.0; spleen, 14.1; and heart, 4.9. In the cat, 24% of the total phospholipid was sphingomyelin and 50.0% of the sciatic nerve was composed of this compound. In beef brain, the white matter was shown to have four times as much sphingomyelin as the grey matter.

6. The Phosphatidic Acids

Chibnall and Channon^{143,144,365,366} first isolated this type of phosphatide from cabbage leaves and also from spinach. Such compounds have a typical phosphatide structure except that no nitrogenous base is present. In plants, the phosphoric acid is usually combined with calcium. When calcium is removed from this product, the corresponding diglyceride phosphoric acid or *phosphatidic acid* is formed. Such acids are thick oils which darken on oxidation, under which condition they are no longer soluble in ether. It is not known how extensively they are distributed in nature, as

³⁵⁹ M. Stern and H. Thierfelder, *Z. physiol. Chem.*, **53**, 370-385 (1907).

³⁶⁰ T. B. Osborne and A. J. Wakeman, *J. Biol. Chem.*, **21**, 539-550 (1915).

³⁶¹ J. Schumacher, *Centr. Bakt. Parasitenk.*, I, *Orig.*, **103**, 193-207 (1928).

³⁶² F. E. Kurtz and G. E. Holm, *J. Dairy Sci.*, **22**, 1011-1015 (1939).

³⁶³ S. J. Thannhauser, J. Benotti, A. Walcott, and H. Reinstein, *J. Biol. Chem.*, **129**, 717-719 (1939).

³⁶⁴ G. Schmidt, J. Benotti, B. Hershman, and S. J. Thannhauser, *J. Biol. Chem.*, **166**, 505-511 (1946).

³⁶⁵ A. C. Chibnall and H. J. Channon, *Biochem. J.*, **21**, 225-232 (1927).

³⁶⁶ A. C. Chibnall and H. J. Channon, *Biochem. J.*, **23**, 176-184 (1929).

thick oil, colored yellow with chlorophyll. The yield in cytoplasm amounts to 0.6–0.97%, while the ether extract amounts to 0.1–0.2% of the fresh leaves. The ether residue is dried *in vacuo* and taken up with anhydrous ether, whereby a small amount remains undissolved. About 40% of the dissolved material, which contains all the phosphorus, is precipitated from this ether solution by the addition of 4 volumes of acetone. The precipitate contains *n*-nonacosane and di-*n*-tetradecyl ketone, which may be extracted with boiling acetone. The undissolved residue contains all the phosphorus, which is present in quite similar proportions in different preparations. It consists of the calcium salt of the phosphatidic acids.

To remove the calcium, the ether solution of the calcium salt is shaken with 0.5–0.25 *N* sulfuric acid, after which the calcium is removed with lead acetate. The ethereal solution of lead salt is washed with water and concentrated *in vacuo*. The lead salt is precipitated with absolute alcohol. It is redissolved in ether and reprecipitated with absolute alcohol, again dissolved in ether, and then shaken with 0.25 *N* hydrochloric acid to remove the lead. The ether solution is washed with water and concentrated *in vacuo* to a syrup. Afterward, through treatment with cold absolute alcohol, a small amount of an insoluble iron-containing impurity is removed, and the free acid remains behind on evaporation of the alcohol.

(3) *Properties of the Phosphatidic Acids*

The phosphatidic acids are liquid olive-brown oils which remain unchanged for long periods at room temperature, but which turn hard and brown on exposure to air and become insoluble in organic solvents. The unchanged acids are soluble in most organic solvents, cold acetone, and especially ether, but are only slightly soluble in water. An alcoholic or acetone solution can, however, be considerably diluted with water without the development of an emulsion.

Phosphatidic acid forms a number of salts. The sodium salt is soluble in water, but only slightly soluble in cold alcohol, and is completely insoluble in ether. The barium, calcium, and lead salts are hard plastic masses which are insoluble in water but very soluble in ether, from which they may be precipitated by alcohol and acetone.

(4) *Hydrolysis of the Phosphatidic Acids*

Channon and Chibnall¹⁴⁴ found that, on hydrolysis with barium hydroxide, a glycerophosphoric acid was formed which had a dextro-rotation. Fatty acids were also recovered to the extent of 93% of the theoretical. These had an iodine number of 136. Proof that the phosphatidic acid is usually a mixture of several types of molecules is found in the fact that, on

separation of the ether-insoluble and ether-soluble lead salts, the iodine numbers of the respective fractions were 44 and 173.

(5) *Enzymic Breakdown of Phosphatides to Phosphatidic Acids*

That the phosphatidic acids may not be primary products in nature but may rather be intermediates in the breakdown of lecithin, phosphatidylethanolamine, or phosphatidylserine, seems possible in view of the recent demonstration of an enzyme present in plants which is capable of splitting off the nitrogenous base from these compounds. Phosphatidic acid would be the residue which remains. This enzyme was first demonstrated by Hanahan and Chaikoff³⁶⁸ in raw carrot. It was later shown³⁶⁹ to have an optimum *pH* between 5.2 and 5.9. The enzyme exhibits a high degree of stability toward heat; it is not completely inactivated when exposed to a temperature of 95°C. for 15 minutes.

The same enzyme has recently been shown to be present in cabbage leaves.³⁷⁰ Hanahan and Chaikoff³⁷⁰ believe that, in the preparation of phosphatidic acid, the enzyme set free by maceration of the leaves hydrolyzes the phospholipid to phosphatidic acid. These investigators were able to prepare phosphatidic acid when they followed the procedure of Chibnall and Channon.³⁶⁵ However, when the enzyme was largely destroyed by steam treatment prior to maceration and extraction, a phospholipid high in choline and nitrogen was isolated from cabbage leaves, in place of phosphatidic acid. One must assume, therefore, that phosphatidic acid probably does not exist as a primary product; however, since it so readily originates by enzymic breakdown of the phospholipids, it must be considered to be of importance as an intermediate.

(6) *Compounds Related to Phosphatidic Acid*

Cardiolipin is a phospholipid present in beef heart which is now believed to be a phosphatidic acid. Pangborn³⁷¹ coined the name for a substance prepared from beef heart which is responsible for the reaction with the sera of syphilitics. Since the work of Noguchi,³⁷² it has been recognized that the antigenic action of beef heart preparations is associated with an acetone-insoluble fraction, but it has been uncertain which phosphatide is involved. Although impure lecithin preparations have been described as

³⁶⁸ D. J. Hanahan and I. L. Chaikoff, *J. Biol. Chem.*, **168**, 233-240 (1947).

³⁶⁹ D. J. Hanahan and I. L. Chaikoff, *J. Biol. Chem.*, **169**, 699-705 (1947).

³⁷⁰ D. J. Hanahan and I. L. Chaikoff, *J. Biol. Chem.*, **172**, 191-198 (1948).

³⁷¹ M. C. Pangborn, *J. Biol. Chem.*, **143**, 247-256 (1942).

³⁷² H. Noguchi, *Z. Immunitäts.*, **9**, 715-749 (1911).

antigenic *in vitro*,^{373,374} Wadsworth *et al.*³⁷⁵ demonstrated that adequately purified lecithin and cephalin are completely ineffective in the test. However, the active principle is also thrown out of solution by cadmium chloride, since beef heart lipids from which all cadmium chloride-precipitable material has been removed were found to be no longer active as antigens in the complement-fixation test for syphilis.³⁷⁶

Pangborn³⁷⁷ demonstrated that a properly balanced mixture of cardioli-
pin, cholesterol, and lecithin functions in the same way as beef heart extract to produce a complement-fixing action with the sera of syphilitics. It was also proved that the system is inactivated if any one of the three components is omitted from the mixture. Cardioli-
pin by itself possesses no activity.

The separation of cardioli-
pin from lecithin offers considerable difficulty. However, it was accomplished by transforming the mixtures to their barium salts,³⁷⁸ from which the barium salt of cardioli-
pin could be separated because of its insolubility in methanol. The use of sodium sulfate in precipitation was later recommended for improving the yield of cardioli-
pin.³⁷⁹ When this modified procedure was employed, 6.4 g. of purified sodium cardioli-
pin was obtained from 5 hearts. A further improvement in procedure for the preparation of cardioli-
pin has been reported more recently.³⁸⁰

Cardioli-
pin has been found to be a viscous yellow oil soluble in acetone or alcohol, as well as in the other usual lipid solvents, but not in water. It has a specific rotation of $+5.8^\circ$ at 25°C . in ethanol. The iodine number was found to be 119³⁷⁸ (106, 112, 126, 99.8, and 112 in later successive preparations),³⁸⁰ and the apparent molecular weight as determined by titration was 726. The phosphorus content was found to be 4.31%, and no chlorine or sodium was detected. Although the compound was originally believed to contain a polysaccharide group,³⁷¹ this report was later found to be in error.³⁷⁸ Cardioli-
pin is believed to be a phosphatidic acid

7. Acetal Phosphatides (Plasmalogens)

Feulgen discovered a new group of phosphatides, as a result of peculiar staining reactions which were observed when fuchsin sulfuric acid was used. This reagent produces a purple color with aldehydes; if it is used after a preliminary treatment of the cells with sulfuric acid, a selective

³⁷³ I. Sakakibara, *J. Biochem. Japan*, *24*, 31-72 (1936).

³⁷⁴ J. W. Wellman and H. P. Lankelma, *Venereal Dis. Inform.*, *22*, 12-14 (1941).

³⁷⁵ A. Wadsworth, E. Maltaner, and F. Maltaner, *J. Immunol.*, *26*, No. 1, 25-48 (1934).

³⁷⁶ F. Maltaner, in *Ann. Rep. Div. Lab. Res., New York State Dept. Health*, Albany, No. 54, 19 (1933).

³⁷⁷ M. C. Pangborn, *Proc. Soc. Exptl. Biol. Med.*, *48*, 484-486 (1941).

³⁷⁸ M. C. Pangborn, *J. Biol. Chem.*, *153*, 343-348 (1944).

³⁷⁹ M. C. Pangborn, *J. Biol. Chem.*, *157*, 691-692 (1945).

³⁸⁰ M. C. Pangborn, *J. Biol. Chem.*, *161*, 71-82 (1945).

staining of the chromatic structures of the nuclei obtains, due to the aldehyde groups which are set free by the hydrolysis of thymonucleic acid.

However, Feulgen noted that his reagent stained not only the nuclei but also the cytoplasm of many tissues, although the cytoplasm retained the dye to a lesser extent. When the tissue had been previously defatted, staining was no longer observed in the cytoplasm, although that in the nuclei was unaffected. Feulgen, therefore, suggested that the unknown carriers of the fuchsin-sulfurous acid in the cytoplasm should be designated as *plasmal*; since the staining was markedly accelerated by a preliminary treatment with acid, this investigator believed that the plasmal was not present in the cell as such but in the form of a precursor which was called *plasmalogen*.³⁸¹

When attempts were made to isolate plasmalogen, it was found that it was invariably present in the phosphatide fraction. When mercuric chloride was added to phospholipid suspensions from brain, muscle, or heart, a strong reaction indicating the presence of plasmal was immediately obtained. Plasmal was first isolated by Feulgen *et al.*³⁸¹ from horse muscle phosphatides, by steam distillation, and by subsequent condensation as the semithiocarbazone. A better method for the preparation of plasmal has recently been proposed by Behrens.³⁸² Employing this method, he was able to prepare 1–1.5 g. of plasmal semithiocarbazone from 10 kg. of horse meat. Anchel and Waelsch³⁸³ have developed a method for the isolation of the plasmals from comparatively small amounts of tissue. Klenk³⁸⁴ found that non-saponifiable material separated from the cephalin fraction of brain responds to the fuchsin test, and has properties identical with those of the acetal phospholipids which were separated in the form of dimethyl acetals.

Feulgen and Bersin²⁷ succeeded in preparing plasmalogen by virtue of its relative stability against alkali. On treatment of the phosphatide emulsion from beef muscle with sodium hydroxide, most of the plasmalogen remained intact, while the contaminating phosphatides were largely removed by saponification. Plasmalogen, together with the fatty acids, can be precipitated at this stage by brucine. After removal of the brucine soaps by extraction with acetone, the plasmalogen is purified by repeated treating with benzene, in which it is insoluble. The plasmalogen is then crystallized from alcohol at room temperature.

(1) Structure of the Plasmalogens

The plasmalogens are acetals of fatty aldehydes which are combined with colamine glycerophosphate.²⁷ Two possible types of compounds are

³⁸¹ R. Feulgen, K. Imhäuser, and M. Behrens, *Z. physiol. Chem.*, 180, 161–179 (1929).

³⁸² M. Behrens, *Z. physiol. Chem.*, 191, 183–186 (1930).

³⁸³ M. Anchel and H. Waelsch, *J. Biol. Chem.*, 145, 605–613 (1942).

³⁸⁴ E. Klenk, *Z. physiol. Chem.*, 281, 25–28 (1944).

it would appear to be a phosphatidic acid formed by the removal of the nitrogenous base, since Feulgen and Bersin²⁷ showed that it contained glycerophosphate and a fatty aldehyde in equivalent amounts.

B. CEREBROSIDES

1. Introduction

The cerebrosides consist of that group of conjugate lipids which contains a nitrogenous base, sphingosine, one of several fatty acids of the C₂₄ series, and a molecule of carbohydrate, which may be either galactose or glucose. They are distinguished from lecithin, phosphatidylethanolamine, and phosphatidylserine by the fact that they contain neither glycerol nor phosphoric acid, while they differ from inositol phosphatides and sphingomyelin in having no phosphoric acid. They are also unique in that they are the only common lipids in which carbohydrate is an essential part of the molecule. Since they are usually associated in nature with the various phosphatides, and since their solubilities in many solvents are similar to those of these substances, the preparation of pure cerebrosides presents some difficulties.

The cerebrosides were first isolated from the brain by Thudichum,¹ who proposed this name for them because of their preparation from the cerebrum. This pioneer worker also demonstrated the presence of two types of cerebrosides, which he called phrenosine and kersasine.³⁸⁷ It was not until many years later that such other representatives as nervone³⁸⁸ and oxynervone³⁸⁹ were first recognized. The fact that there are two general groups of cerebrosides, one of which contains galactose and the second of which has the glucose molecule as the component part, has only recently been conclusively demonstrated.³⁹⁰

Although the cerebrosides are found in the highest concentration in the white matter of the nervous tissues,³⁹¹ they also occur in the gray matter. In addition to being present in the brain of man and of other mammals, they have been found in the brain of birds.³⁹² They occur in only extremely small amounts, or are entirely absent, in the brain tissue of such lower forms as codfish and shellfish.³⁹² However, cerebrosides have been isolated from sturgeon brain (*Acipenser sturio*).³⁹³

³⁸⁷ The original German spelling "kerasin" has been subsequently modified to "cersasine" to be more in line with current American practice.

³⁸⁸ E. Klenk and R. Härle, *Z. physiol. Chem.*, 189, 243-253 (1930).

³⁸⁹ E. Klenk, *Z. physiol. Chem.*, 157, 291-298 (1926).

³⁹⁰ N. Halliday, H. J. Deuel, Jr., L. J. Tragerman, and W. E. Ward, *J. Biol. Chem.*, 132, 171-180 (1940).

³⁹¹ A. Noll, *Z. physiol. Chem.*, 27, 370-397 (1899).

³⁹² A. Argiris, *Z. physiol. Chem.*, 57, 288-295 (1908).

³⁹³ A. Kossel and F. Freytag, *Z. physiol. Chem.*, 17, 431-456 (1893).

There is some indication that the cerebrosides may also be found in tissues other than the brain. In Gaucher's disease, their appearance in the liver and especially in the spleen in relatively large amounts has been repeatedly noted. Moreover, Thierfelder and Klenk⁴ are of the opinion that the cerebrosides are probably to be considered as general cell constituents under normal conditions, since their appearance in a wide variety of normal organs and tissues has been reported. The normal tissues in which they have been found include the adrenal,³³⁰ the kidney,^{394,395} the spleen,^{396,397} the liver,³⁹⁵ leukocytes from the thymus,³⁹⁸ the lung,³⁹⁹ and the retina.⁴⁰⁰ Their relationship to the reproductive organs is indicated by the demonstration of their presence in egg yolk³⁹⁵ and fish sperm.^{326,393} Cerebrosides have also been noted in the red blood cells,^{401,402} while their occurrence in white cells is evident from their early demonstration in pus.^{393,396} Müller⁴⁰³ has also reported their presence in sputum. The finding of cerebrosides under pathological conditions such as hypernephroma⁴⁰⁴ and atherosclerosis of the aorta⁴⁰⁵ is of considerable interest.

The occurrence of cerebrosides in plant sources has frequently been reported. These include the fungi,⁴⁰⁶⁻⁴¹¹ such seeds as oats and rice,^{412,413} and oak wood⁴¹⁴; however, the cerebrosides have not been prepared in pure form. It is possible that impure preparations of phosphatides have been mistaken for cerebrosides.

There are certain properties which are more or less common to the various cerebrosides. In general they are amorphous white substances, but when pure they can be prepared in crystalline form. The properties and composition of the different cerebrosides correspond closely; therefore, their separation and preparation in pure form are exceedingly difficult. They

³⁹⁴ O. Rosenheim and H. MacLean, *Biochem. J.*, **9**, 103-109 (1915).

³⁹⁵ P. A. Levene and C. J. West, *J. Biol. Chem.*, **31**, 649-654 (1917).

³⁹⁶ F. Hoppe-Seyler, *Med.-Chem. Untersuch.*, **4**, 486-501 (1871).

³⁹⁷ E. Walz, *Z. physiol. Chem.*, **166**, 210-222 (1927).

³⁹⁸ L. Lillienfeld, *Z. physiol. Chem.*, **18**, 473-486 (1894).

³⁹⁹ U. Sammartino, *Biochem. Z.*, **124**, 234-243 (1921).

⁴⁰⁰ A. Cahn, *Z. physiol. Chem.*, **5**, 213-232 (1881).

⁴⁰¹ O. Pascucci, *Beitr. chem. Physiol. Path.*, **6**, 543-551 (1905).

⁴⁰² I. Bang and I. Forssman, *Beitr. chem. Physiol. Path.*, **8**, 238-275 (1906).

⁴⁰³ F. Müller, *Berlin. klin. Wochschr.*, **35**, 75-76 (1898).

⁴⁰⁴ R. Schönheimer, *Z. physiol. Chem.*, **168**, 146-151 (1927).

⁴⁰⁵ R. Schönheimer, *Z. physiol. Chem.*, **177**, 143-157 (1928).

⁴⁰⁶ M. Bamberger and A. Landsiedel, *Monatsh.*, **26**, 1109-1118 (1905).

⁴⁰⁷ J. Zellner, *Monatsh.*, **32**, 133-142 (1911).

⁴⁰⁸ J. Zellner, *Monatsh.*, **32**, 1057-1063 (1911).

⁴⁰⁹ R. Rosenthal, *Monatsh.*, **43**, 237-253 (1922).

⁴¹⁰ E. Hartmann and J. Zellner, *Monatsh.*, **50**, 193-200 (1928).

⁴¹¹ N. Fröschl and J. Zellner, *Monatsh.*, **50**, 201-210 (1928).

⁴¹² G. Trier, *Z. physiol. Chem.*, **86**, 153-173 (1913).

⁴¹³ G. Trier, *Z. physiol. Chem.*, **86**, 407-414 (1913).

⁴¹⁴ M. X. Sullivan, *Chem. Zentr.*, **1918**, *II*, 632-633.

are insoluble in water, as well as in diethyl ether and petroleum ether. They are readily soluble in hot alcohol, but separate from it on cooling. Pyridine is an excellent solvent when cold as well as when heated. Cerebrosides can be precipitated from alcoholic or pyridine solutions by the addition of paraldehyde.⁶⁸ They are optically active.

These compounds have been referred to in the literature under a number of designations, such as cerebrosides (Thudichum), galactolipins (Leathes and MacLean), galactosides (Rosenheim), and sometimes glycolipids. Since glucose-containing cerebrosides as well as galactose-containing cerebrosides are known, it has been suggested that the term "galactolipid" be reserved for those which contain galactose, while "glycolipid" be used to designate only those members of the group which contain glucose.³⁹⁰

2. Structure of the Cerebrosides

The cerebrosides are believed to have a uniform structural pattern in which the variations in the individual members involve only the substitution of different fatty acid molecules or the replacement of galactose by glucose. When cerebrosides are hydrolyzed, sphingosine, one molecule of sugar (*d*(+)-galactose or *d*(+)-glucose), and one fatty acid are set free. The fact that the amino group of sphingosine is in combination, and that the carboxyl grouping of the fatty acid cannot react, leads to the belief that these components are joined together in a NH—CO linkage similar to that which exists in the sphingomyelins. This is further confirmed by the fact that the galactose can be split off, leaving a sphingosine fatty acid compound or ceramide of which lignoceryl sphingosine is an excellent example. The galactose (or glucose) is combined with the ceramide fraction through the aldehyde group of the sugar, since the cerebroside is a non-reducing compound. That such a combination between the carbohydrate and the ceramide occurs with the sphingosine rather than with the fatty acid is indicated by the fact that a non-reducing derivative, *psychosine*, has been prepared which consists of one molecule of sphingosine and one of galactose, but from which the fatty acid residue is absent. In contradistinction to the cerebrosides, or in fact to the ceramides, the amino group of psychosine is free; this constitutes further evidence that the union of fatty acid to the sphingosine is through the amino group. The combination of the carbohydrate with sphingosine must be through one of the hydroxyls of the latter molecule. It is not known whether such a union occurs with the hydroxyl on C₁ or on C₃.

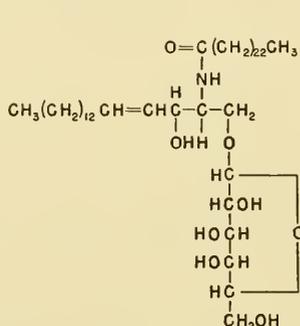
Four different cerebrosides of the galactolipid type are known. These consist of the following:

Cerasine (referred to also as kersasine), which was discovered by Thudichum¹ in 1874, and which contains lignoceric acid ($\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$) as the component acid.

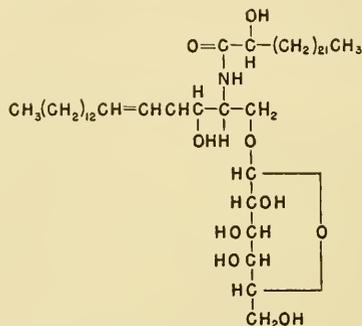
Phrenosine (also called *cerebron* by Thierfelder),⁴¹⁵ which was also discovered and named by Thudichum¹ in 1874. This contains cerebronic acid as a constituent acid, which has been shown by Klenk⁴¹⁶ to be α -hydroxylignoceric acid ($\text{CH}_3(\text{CH}_2)_{21}\text{CHOHCOOH}$).

Nervone, discovered in 1925 by Klenk,⁴¹⁷ in which the acid, nervonic acid, was shown to be 15,16-tetracosenic acid ($\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_{13}\text{COOH}$).

Oxynervone, also discovered by Klenk,⁴¹⁸ on the basis of separation of oxynervonic acid, which is α -hydroxynervonic acid ($\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_{12}\text{CHOHCOOH}$). This cerebroside has not been prepared in pure form.

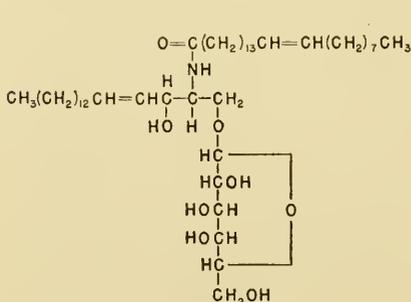


Cerasine

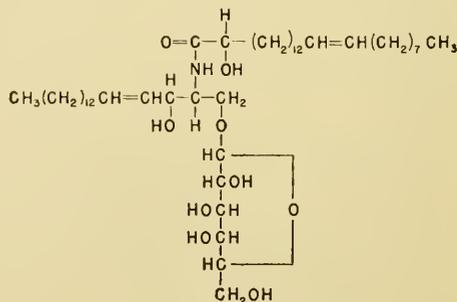


Phrenosine

It seems probable that similar cerebrosides of the glycolipid type exist. Of the glycolipids which have been studied, only the cerasine type has been reported.³⁹⁰ It would also appear that isomeric cerebrosides might exist where the galactoside or glucoside attachment to sphingosine occurs through the hydroxyl group on carbon 3 instead of on carbon 1.



Nervone



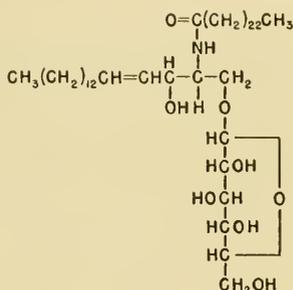
Oxynervone

⁴¹⁵ H. Thierfelder, *Z. physiol. Chem.*, **43**, 21-31 (1904).

⁴¹⁶ E. Klenk, *Z. physiol. Chem.*, **179**, 312-319 (1928).

⁴¹⁷ E. Klenk, *Z. physiol. Chem.*, **145**, 244-260 (1925).

⁴¹⁸ E. Klenk, *Z. physiol. Chem.*, **174**, 214-232 (1928).



Glucosidocerasine

3. Preparation of the Cerebrosides

(1) *Thierfelder Method for the Preparation of Phrenosine*^{419,420}

After removal of the blood and connective tissue from the brain, the tissue is macerated and extracted in 2.5-kg. batches with 3 liters of acetone. This is allowed to stand at room temperature, with frequent shaking, for 24 hours after which it is filtered. This treatment is then repeated. The residue, which is now largely dehydrated, is extracted with 1500 ml. of ether for 24 hours, during which the flask is shaken frequently. This extraction is repeated until the extract is no longer colored and a minimum of the material is left after concentration of the filtrate. This usually requires 4 or 5 successive extractions. About 350 g. of defatted tissue remains (13–14% of the original). After this residue is pulverized, it is heated with 5 volumes of 85% ethyl alcohol and is filtered hot. The crude cerebroside separates on cooling. The filtrate is used for a second extraction of the tissue. Similar extractions are continued, making use of the filtrate each time until no more cerebroside separates on cooling. The precipitate is shaken with ether for the removal of any adherent ether-soluble material, and is then separated by centrifugation.

This so-called *protagon* is a snow-white mass which amounts to about 3–4% of the fresh brain (80–100 g.). For further purification it is dissolved in methanol containing 75% chloroform. It is readily soluble, and 100 g. will dissolve easily in 500 ml. of the solvent when it is warmed slightly. After the protagon has dissolved, the solution is filtered and is allowed to stand in a closed flask at room temperature. Within a day, a hard thick crust forms on the surface. This is filtered off, the filtrate is cooled, and the precipitate is filtered. The precipitates are recrystallized from a fresh methyl alcohol-chloroform mixture. The mother liquor is evaporated to dryness and the residue is crystallized from the same solvent. The precipitates are combined and dissolved in 25–30 volumes of methanol

⁴¹⁹ H. Loening and H. Thierfelder, *Z. physiol. Chem.*, 68, 464–470 (1910).

⁴²⁰ F. Kitagawa and H. Thierfelder, *Z. physiol. Chem.*, 49, 286–292 (1906).

containing 20% chloroform. The precipitate separating on cooling still contains small amounts of phosphorus-containing compounds.

In order to remove the impurities, a zinc reagent— $Zn(OH)_2 + NH_3$ (in methanol)—is added to a methyl alcohol solution containing 10% of chloroform and the mixture is treated until the precipitate flocculates. The precipitate chiefly contains compounds rich in phosphorus. The precipitate originating on cooling the residue is filtered off, and it is again dissolved in a mixture of methyl alcohol and 10% chloroform. Phrenosine separates from the cooled filtrate chiefly in the form of glistening platelets or in an amorphous state.

(2) *Preparation of Phrenosine and Cerasine by the Rosenheim Method*^{421,422}

a. Separation of Crude Cerebrosides. In order to prepare the crude cerebroside mixture, 10 kg. of macerated brain is mixed with 10 liters of acetone and is allowed to stand for 24 hours, with frequent stirring. The water-containing acetone is removed by pouring through fine muslin, and the brain is treated repeatedly with fresh acetone until all the cholesterol has been removed. After this treatment is completed, the acetone is removed from the residue, and the latter is dried in thin layers on a warm plate. It is then exhaustively extracted with petroleum ether as long as any material is being removed. The residue is again dried as above. It is ground to a fine powder, which amounts to about 13% of the fresh brain (1300 g.). Batches of 500 g. of this powdered residue are extracted with 1500 ml. of pyridine (b.p., 115°C.) by warming for about 20 minutes to 45–50°C. After cooling, the filtrate is concentrated to a small volume, and the galactosides are precipitated by the addition of 3 or 4 volumes of acetone and by cooling to 0°C. After the residue has been filtered through a glass filter without pressure, the residue is suspended in acetone, filtered by suction, and the acetone is removed from the precipitate by drying *in vacuo*.

Further elimination of ether-soluble impurities is effected by extraction with ether in a Soxhlet apparatus. The crude cerebroside is a yellow powder which contains only about 0.5% phosphorus. The yield is about 2% of the fresh brain.

b. Removal of Phosphorus-Containing Impurities. The phosphatides and other similar contaminants are readily removed by recrystallizing them twice from alcohol containing 67% chloroform. The purified cerebroside mixture is a white powder containing only a trace of phosphorus (0.08%).

c. Separation of Phrenosine and Cerasine.⁴²² Phrenosine and cerasine

⁴²¹ O. Rosenheim, *Biochem. J.*, 7, 604–610 (1913).

⁴²² O. Rosenheim, *Biochem. J.*, 8, 110–120 (1914).

can be separated because of a differential solubility in acetone. 50 g. of the purified cerebroside mixture is suspended in 3500 ml. of 90% acetone and heated on a water bath at 56°C. The insoluble residue (about 15%) is filtered off and the filtrate is allowed to stand at 37°C. After 16 to 20 hours, the precipitate, which adheres to the walls of the flask and which contains the phrenosine fraction, is separated by filtration through a filter heated to 37°C. The filtrate is allowed to stand for at least 24 hours in the refrigerator, after which time the cerasine is largely precipitated. This cerebroside starts to precipitate when the solution temperature falls below 28°C. It is filtered by suction, washed with acetone, and dried *in vacuo*. The phrenosine fraction amounts to about 50% of the total crude cerebroside, while the cerasine fraction is much smaller.

d. Purification of Phrenosine. The crude phrenosine (32.5 g.) is pulverized, dissolved in 120 ml. of chloroform at 60°C., and 180 ml. of acetic acid heated to 60°C. is added to it. The phrenosine precipitates after standing overnight at 37°C.; it is then filtered and washed with a mixture of 3:2 acetic acid and chloroform at 37°C. The moist precipitate is treated with 200 ml. of the same mixed solvents; it is then filtered and dried *in vacuo*. About 6.6 g. of precipitate is obtained from the first 2 mother liquors when the solution stands at room temperature; this precipitate is worked up with the cerasine. The phrenosine preparation, which still contains traces of cerasine, is purified by solution in 40 volumes of chloroform, to which is added 60 volumes of warm acetone; the precipitate of phrenosine which originates when the solution stands at 37°C. is filtered, and the reprecipitation is repeated twice. The preparation is found to be cerasine-free. The final purification employed involves crystallization from acetone containing 50% pyridine and acetone containing 10% of water.

e. Purification of Cerasine. For the purification of the cerasine, the crude sample (10 g.) is dissolved in 40 ml. of chloroform at 50°C., and 60 ml. of acetic acid heated to 60°C. is added to it. The solution remains clear until the temperature drops to about 40°C., after which the phrenosine present separates out. The solution is kept at 37°C. for some time and the precipitate is removed. On cooling of the filtrate to 26°C., separation of a gelatinous precipitate of cerasine occurs. This is filtered and washed with a 3:2 acetic acid-chloroform mixture. The precipitate is then suspended in acetone and is again filtered. A second purification is carried out, using 50 ml. of the acetic acid-chloroform mixture, at which time about 0.5 g. of phrenosine separates at 37°C. When this is repeated once more, no additional phrenosine precipitates from the mixture on cooling to 37°C., even if the solution stands for many hours. If the precipitate still gives a qualitative test for phrenosine, the precipitate can be further purified by dissolving it in 10 volumes of warm pyridine, and by adding the same volume of acetone warmed to 45°C. Any phrenosine which precipi-

tates at 37°C. is removed, and the precipitate of cerasine which forms at room temperature is removed. This purification may be repeated. The final purification involves recrystallization from a large amount of 90% acetone containing 2% pyridine. On an average, only 1.36 g. of pure material is obtained from 10 g. of crude cerasine.

(3) *Loening and Thierfelder Method for Removing Phosphatides by Use of Barium Hydroxide*⁴²³

The most difficult procedure in the purification of the cerebrosides is the removal of the phosphatide impurities. Loening and Thierfelder have accomplished this by hydrolysis of these compounds with saturated barium hydroxide; this procedure leaves the cerebroside unaltered.

Saturated barium hydroxide is added to 30 g. of the "protagon" obtained by the Thierfelder method (Method 1), and the mixture is reduced to a fine emulsion which is free from large particles. The volume is made up to 1100 ml. and the mixture is heated in a boiling water bath for 70 minutes, with constant agitation. After cooling, the precipitate is filtered and washed with water to remove the barium and then with acetone to remove the water. The residue is ground with acetone and is then heated at boiling temperature with 1000 ml. of acetone for 3 minutes with shaking. It is then filtered through a hot-water funnel. After cooling in a refrigerator, the precipitate is filtered and the filtrate is used for further extraction. The digestion of the precipitate with acetone is repeated a number of times, first for only a few minutes, then for 15 minutes, for 30 minutes, and finally for a number of hours. The first precipitates are phosphorus-free, while the later ones contain small amounts of phosphorus. Additional phosphorus-free material can be obtained from the impure precipitates by a new extraction with acetone. About 25 g. of the partially purified cerebrosides is dissolved in 70 volumes of absolute alcohol, and the solution is allowed to stand overnight at 29°C. The precipitate is removed by filtration through a hot-water funnel. The precipitate is cerebroside and accounts for about 70% of the total cerebroside. It consists chiefly of phrenosine. The further purification and separation of the cerebrosides into phrenosine and cerasine can be carried out from this point as described earlier.

(4) *Preparation of Nervone According to Method of Klenk*⁴¹⁷

a. Separation of Crude Nervone. The brain is macerated, dehydrated, and prepared for extraction according to the Thierfelder method.^{419,420} A petroleum ether extract of 10 human brains is concentrated to 3 liters and refrigerated overnight. The precipitated protagon is removed, the filtrate is reduced to 500 ml., and 2.5 liters of absolute alcohol is slowly added, with continual stirring. The clear supernatant fluid is poured off from the sticky

⁴²³ H. Loening and H. Thierfelder, *Z. physiol. Chem.*, 74, 282-289 (1911).

precipitate of cephalin which forms on the sides and bottom of the flask. The precipitate is washed with absolute alcohol. This is added to the main solution. The petroleum ether is removed by reducing the volume of the solution under reduced pressure. The remaining liquid is brought to the original volume with absolute alcohol. A hot saturated alcoholic ammoniacal lead acetate solution is added, and the precipitate is filtered after the solution cools. A second precipitate was obtained when the process was repeated. In the material from the 10 brains, 21 g. of the crude precipitate separated on the first precipitation, and 4.5 g. on the second treatment.

The lead precipitate (21 g.) is decomposed with hydrogen sulfide by digesting a suspension in hot 96% ethanol (350 ml.) on a water bath for 90 minutes. After removal of the precipitate of lead sulfide, the filtrate is refrigerated overnight. In the experiment described, a copious precipitate of crude nervone was obtained. On concentration of the filtrate *in vacuo*, an additional small crop of crystals separated.

The phospholipid and other sugar-free impurities are removed by washing the precipitate with acetone acidified slightly with hydrochloric acid. A further purification from the phosphorus-containing compounds involves precipitation with cadmium acetate from a methanol solution.

The crude nervone was found to be phosphorus-free and, on hydrolysis with acid, it yielded galactose, sphingosine, and a mixture of saturated and unsaturated acids. Nervone is believed to be mixed with another cerebroside. The iodine number is 50.7–51.8, compared with a value for the pure nervone of about 63. Cerebron and cerasine both have iodine numbers of 31.

b. Purification of Nervone. Since it was assumed that the saturated fatty acids which occurred in the crude nervone preparation were impurities present in cerebron or cerasine, the new cerebroside (nervone) was necessarily in the fraction with the higher iodine number. The determination of the iodine number was used by Klenk to follow the progress of the purification. Several fractional crystallization procedures were employed.

(a) *First Method of Fractional Crystallization.*⁴¹⁷ The phosphorus-free crude nervone obtained above was dissolved in 15 volumes of methanol with heating. On cooling to 40°C., a granular precipitate separated which was filtered off (Fraction 1). When the filtrate was allowed to cool further, a second crystallization started at 30°C. After cooling to 0°C., the precipitate was filtered (Fraction 2). This was refractionated in the same manner; most of the material appeared in the fraction precipitating at the lower temperature (Fraction 2a). A further refractionation gave no further purification.

	Iodine number
Fraction 1.....	47.1, 46.5
Fraction 2.....	58.7

(b) *Second Method of Purification.*⁴¹⁷ The crude nervone was taken up by warming in 20 volumes of a 1:2 mixture by volume of chloroform and methanol. The following fractions were obtained:

	Iodine number
Fraction 3, by cooling at room temperature.....	55.8
Fraction 4, by refrigeration.....	60.0
Fraction 5, by recrystallization of the residue from the mother liquor from methanol.....	61.0

The third fraction was considered to be the purest.

(c) *Purification as the Acetyl Compound.* After acetylation by Thierfelder's method,⁴²⁴ fractionation of a methyl alcohol solution was employed. One fraction (6) was obtained on cooling while the other fraction (7) was obtained from the filtrate. After removal of the acetyl groups by boiling for 30 minutes with 0.5 *N* methanol-sodium hydroxide, the two fractions had the following iodine numbers:

Fraction 6.....	52.1
Fraction 7.....	62.0, 62.6

It is obvious that Fraction 7 is the purest preparation of nervone obtained. The purer fractions of nervone comprised only about 10% of the original crude nervone. Most of the nervone remained in the less pure fractions.

4. Tests for Purity of the Cerebrosides

Three tests are useful in establishing the purity of phrenosine and cerasine.

(1) *Proof of Absence of Phosphatides*

The phosphatides are the impurities most frequently present. A pure preparation in fairly large amounts (0.3 g.) should give no phosphorus reaction after incineration to an ash with sodium carbonate and potassium nitrate with ammonium molybdate.

(2) *Crystallization Test*

When dissolved in a mixture of methyl alcohol and 10% chloroform by treatment over a water bath, the clear solution changes to a mass of crystals which resemble those of cholesterol.⁴²³

(3) *Selenite Plate Test*

To determine whether or not cerasine is mixed with phrenosine, the Rosenheim test⁴²² is most useful. This depends upon the variation in properties of the two cerebrosides in polarized light. One dissolves 8-10

⁴²⁴ H. Thierfelder, *Z. physiol. Chem.*, 89, 248-250 (1914).

mg. in a small beaker (0.5×4 cm.) in 2 drops of pyridine at about 37°C . This is transferred by means of a capillary pipette to a warmed microscope slide, covered with a cover slip, and allowed to cool slowly. Spherocrystals separate out first along the edges, preventing the further evaporation of the solution. Since the crystals have the same refractive index as pyridine, they are barely visible under the microscope in ordinary light. However, when observed in polarized light with the crossed Nicol prisms, they stand out from the dark background and exhibit well-defined crosses. If the selenite plate with red I filter is placed directly over the polarizer, so that its axis lies diagonal to the polarization planes of the crossed Nicol prisms, characteristic differences are noted between phrenosine and cerasine. Against the red background, the crystals appear divided into quadrants; 2 opposite quadrants with the addition color blue are contrasted with the other 2 with the subtraction color yellow. The relative position of the crossed axes of the spherocrystals and of the α -axis of the selenite plate in phrenosine, *i.e.*, the upper right and the lower left quadrants being blue, is reversed in cerasine, in which the upper left and the lower right quadrants are blue. The axis direction of the selenite plate described by Rosenheim⁴²² is the γ -axis.

In the case of phrenosine, the spherocrystals are frequently replaced by dense tufts of crystals, probably made up of individual needles arranged side by side, with a feathery appearance. The tufts in which the vertical axis runs parallel to the α -axis of the selenite plate are yellow.

In the case of nervone, the separation always takes the form of well-developed tufts of needles, which become integrated like felt after a few hours. The color arrangement is opposite to that observed in cerasine, *i.e.*, the needles with a vertical axis parallel to the α -axis are blue.

According to Rosenheim, if the mixture is made up of various cerebroside, the phrenosine form appears first on the test. A few hours later, forms with the opposite optical characteristics appear, and frequently surround and envelop the phrenosine.

Rosenheim⁴²² has used the test with success. It is applicable only when the substance to be tested is completely free from phosphorus. Experience has shown that, even in this case, occasional irregularities occur. In a few instances, forms characteristic of cerasine have been observed to predominate in preparations of cerebrin which were not entirely pure but which were free from phosphorus. In fact, the separation products consisted exclusively of these cerasine forms at first, and the opposite form did not appear until later.

Another test is the determination of the iodine number, preferably by the procedure of Rosenmund, Kuhnhehn *et al.*⁴²⁵ The iodine numbers are:

⁴²⁵ K. W. Rosenmund, W. Kuhnhehn, D. V. Rosenberg-Gruszynsky, and H. Rosetti, *Z. Untersuch. Nahr. Genussm.*, 46, 154-159 (1923); *Chem. Abst.*, 18, 477 (1924).

cerebron, 30.7; cerasine ($C_{48}H_{93}NO_8H_2O$), 30.6; nervone, 62.7. The specific rotation is also of use in determining the purity of the sample. This latter criterion and the determination of the iodine number can, of course, be used only if the amounts of material are relatively large.

5. Properties of the Cerebrosides

The cerebrosides in general exhibit similar chemical properties. They are all white powders which are more or less wax-like; they separate from alcohol in small particles which resemble crystal balls, but they probably are not truly crystalline, although it is claimed that phrenosine has been prepared in crystal form. Under certain conditions they form liquid crystals, and this fact may account for discrepancies in the literature as to the melting point. The cerebrosides are all insoluble in water, as is also the case with diethyl ether and petroleum ether. On the other hand, pyridine, acetone, chloroform, and acetic acid act as solvents, as does alcohol to a lesser extent. All of the cerebrosides are optically active. None of them reacts with nitrous acid, since in all cases the amino group is in combination with the fatty acid residue.

(1) *Phrenosine*

Phrenosine is soluble in hot and cold pyridine and in hot solutions of alcohol, benzene, acetic acid, ethyl acetate, acetone containing 10% water, and chloroform. When the hot solutions are cooled, phrenosine is usually precipitated in lumps which on drying appear more or less like cholesterol crystals.

The so-called liquid crystals were first described by Rosenheim.⁴²⁶ When some dry powder is heated until it is melted, on a slide in the Lehmann's polarization microscope, it becomes completely isotropic. On cooling, numerous anisotropic, needle-like, liquid crystals appear. Such crystals contain one molecule of water of crystallization,^{342,426} which can be removed *in vacuo* over sulfuric acid, as well as when heated to 105°C.

On heating, phrenosine assumes a moist appearance at 130°C. and melts at 212°C.⁴²⁷ This property is explained on the assumption that it first enters the liquid crystalline state and later the liquid state. At 95°C. it begins to go into the anisotropic liquid state, and this is completed at 130°C. The change from the liquid crystalline state to the liquid occurs at 212–215°C.^{426,427} A marked decrease in viscosity results concomitantly. This has been referred to as the "clearing point."

When a fine aqueous suspension of phrenosine is heated, it shows a tend-

⁴²⁶ O. Rosenheim, *Biochem. J.*, 8, 121–127 (1914).

⁴²⁷ E. Wörner and H. Thierfelder, *Z. physiol. Chem.*, 80, 542–551 (1900).

ency to undergo myelin formation.^{426,428} Further description of myelinization is given by Lehmann.⁴²⁹ Phrenosine is dextro-rotatory, but the data obtained by various investigators differ greatly. In a 3:1 chloroform-methyl alcohol solution, the values for the specific rotation are as follows: +8.1 (50°C.)⁴³⁰; +8.03, 6.3, 8.4 (50°C.)⁴²⁰; +7.40 (40°C.), +10.4 (45°C.)³⁴²; +9.5 (3.6% solution), +10.7 (6.7% solution).⁴³¹ In 1:1 chloroform-methyl alcohol solution, Levene and Taylor⁴³² report values at 20°C. of +11.2° and +9.7°. The rotation in pyridine was found to be considerably lower. Rosenheim³⁴² reports values of +3.70 (18°C.), +3.70 (20°C.), +3.78 (20°C.), and +4.30 (30°C.), while Levene and Taylor⁴³³ give the value at 20°C. as +5.0°. Thierfelder and Klenk⁴ criticize the data of Levene on the hypothesis that sufficient proof is not given to establish the purity of their preparations. An iodine number of 30.7 has been reported.⁴

Phrenosine is not acted on by pancreatic lipase or by commercial emulsin. It has been suggested by Jungmann and Kimmelstiel⁴³⁴ that galactose is split from the cerebroside molecule after death, although the degree of hydrolysis may be quite small.⁴³⁵

Phrenosine forms relatively few derivatives. Those that are known include the dibromo compound,^{393,436} which is soluble in benzene and slightly soluble in ether and alcohol. Other compounds include those listed below.

Hexaacetylphrenosine (m.p., 40–41°C.) is insoluble in water, and soluble in most organic solvents; $[\alpha]_D^{17} = -3^\circ$.⁴²⁴

Tribenzoylphrenosine separates as an oil or as crystals when a methyl alcohol solution is cooled, depending upon the concentration. It dissolves readily in acetone, benzene, petroleum ether, chloroform, acetic acid, and pyridine. It melts at 65–66°C., and has a specific rotation (7% solution) of +21.1°.

Methylphrenosine, which contains 5 to 6 methyl groups, is formed when phrenosine is treated with methyl iodide and silver oxide. It is a white amorphous powder, melting at 35–40°C., which has a specific rotation at 16°C. of 7.5°.⁴³⁷ Phrenosine is precipitated quantitatively from an alcoholic solution on the addition of ammoniacal lead acetate.⁴¹⁷

⁴²⁸ J. L. Smith and W. Mair, *J. Path. Bact.*, *15*, 122–123 (1910).

⁴²⁹ O. Lehmann, *Biochem. Z.*, *63*, 74–86 (1914).

⁴³⁰ H. Thierfelder, *Z. physiol. Chem.*, *85*, 35–58 (1913).

⁴³¹ P. A. Levene, *J. Biol. Chem.*, *15*, 359–364 (1913).

⁴³² P. A. Levene and F. A. Taylor, *J. Biol. Chem.*, *52*, 227–240 (1922).

⁴³³ P. A. Levene and F. A. Taylor, *J. Biol. Chem.*, *80*, 227–230 (1928).

⁴³⁴ H. Jungmann and P. Kimmelstiel, *Biochem. Z.*, *212*, 347–358 (1929).

⁴³⁵ P. Kimmelstiel, *Biochem. Z.*, *212*, 359–362 (1929).

⁴³⁶ P. A. Levene and W. A. Jacobs, *J. Biol. Chem.*, *12*, 381–388 (1912).

⁴³⁷ J. Pryde and R. W. Humphreys, *Biochem. J.*, *18*, 661–664 (1924); *20*, 825–828 (1926).

Several tests have been employed to detect phrenosine, all of which depend upon the carbohydrate group. These reactions are not specific for phrenosine, however, but will be given by all cerebrosides. These include the reaction with concentrated sulfuric acid (yellow, then purple coloration),²⁶² which depends upon the presence of galactose and sphingosine, the Molisch reaction (violet coloration when pure concentrated sulfuric acid is added to an α -naphthol solution of cerebroside), and the orcin test (green-blue-green coloration on the addition of orcin and hydrochloric acid containing ferric chloride), which depends upon the galactose.⁴³⁸

(2) *Cerasine*

The solubility of cerasines is similar in all cases to that of the phrenosines, except that in most instances it is somewhat greater. This differential solubility, particularly that obtained in acetone containing 10% water, and in a 3:2 acetic acid-chloroform mixture, has been widely employed for the separation of phrenosine from cerasine. The phrenosines will almost completely precipitate when the solution is maintained at a temperature of 37°C., while the cerasines remain in solution. When the temperature is lowered, cerasine will precipitate.

Cerasine forms liquid crystals, in the same way as does phrenosine; these crystals melt with further heating at 180°C. (clearing point)⁴²⁶ or 185–187°C.⁴³⁹ Myelin formation occurs less readily with cerasine than with phrenosine.⁴²⁶

In contradistinction to phrenosine, which is dextro-rotatory, cerasine preparations have uniformly been found to be levo-rotatory. The values reported in the several solvents are quite variable. Thus, in pyridine, the following specific rotations have been reported: -9.03° (18°C.)⁴³⁹; -2.78° , -2.50° (18°C.), -2.74° (20°C.), -3.71° , -3.78° (25°C.)³⁴²; and -9.12° (15°C.).³⁹⁷ The value found in a 1:1 pyridine-acetone mixture was -4.58° (50°C.),³⁴² while in chloroform with 10% pyridine the following specific rotations have been noted: -10.99° (57°C.)⁴⁴⁰; -9.18° (57°C.)⁴³⁹; -8.76° (57°C.)³⁹⁷; and -5.08° (50°C.).³⁴² In chloroform solution rotations of -11.59° (56°C.)⁴³⁹ and -9.18° (57°C.)⁴³⁹ have been reported, while the results in absolute alcohol at 59°C. are given as -3.89° ⁴³⁹ and -4.52° .⁴⁴⁰ The iodine value, 31.3, is quite similar to that for phrenosine. It is unchanged by heating for one hour with saturated barium hydroxide.

Cerasine forms several derivatives which correspond to those of phrenosine. *Cerasine dibromide* has been investigated by Kossel and Freytag³⁹³ and also by Levene and Jacobs.⁴³⁶ Cerasine forms a *pentacetyl* derivative instead of the hexaacetyl derivative yielded by phrenosine. The solubility

⁴³⁸ S. Fränkel and K. Linnert, *Biochem. Z.*, 26, 41–43 (1910).

⁴³⁹ H. Lieb and M. Mladenovic, *Z. physiol. Chem.*, 181, 208–220 (1929).

⁴⁴⁰ H. Lieb, *Z. physiol. Chem.*, 140, 305–313 (1924).

of pentaacetylcerasine is similar to that of the corresponding phrenosine compound except that the former is less soluble in methyl alcohol.⁴²⁴ Pentaacetylcerasine melts at 54–56°C.,⁴⁴¹ and has a specific rotation of $-16.46^{\circ 441}$ at 20°C. in a 1:1 mixture of chloroform and methyl alcohol.

Pentamethylcerasine originates on methylation of the parent compound with methyl iodide and silver iodide. It is a white amorphous compound melting at 73°C.⁴³⁷ *Benzoylcerasine* dissolves in cold methyl alcohol. This reaction serves as a means of separation of cerasine from phrenosine, since the corresponding cerasine derivative is insoluble in this solvent.

The same non-specific chemical tests as those used for phrenosine, which are based upon the presence of the galactose molecule, also serve equally well for the detection of cerasine.

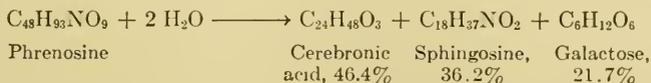
(3) Nervone

Nervone corresponds closely to the other cerebrosides as regards its solubilities. Like cerasine, its solubility somewhat exceeds that of phrenosine. It behaves like cerasine under the polarization microscope and in the selenite plate test. It melts at 180°C. but changes at a lower temperature to a semiliquid state. It is levo-rotatory ($[\alpha]_D^{16}$ pyridine = -4.33°). The iodine number is 62.7.

The only known compound of nervone is the lead salt. This precipitates when alcoholic lead acetate and ammonia are added to a hot alcoholic solution of the cerebroside.⁴¹⁷

6. Hydrolysis of the Cerebrosides

On hydrolysis, the cerebrosides are split into sphingosine, a fatty acid, and galactose or glucose. The hydrolysis of galactosidophrenosine proceeds as follows:



The reaction can be brought about in aqueous solution,^{262,415,442} and in ethyl alcoholic sulfuric acid,^{342,442–444} but the best results are obtained with methyl alcoholic sulfuric acid.^{443,445} In the latter case, one obtains the methyl ester of cerebronic acid, methyl sphingosine, and methyl galactoside. For the hydrolysis, about 3 g. of the cerebroside is refluxed with 150

⁴⁴¹ P. A. Levene and C. J. West, *J. Biol. Chem.*, *31*, 635–647 (1917).

⁴⁴² P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, *31*, 627–634 (1917).

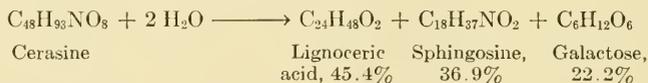
⁴⁴³ H. Thierfelder, *Z. physiol. Chem.*, *44*, 366–370 (1905).

⁴⁴⁴ O. Riesser and H. Thierfelder, *Z. physiol. Chem.*, *77*, 508–510 (1912).

⁴⁴⁵ J. L. W. Thudichum, *Further Researches on the Chemical Constitution of the Brain*. Report of the Medical Officer, Privy Council, Local Government Board, n.s., *8*, No. 6, London, 1876, pp. 117–150.

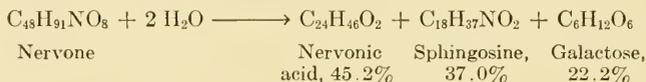
ml. of methyl alcohol containing 10% sulfuric acid (by weight) for 3 or 4 hours over the water bath.

The hydrolysis of cerasine proceeds in a similar manner:



The hydrolysis of cerasine proceeds most satisfactorily with methyl alcohol containing 10% of sulfuric acid; however, a period of 7 or 8 hours is required to complete the hydrolysis.

In the case of nervone, the hydrolysis results in the following reaction:



Details of the separation of the various components from the hydrolysis mixture are reported by Thierfelder and Klenk.⁴

Barium hydroxide is a poor hydrolyzing agent. When cerebrosides are heated at boiling temperature with saturated barium hydroxide for several hours, no decomposition of the cerebroside occurs.⁴²³ This resistance to hydrolysis is made use of in the removal of phosphatide impurities by saponification.

7. Chemistry and Properties of Hydrolysis Products of the Cerebrosides

(1) *Psychosine*

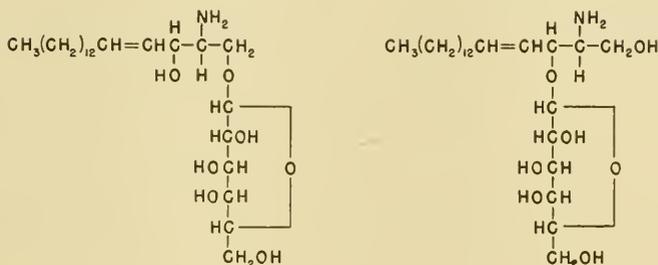
Thudichum^{445,446} first described a product of the incomplete hydrolysis of phrenosine which was formed when the fatty acid was split from the cerebroside molecule. This substance was called *psychosine*. Thudichum²⁶² later obtained an impure preparation of this substance from cerasine as well. These findings were later confirmed by Klenk,⁴⁴⁷ who showed that psychosine could be prepared by hydrolysis of cerebrosides with barium hydroxide. Psychosine can likewise be prepared from a mixture of cerebrosides containing largely nervone and hydroxynervone.³⁴⁷ It would therefore appear that the sugar-sphingosine complex, psychosine, is present in all types of cerebrosides; in fact, the structure of the cerebrosides which we now accept is, in all cases, based upon this type of structural relationship of the sugar and sphingosine. In view of the new structure of sphingosine established by Carter *et al.*,^{312,448} the formula of *galactopsychosine* as postu-

⁴⁴⁶ J. L. Thudichum, *Further Researches in Chemical Biology*, Seventh Annual Report of the Local Government Board, 1877-1878. Suppl. Abt. B, No. 2, containing Report of the Medical Officer for 1877, London, pp. 281-301.

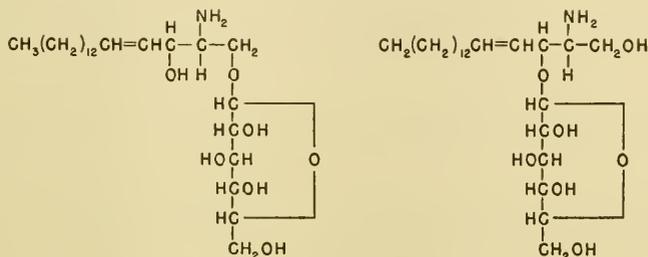
⁴⁴⁷ E. Klenk, *Z. physiol. Chem.*, 153, 74-82 (1926).

⁴⁴⁸ H. E. Carter, F. J. Glick, W. P. Norris, and G. E. Phillips, *J. Biol. Chem.*, 170, 285-294 (1947).

lated by Thierfelder and Klenk⁴ must be modified to one of the following:



It is now certain that similar psychosine molecules occur as a result of the partial hydrolysis of glucosidocerebrosides. The possible formulas for the *glucosidopsychosines* are:



a. Structure of Psychosine. The combination of sphingosine and galactose has been shown to be a glucosidic one. The fact that psychosine cannot reduce the ordinary aldehyde reagents indicates that the aldehyde group in the sugar exists in combination. The galactose cannot be combined with the amino group of sphingosine, since psychosine reacts with nitrous acid and the amino group must therefore certainly be unattached. The proof that galactose is combined with only one of the hydroxyl groups of sphingosine in a true glucoside linkage was furnished by Klenk and Härle,³⁴⁷ who isolated a monomethoxydihydro-sphingosine when dihydro-psychosine, which had previously been methylated, was hydrolyzed with dilute sulfuric acid. A further proof of the glucosidic type of union is afforded by the demonstration by Pryde and Humphreys⁴³⁷ that galactose exists in psychosine with an oxygen bridge from carbons 1 to 5. Such a cyclic structure is essential if one is to assume a glucoside structure. It was shown that, on hydrolysis of a methylated cerebroside mixture, 2,3,4,6-tetramethylgalactose was present in the hydrolysis mixture.⁴³⁷ Klenk and Härle believe that the combination between galactose and sphingosine occurs through the hydroxyl on carbon 2 of the base. However, in view of the recent revision in the structure of sphingosine according to which the hydroxyl groups

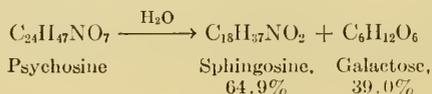
are placed on carbons 1 and 3, it is not yet certain which particular hydroxyl of the sphingosine is involved.

b. Preparation of Psychosine. Psychosine can be prepared by the Klenk method⁴⁴⁷ by the hydrolysis of phrenosine with barium hydroxide. In this method, 4 g. of the pure cerebroside is heated with 80 ml. of 10% barium hydroxide for 7 hours in a boiling water bath. After cooling, the precipitate, consisting chiefly of unhydrolyzed phrenosine, is filtered off, washed, and dried *in vacuo*; the residue is pulverized and boiled with 50 ml. of 96% alcohol. The unchanged phrenosine which precipitates on cooling is filtered off, and the process is repeated. About 1 g. of phrenosine remains unhydrolyzed. The combined alcoholic extracts are condensed to about 30 ml. under reduced pressure. Psychosine is precipitated as the sulfate by the addition of dilute alcoholic sulfuric acid. Care must be taken to avoid an excess of the acid. The psychosine sulfate which precipitates is purified by recrystallization from 500 ml. of absolute alcohol. The yield of psychosine from 4 g. of phrenosine amounts to about 1.65 g. The separation of the psychosine from cerebroside mixtures containing appreciable amounts of unsaturated acids is somewhat more complicated.

The free base can be prepared from the sulfate by hydrolysis of an aqueous solution with barium hydroxide, followed by filtration of the precipitate, which is a mixture of psychosine and barium sulfate. After washing and drying, the precipitate is treated with hot alcohol, and the insoluble barium sulfate is filtered off. Psychosine precipitates from the alcoholic solution after it is concentrated to a few milliliters, and is allowed to stand in the cold.

c. Properties of Psychosine. Psychosine is a white powder which is soluble in ethyl and methyl alcohol, but which is insoluble in diethyl ether and petroleum ether. It swells up to form a gelatinous mass in hot benzene or chloroform. Psychosine is difficult to obtain in crystalline form. However, it can be prepared in long thin needle-like crystals by the following procedure: A solution in 20 parts of a mixture of equal parts of chloroform and ethyl alcohol is mixed with 100 volumes of petroleum ether, and the mixture is warmed carefully until the precipitate is dissolved. When the solution is cooled, psychosine crystallizes practically quantitatively and almost immediately in a homogeneous needle-like form. The crystals sinter at 110°C., turn yellow at 160°C., melt at about 215°C., and decompose at 223°C.³⁴⁷

Psychosine reacts quantitatively with the Van Slyke reagent for amino nitrogen.⁴⁴⁷ It produces a purple color with concentrated sulfuric acid,²⁶² and a positive Molisch reaction. On hydrolysis, it gives rise to sphingosine and galactose:



Psychosine sulfate ($C_{24}H_{47}NO_7 \cdot 1/2H_2SO_4$) is easily soluble in water and benzene. It dissolves in hot methanol (1 part in 40 parts), from which it slowly separates in wart-like crystals on the side of the flask when the solution is cooled. It is less soluble in ethyl alcohol and insoluble in diethyl ether. The aqueous solution shows a great tendency to foam. The crystals sinter at $170^\circ C.$ and begin to turn brown. They melt at $225^\circ C.$ with decomposition.⁴⁴⁷

Since psychosine contains several asymmetric carbon atoms, it is optically active. Klenk and Härle³⁴⁷ report the following values for specific rotation of the sulfate in pyridine solution: preparation from phrenosine, -16.6° ($19^\circ C.$); preparations from cerebroside mixture, -16.0° ($19^\circ C.$), and -16.5° ($15^\circ C.$).

Psychosine phosphate can be formed by the addition of alcoholic phosphoric acid to an alcohol solution of psychosine. It is soluble in hot water, but separates as a gelatinous precipitate on cooling. When dissolved in chloroform which contains alcohol, psychosine phosphate crystallizes in long needles which are apparently a mixture of the mono- and the diphosphate.

Psychosine picrate may be prepared from an aqueous solution of the sulfate by the addition of an equivalent amount of sodium picrate.³⁴⁷ It separates out as a resinous mass.

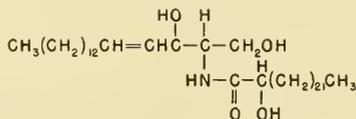
Dihydropsychosine ($C_{24}H_{49}NO_7$) is formed by hydrogenation of psychosine in the presence of palladium. It contains two additional hydrogens which are used to saturate the double bonds on carbons 4 and 5 of sphingosine. Dihydropsychosine is insoluble in water, diethyl ether, and petroleum ether, but it dissolves readily in hot alcohol or acetone; on warming with chloroform, it forms a gelatinous mass. Dihydropsychosine sulfate is difficultly soluble in alcohol.

(2) *The Ceramides*

Thudichum²⁶² separated a second compound derived from the partial hydrolysis of cerebroside, which he called *aesthesine*. The product was similar to that of the original cerebroside molecule without the sugar molecule. Klenk⁴⁴⁷ was unable to obtain aesthesine by the method employed by Thudichum. More recently these compounds have been prepared by the partial breakdown of sphingomyelins: they are referred to as ceramides. The best example of these compounds is lignocerylsphingosine, which has been extensively discussed earlier (see pages 460–461). Moreover, Klenk⁴⁴⁷ has been able to prepare cerebrosyl-*N*-sphingosine from cerasine by a method differing from that of Thudichum.

a. Structure of Cerebrosyl-*N*-Sphingosine. Cerebrosyl-*N*-sphingosine has the same general formula as lignocerylsphingosine. It is a

member of the group referred to as ceramides. Since it gives a neutral reaction, it is obvious that both the amino and the carboxyl groups must be combined. The evidence indicates that a $\text{NH}-\text{CO}$ union occurs, as in the case of lignocerylsphingosine.



Cerebronyl-*N*-sphingosine

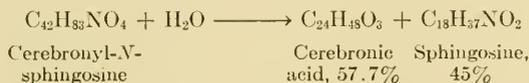
b. Preparation of Cerebronyl-*N*-Sphingosine. According to Klenk,⁴⁴⁷ the ceramide can be prepared from phrenosine. 10 g. of the cerebroside is dissolved by warming in a mixture of 270 ml. of acetic acid and 30 ml. of 10% sulfuric acid. The mixture is heated for 42 minutes over a boiling water bath. On cooling, 500 ml. of water is added to the flask without any attention being paid to the precipitate which has appeared, and enough 33% sodium hydroxide is introduced to render the reaction only slightly acid. It is shaken with about 2 liters of diethyl ether, whereby the precipitate is dissolved and the galactose which has been split off remains in the aqueous layer. The ether solution is now shaken with an excess of dilute sodium hydroxide solution, as a result of which a voluminous precipitate forms. This is separated by centrifugation. There are 3 layers, *i.e.*, the aqueous layer on the bottom, the precipitate lying above, and the ether layer on top. After removal of the clear ether layer (A), the precipitate (B) can easily be removed from the alkali below.

Most of the ceramide is present in the ether layer. After removal of the ether by distillation, the residue (2.7 g.) is dissolved by warming in acetone. The small amount of substance which fails to dissolve is removed by filtering it hot. When the solution is cooled in the refrigerator, the cerebronyl-*N*-sphingosine crystallizes. In order to purify this product, it is dissolved in 15 ml. of hot methanol and a little lead acetate solution in methanol; some ammoniacal methanol is added. It is heated and filtered while still hot. To a great extent the ceramide separates from the methanol solution on cooling. It is dissolved in acetone and the insoluble material is discarded. The residue remaining after evaporation of the acetone is again dissolved in methanol (15 ml.), and any remaining lead is removed with hydrogen sulfide. The lead sulfide is filtered, and in case of necessity diatomaceous earth (kieselguhr) is added, to remove any colloidal precipitate. The precipitate which originates on cooling is recrystallized from acetone. Klenk obtained 0.57 g. from this fraction. About 0.9 g. was prepared from the precipitate (B) by similar procedures.

c. Properties of Cerebronyl-*N*-Sphingosine. Cerebronyl-*N*-sphingosine is soluble in warm methanol and acetone; it is difficultly soluble in

diethyl ether at room temperature, but dissolves more readily on warming. When it is crystallized from acetone, the crystals are spherulithic, with a structure consisting of radiating fibers and with a glistening appearance. The crystals melt at 83–84°C. and have an iodine number of 38.8 (theoretical 38.1). The compound forms tri-*m*-nitrobenzoylcerebronyl-*N*-sphingosine when treated with *m*-nitrobenzoyl chloride. This derivative melts at 96–97°C. and is easily soluble in diethyl ether and acetone; it can be purified by separation from absolute alcohol, in the form of needle-like crystals.

On hydrolysis, cerebronyl-*N*-sphingosine splits to yield one molecule each of cerebronic acid and sphingosine:



(3) *Sphingosine*

The chemistry of sphingosine has been considered earlier in the discussion on sphingomyelin (see page 461).

(4) *Fatty Acids*

a. Cerebronic Acid. (a) *Structure.* Cerebronic acid is α -hydroxylignoceric acid. The presence of the hydroxyl group was established by Thierfelder,⁴¹⁵ who prepared the acetyl derivative. Up to recently it was believed that cerebronic acid had 25 carbon atoms. This suggestion was especially unique, since all the natural fatty acids which have been prepared have an even number of carbon atoms. However, the recent work of Klenk has furnished proof that cerebronic acid belongs to the C_{24} series.

Levene and Jacobs⁴³⁶ based their opinion that cerebronic acid was a C_{25} acid on the fact that, on oxidation with potassium permanganate, lignoceric acid ($\text{C}_{24}\text{H}_{48}\text{O}_2$) was obtained. However, by means of a similar reaction, Klenk⁴¹⁸ isolated an acid which he believed was triccsanic acid ($\text{C}_{23}\text{H}_{46}\text{O}_2$) in a yield of 74% of the theory for the crude acid and 43% for the purified product. Klenk's product melted at 78.5°C.; Levene and Taylor⁴⁴⁹ state that it should melt at 80–81°C., although 76.5–77.5°C. was later given as the correct figure.⁴⁵⁰

Klenk⁴¹⁸ obtained further data to prove his contention by determining the products of ozonization of α -hydroxynervonic acid, $\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_{12}\text{CHOHCOOH}$. This acid was known to be closely related to cerebronic acid since, on careful reduction, it was converted to the latter. When split at the double bond under conditions of oxidation, pelargonic

⁴⁴⁹ P. A. Levene and F. A. Taylor, *J. Biol. Chem.*, 59, 905–921 (1924).

⁴⁵⁰ F. A. Taylor and P. A. Levene, *J. Biol. Chem.*, 80, 609–613 (1928).

acid, $\text{CH}_3(\text{CH}_2)_7\text{COOH}$, and a dicarboxylic acid with 14 carbons, presumably $\text{COOH}(\text{CH}_2)_{12}\text{COOH}$, were obtained. This would indicate that the oxidation product of cerebronic acid must have been tricosanic rather than tetracosanic (lignoceric) acid. If the product had been lignoceric acid, then the second product must necessarily have been a C_{15} dicarboxylic acid.

The most cogent proof that cerebronic acid is α -hydroxylignoceric acid is the fact that Klenk⁴¹⁶ succeeded in reducing it to lignoceric acid. Although the figures in the elementary analyses favor the C_{25} formula, it is believed that this may be related to the presence of small amounts of anhydride which would give values somewhat too high.⁴¹⁸

(b) *Properties.* Cerebronic acid is a white crystalline powder, insoluble in water, but soluble in ether, pyridine, warm alcohol, and acetone. When the alcoholic solutions are cooled, cerebronic acid precipitates in more or less oval forms which can be recognized by their fine radiating stripes⁴¹⁵; these sometimes appear yellow, according to Thierfelder and Klenk.^{4,415} The crystals occur in two forms,⁴¹⁶ which Thudichum³³⁵ designated as wart-like and cauliflower-type. Cerebronic acid appears under the polarizing microscope as a conglomerate of irregular spherocrystals which give the same selenite plate reaction as does phrenosine.

Various melting points have been reported for this acid. These range from values as high as 130°C .⁴³³ to values as low as 81°C .,^{436,451,452} while Levene and Jacobs⁴³⁶ and Rosenheim³⁴² have both reported 108°C . However, Thierfelder and Klenk⁴ state with emphasis that the cerebronic acid obtained in their laboratory from the hydrolysis of pure phrenosine, as well as from a cerebroside mixture, always shows dextro-rotation and melts at 100 – 101°C . Moreover, cerebronic acid prepared by saturation of hydroxynervonic acid also has the same melting point.⁴¹³

Cerebronic acid contains an asymmetric carbon atom in the α -position and so would be expected to exhibit optical activity. Most investigators have obtained specific rotations of $+3^\circ$ to $+4^\circ$ in pyridine, although some of the low-melting samples failed to show any appreciable optical activity. On the other hand, cerebronic acid exhibits a levo-rotation when dissolved in chloroform. Some of these values are included in Table 12.

On oxidation, tricosanic acid is obtained,⁴¹³ while on reduction with hydriodic acid in acetic acid, lignoceric acid melting at 81 – 81.5°C . is formed.⁴¹⁶ Levene and Jacobs,⁴³⁶ as well as Levene and West,⁴⁵¹ obtained hydrocarbons with melting points of 53 – 54°C ., on more drastic reduction with hydriodic acid.

Derivatives of cerebronic acid include the sodium salt, the sodium salt of acetylcerebronic acid, and the methyl ester (m.p., 65°C .), all of which can be crystallized from hot alcohol.^{415,443} Lithium cerebronate precipitates

⁴⁵¹ P. A. Levene and C. J. West, *J. Biol. Chem.*, *14*, 257–265 (1913).

⁴⁵² P. A. Levene and C. J. West, *J. Biol. Chem.*, *26*, 115–120 (1916).

TABLE 12
SPECIFIC ROTATION OF CEREBRONIC ACID SAMPLES AS RELATED TO SOLVENT AND
PURITY AS DETERMINED FROM MELTING POINT

M.p., °C.	Concn., g./110 ml.	Temp., °C.	Tube length, dm.	$[\alpha]_D$ (obs.)	$[\alpha]_D$	Ref.
Pyridine Solution						
130	0.3	25	1	0.14°	4.66°	a
106	0.4458	20	2	0.37°	4.16°	b
105-106	0.2849	20	1	0.11°	3.86°	c
100-101	0.5566	17	2	0.38°	3.41°	d
100-101 ^e	0.570	19	2	0.38°	3.33°	d
99-100	0.594	20	1	0.16°	2.6°	f
99	0.83	20	1	0.29°	3.5°	g
99.5	0.87	20	1	0.33°	3.8°	g
91-93	0.563	20	—	—	3.55°	f
86	—	20	—	—	1.5°	f
82-84	—	—	—	—	0	b
Chloroform Solution						
—	0.455	50	1	-0.08°	-1.76°	d

^a P. A. Levene and F. A. Taylor, *J. Biol. Chem.*, **80**, 227-230 (1928).

^b P. A. Levene and W. A. Jacobs, *J. Biol. Chem.*, **12**, 381-388 (1912).

^c O. Rosenheim, *Biochem. J.*, **10**, 142-159 (1916).

^d E. Klenk, *Z. physiol. Chem.*, **174**, 214-232 (1928).

^e Sample obtained by hydrogenation of hydroxynervonic acid.

^f P. A. Levene and C. J. West, *J. Biol. Chem.*, **26**, 115-120 (1916).

^g P. A. Levene and F. A. Taylor, *J. Biol. Chem.*, **52**, 227-240 (1922).

from hot alcohol as a voluminous, amorphous mass on the addition of lithium acetate.⁴⁵³ Magnesium cerebionate is another salt which is insoluble in hot alcohol.⁴⁵³

b. Lignoceric Acid. (a) *Structure.* Lignoceric (tetracosanic) acid, $\text{CH}_3\text{-(CH}_2\text{)}_{22}\text{COOH}$, is the fatty acid separating on hydrolysis of cerasine. Synthetic lignoceric acid melts at 85-86°C.^{454,455} Brigl and Fuchs⁴⁵⁶ obtained two modifications of lignoceric acid from beech tar, one of which was identical with the synthetic product while the second form, which was probably a polymorphic modification, melted about 11°C. lower. The first product corresponded in all properties to synthetic lignoceric acid. Further proof that the fatty acid of cerasine is identical with lignoceric acid was brought forward by Levene, Taylor, and Haller,⁴⁵⁷ who demonstrated that no fractionation could be obtained when the methyl esters of lignoceric acid prepared from peanut oil and from cerasine were mixed and distilled *in vacuo*.

⁴⁵³ E. Klenk, *Z. physiol. Chem.*, **166**, 268-286 (1927).

⁴⁵⁴ H. Meyer, L. Brod, and W. Soyka, *Monatsh.*, **37**, 1113-1142 (1913).

⁴⁵⁵ P. Brigl, *Z. physiol. Chem.*, **95**, 161-194 (1915).

⁴⁵⁶ P. Brigl and E. Fuchs, *Z. physiol. Chem.*, **119**, 280-311 (1922).

⁴⁵⁷ P. A. Levene, F. A. Taylor, and H. L. Haller, *J. Biol. Chem.*, **61**, 157-161 (1924).

Klenk⁴⁵³ furnished additional proof by obtaining a pure sample of lignoceric acid from cerasine, with a melting point of 83–84°C., and by also showing its identity with hydrogenated nervonic acid.

(b) *Properties.* Lignoceric acid is a component not only of cerasine but also of sphingomyelin. It also occurs in peanut oil, and it makes up as much as 25.5% of the oil of the sandal bead-tree nut⁴⁵⁸ (*Adenanthera pavonina*).

Lignoceric acid dissolves readily in diethyl ether, petroleum ether, warm alcohol, acetone, benzene, carbon disulfide, acetic acid, chloroform, and pyridine. It crystallizes from alcohol on cooling, in glistening platelets. It contains no asymmetric carbon atoms and is therefore optically inactive. On reduction, a hydrocarbon is formed which melts at 51°C.,⁴⁵² while on oxidation an oxy-acid melting at 94–95°C. originates.⁴⁵⁰

Lithium lignocerate is precipitated from hot alcohol when lithium acetate is added. Magnesium lignocerate is formed in the same manner when magnesium acetate is added and the alcohol solution is allowed to cool. The silver and lead salts (m.p., 117°C.) are known.⁴⁵³ The methyl ester (m.p., 57–58°C.) and the ethyl ester (m.p., 56°C.) have similar properties when prepared from synthetic or natural sources.

c. Nervonic Acid. (a) *Structure.* This is a normal acid, as it converts to *n*-tetracosanic acid on hydrogenation. The methyl ester of hydrogenated nervonic acid⁴⁵⁹ is identical with that of synthetic tetracosanic acid.^{449,454,456} On ozonization, pelargonic acid and a *n*-dicarboxylic acid, C₁₅H₂₈O₄, are obtained.^{418,460} The structural formula of nervonic acid must therefore be CH₃(CH₂)₇CH:CH(CH₂)₁₃COOH.

(b) *Properties.* Nervonic acid is present, not only in the cerebroside nerve, but also in sphingomyelin. Furthermore, the acid described by Tsujimoto^{461,462} as selacholeic acid, which is obtained from shark liver oil, is apparently nervonic acid.

Nervonic acid is a white crystalline powder which is easily soluble in diethyl ether, alcohol, and acetone. It crystallizes from alcohol when the solution is cooled to a low temperature. Nervonic acid melts at 40–41°C. The iodine number is 69.3. On treatment with nitric acid, it is converted to its geometric isomer, which is very difficultly soluble and melts at 59°C.⁴⁵⁴

The sodium salt dissolves in hot alcohol, but it precipitates on cooling. It dissolves better in cold methyl alcohol. The lead salt of lignoceric acid is

⁴⁵⁸ S. M. Mudbidri, P. R. Ayyar, and H. E. Watson, *J. Indian Inst. Sci.*, A11, Pt. 14, 173–180 (1928).

⁴⁵⁹ E. Klenk, *Z. physiol. Chem.*, 157, 283–290 (1926).

⁴⁶⁰ E. Klenk, *Z. physiol. Chem.*, 166, 287–293 (1927).

⁴⁶¹ M. Tsujimoto, *Z. deut. Öl- u. Fett-Ind.*, 46, 385–388 (1926); *Chem. Zentr.*, 1928, I, 1385; *J. Soc. Chem. Ind. Japan*, 29, 67–71 (1926); *Chem. Abst.*, 20, 2421 (1926).

⁴⁶² M. Tsujimoto, *J. Soc. Chem. Ind. Japan*, 30, suppl., 868–873 (1927); *Chem. Zentr.*, 1928, I, 1385; *Chem. Abst.*, 22, 1326 (1928).

slightly soluble in cold ether (Twitchell separation) but more soluble in warm ether. Nervonic acid cannot be precipitated from alcoholic solution by magnesium.

d. Hydroxynervonic Acid. (a) *Structure.* The conversion of hydroxynervonic to cerebronic acid by hydrogenation indicates that it is an α -hydroxy-acid of the C_{24} series. Inasmuch as, on decomposition with ozone, it yields pelargonic acid, the double bond must be present between carbons 15 and 16. The structure which is assigned to it is $CH_3(CH_2)_7CH:CH(CH_2)_{12}CHOHCOOH$.

(b) *Properties.* Klenk³⁸⁹ first prepared hydroxynervonic acid from a cerebroside fraction partially soluble in petroleum ether. It is a white crystalline solid, readily soluble in diethyl ether, chloroform, alcohol, and acetone. It can be crystallized in needles (mostly in clusters) from 75% alcohol. It melts at 65°C.

Since the α -carbon atom is asymmetric, hydroxynervonic acid is optically active. It is similar to cerebronic acid in showing dextro-rotation in pyridine ($[\alpha]_D^{20} +2.87^\circ$),³⁸⁹ and levo-rotation in chloroform.⁴¹⁸ The iodine number is 66.4.

Sodium hydroxynervonate dissolves only in warm methyl or ethyl alcohols. The salt also dissolves in warm water. On cooling, it separates as a voluminous precipitate. The acid is precipitated quantitatively from hot alcoholic solution by magnesium acetate. Lead hydroxynervonate is insoluble in hot ether.

e. *n*-Hexacosenic Acid. A third unsaturated acid, *n*-hexacosenic acid, has recently been isolated from the hydrolysate of brain cerebroside by Klenk and Schumann.⁴⁶³ These workers believe it to be one of the acids normally present in cerebroside. The empirical formula is $C_{26}H_{50}O_2$, and it possesses one unsaturated linkage. The melting point is 45–45.5°C., and the iodine number of the preparations is approximately theoretical (64.3).

n-Hexacosenic acid has likewise been reported in sphingomyelin. Although it was present to the extent of only 3% in the cerebroside fatty acids, it is not believed that this can be ascribed to an impurity in the sphingomyelin.

f. Methods for Separation of the Acids. Klenk^{453, 459} has proposed several methods for the separation of the fatty acids from the hydrolysis products of cerebrosides. If reliable results are to be obtained, it is necessary that the cerebroside which is being analyzed be in as pure a state as possible, and especially that it be free from phosphatides.

After the cerebroside is hydrolyzed by boiling with methyl alcohol containing sulfuric acid, the fatty acids precipitate on cooling in a refrigerator. These are separated and washed with ice-cold methyl alcohol. They consist chiefly of the methyl esters of the saturated fatty acids.

⁴⁶³ E. Klenk and E. Schumann, *Z. physiol. Chem.*, 272, 177–188 (1941–1942).

(a) *Separation of the Saturated Fatty Acids.* The fatty acids remaining in solution are extracted by repeated treatment with petroleum ether. The methyl alcohol present, which is extracted by the petroleum ether, can be removed by washing the petroleum ether solution with water. The residue remaining after removal of the petroleum ether consists largely of the methyl esters of unsaturated acids and small amounts of the methyl esters of the saturated acids. The total amount recovered in the two fractions is approximately theoretical. Two procedures may be employed from this point for the separation of the saturated fatty acids:

a'. Thierfelder Procedure:⁴³⁰ The first procedure involves solution of the solid methyl esters in ether, followed by treatment with an excess of 0.5 *N* alcoholic sodium hydroxide. The sodium salts which precipitate are filtered; they are next suspended in ether, filtered again, and washed further with ether. They are decomposed by shaking in mineral acid and petroleum ether. The acids which are formed dissolve in the petroleum ether. This solution is washed with water and dried with sodium sulfate; the ether is then distilled off. The residue is cerebronic acid.

The petroleum ether filtrate which contains the methyl ester of lignoceric acid is shaken with dilute sulfuric acid and then with water. The ester remaining after distillation of the ether is saponified with 30 volumes of 0.5 *N* methanolic sodium hydroxide for 1 hour. The sodium salt of lignoceric acid, which precipitates at 0°C., is filtered off and obtained as the acid by the process used above for the preparation of cerebronic acid.

b'. Thierfelder-Klenk Procedure:⁴ The second method for the separation of cerebronic and lignoceric acids also requires a saponification of the methyl esters. The fatty acid mixture is dissolved in 70 parts of ethyl alcohol. The solution is heated to boiling, and an excess of a hot saturated solution of magnesium acetate in methyl alcohol is added. The precipitate, which is the magnesium salt of cerebronic acid, is filtered from the solution while hot; the salt is then suspended in diethyl ether and is changed to the acid by the addition of a drop of concentrated sulfuric acid and by shaking the mixture. After thorough washing, the ether solution is dried with sodium sulfate and the cerebronic acid is obtained by evaporation of the ether.

The hot filtrate obtained after removal of the magnesium cerebronate is cooled, and magnesium lignocerate crystallizes. This precipitate is filtered off and the free acid is obtained from this product by a procedure similar to that described above for cerebronic acid.

(b) *Separation of the Unsaturated Acids.* The ester mixture which remains in solution after the precipitation of the esters of the saturated acids in the cold consists largely of methyl esters of the unsaturated fatty acids. This is saponified with 35 volumes of 0.5 *N* methanolic sodium hydroxide by refluxing for 1 hour. The mixture, after cooling, is diluted with 2

volumes of water without removal of the precipitate which forms. After acidification with sulfuric acid, the fatty acid is extracted with diethyl ether. After removal of the ether by distillation, the acid residue is dissolved in 15 volumes of alcohol; the hydroxy-acids are precipitated from the boiling solution by the addition of a hot saturated methanolic solution of magnesium acetate. The precipitate which forms immediately in the hot solution is the magnesium salt of hydroxynervonic acid. It is filtered from the hot solution and the hydroxynervonic acid is obtained from it by the general method employed above.

When the filtrate is cooled, after removal of the magnesium hydroxynervonate, a small amount of a magnesium salt precipitates at room temperature; this is filtered off and discarded. The alcoholic solution is mixed with an equal volume of water acidified with sulfuric acid. The fatty acid set free is extracted with petroleum ether. The latter fraction is then washed with water and the nervonic acid present is recovered by removal of the petroleum ether by distillation.

(c) *Calculation of Amount of Each Fatty Acid.* The amount of unsaturated fatty acids can be calculated from the iodine number of the crude fraction of the unsaturated fatty acids worked up in (b). In the case of the hydroxynervonic acid fraction, the remaining fatty acid can be considered to be cerebronic acid. This is added to the amount of the cerebronic acid isolated under (a). The value for the saturated acid present in the nervonic acid fraction is much smaller; the acid cannot be considered as lignoceric acid without further analysis.⁴

(5) Carbohydrate Component

The sugar component of cerebrosides may be either galactose or glucose. Thudichum,^{335,445} who originally discovered that a sugar is set free when the cerebroside is hydrolyzed, believed that the carbohydrate was an entirely new hexose, for which he coined the term "cerebrose." However, Thierfelder⁴⁶⁴ and almost simultaneously Brown and Morris⁴⁶⁵ proved that cerebrose is identical with *d*(+)-galactose. The fact that the sugar in some cases may be glucose instead of galactose has only recently been demonstrated.³⁹⁰

Galactose may best be prepared from phrenosine after hydrolysis with 7% aqueous sulfuric acid by heating on a water bath over a prolonged period. The insoluble material is filtered off, the excess sulfuric acid is removed with baryta, and the excess barium with carbon dioxide. After concentration and filtration, galactose separates in crystalline form from the filtrate.⁴¹⁵ The carbohydrate has been characterized as galactose be-

⁴⁶⁴ H. Thierfelder, *Z. physiol. Chem.*, 14, 209-216 (1890).

⁴⁶⁵ H. T. Brown and G. H. Morris, *J. Chem. Soc.*, 57, 57-59 (1890).

cause of its conversion to mucic acid and because it forms the expected hydrazone. The presence of *d*(+)-galactose in pyranose form has been proved by Pryde and Humphreys.⁴³⁷ As discussed earlier, it is known that the galactose is combined in glucosidic linkage with sphingosine through one of the hydroxyl groups of the base.

The discovery that glucose-containing cerebrosides, also, may occur resulted from a study of the composition of the cerebroside which is deposited in the spleen in Gaucher's disease. Halliday, Deuel, Tragerman, and Ward³⁹⁰ found that the sugar obtained by the hydrolysis of cerasine prepared from a Gaucher spleen is completely fermentable by yeast, in contrast to the non-fermentability of the sugar fraction from the brain cerebroside. It was further shown that the osazone is identical with glucosazone. In fact, the application of bacteriological tests to differentiate among mannose, fructose, and glucose (all of which are fermentable with yeast and yield glucosazone) proved that the component sugar was glucose. The presence of glucose in the Gaucher spleen was later confirmed by Klenk and Rennkamp,^{466,467} Danielson, Hall, and Everett,⁴⁶⁸ and others.⁴⁶⁹ Two new cerebroside preparations from Gaucher spleens recently examined in the author's laboratory have also been shown to contain glucose. One may conclude that the glucose-containing cerebroside is the form most frequently found when abnormal deposits of cerebrosides occur in the spleen. However, the data of Lieb,⁴⁴⁰ as well as those of Lieb and Mladenović⁴³⁹ based upon studies made many years ago on the sugar component of the spleen cerebroside, are quite definite in proving the carbohydrate to be galactose. A re-examination of the same material made later by Lieb⁴⁷⁰ has again demonstrated that galactose is the component sugar.⁴⁷¹ It would therefore seem probable that two types of cerebroside may be laid down in Gaucher's disease—the galactose-containing type and the glucose-containing one. This view is also held by Klenk and Rennkamp,⁴⁶⁷ who believe that the latter type is much more common. All samples of glucose cerebrosides reported to date have been of the cerasine type.

The question which naturally arises is whether the glucose cerebroside occurs only under abnormal conditions attendant upon Gaucher's disease, or whether it actually may be classed as a normal cell constituent. Klenk and Rennkamp⁴⁷² have shown that the second possibility is the correct one,

⁴⁶⁶ E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, **267**, 128–144 (1940).

⁴⁶⁷ E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, **272**, 280–282 (1942).

⁴⁶⁸ I. S. Danielson, C. H. Hall, and M. R. Everett, *Proc. Soc. Exptl. Biol. Med.*, **49**, 569–571 (1942).

⁴⁶⁹ Aghion, "La Maladie de Gaucher dans l'enfance," *Thesis*, Paris, 1934. Cited by E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, **273**, 253 (1942).

⁴⁷⁰ H. Lieb, *Z. physiol. Chem.*, **170**, 60–67 (1927).

⁴⁷¹ H. Lieb and V. Günther, *Z. physiol. Chem.*, **271**, 211–213 (1941).

⁴⁷² E. Klenk and F. Rennkamp, *Z. physiol. Chem.*, **273**, 253–268 (1942).

since the cerebrosides obtained from all the normal and pathological organs examined contained small amounts of the glucose type of cerebroside. This would indicate that it is a normal cell constituent. The problem still unsolved is why the glucose form should be deposited exclusively in the spleen in most cases of Gaucher's disease, while the normal galactose type predominates in the brain.⁴⁶⁶ One also wonders whether the galactose and glucose residues may be interchanged without a complete rupture of the molecule.

8. Methods for Determination of the Cerebrosides

Although it is possible to determine the cerebroside content by ascertaining the amount which can be isolated from a tissue, this procedure is far from quantitative and is exceedingly time-consuming. Moreover, it cannot be effectively applied where the concentrations of cerebrosides are small. Most of the quantitative methods have been based upon the determination of the sugar present in a sample of cerebroside after hydrolysis.

Noll³⁹¹ first employed an acid hydrolysis of the lipid mixture followed by the determination of the liberated sugar by means of Fehling copper reagent. A somewhat similar procedure was used by Koch⁴⁷³ and also by Winterstein and Hirschberg,⁴⁷⁴ who used the Bertrand method. In these procedures the total amount of reducing substance found after hydrolysis was considered to be galactose. Noll³⁹¹ reported that no reduction occurs with the unhydrolyzed lipid mixture. Kirk⁴⁷⁵ reports, however, that when the Somogyi reagent was used for the determination of the reducing sugar, the alcoholic brain extracts invariably showed a definite reduction before hydrolysis. This author interprets this as indicating that the results obtained by the earlier methods are too high because of the failure to correct for the original reducing action of components in the tissue.

The first method introduced by Kimmelstiel⁴⁷⁶ offered a considerable improvement over the earlier ones. The Hagedorn-Jensen ferricyanide method was employed for the determination of the reducing substances, and the initial reduction values of the lipid mixture were subtracted from the results obtained after hydrolysis. However, the values used for correction were about 50% of the totals obtained after hydrolysis; it is therefore evident that they cannot be very accurate. In his second procedure, Kimmelstiel⁴³⁵ modified his technique by the introduction of a preliminary precipitation with zinc hydroxide, which considerably lowered the reducing value on the unhydrolyzed sample. However, Kirk⁴⁷⁵ points out that an

⁴⁷³ W. Koch, *Am. J. Physiol.*, *11*, 303-329 (1904).

⁴⁷⁴ H. Winterstein and E. Hirschberg, *Biochem. Z.*, *159*, 351-369 (1925).

⁴⁷⁵ E. Kirk, *J. Biol. Chem.*, *123*, 613-621 (1938).

⁴⁷⁶ P. Kimmelstiel, *Mikrochemie (Pregl Festschrift)*, 165-177 (1929).

even greater error was then introduced by omitting the zinc hydroxide precipitation on the hydrolyzed sample. Kirk,⁴⁷⁵ by using a modification of the second Kimmelstiel procedure, whereby the zinc hydroxide precipitation was also used on the hydrolyzed sample, was able to obtain duplicates within 4% when the cerebroside were present to the extent of only 0.3 to 1.3 mg.

Brand and Sperry⁴⁷⁷ introduced a new method which gives excellent quantitative recoveries. Recognizing that the hydrolysis procedure of Kirk is not sufficiently drastic to insure complete hydrolysis, the concentration of the hydrochloric acid used was increased to 3 *N*, and the reaction was carried out in tightly stoppered flasks. Although Brand and Sperry recognize the danger of destruction of the galactose when the acid concentrations are too high, no destruction occurred at the concentration of 3 *N* over a 45-minute period. This sufficed for the complete hydrolysis of the cerebroside. The determination of the galactose set free was made by the ceric sulfate method of Miller and Van Slyke.⁴⁷⁸

Edman⁴⁷⁹ suggested a new method based upon the colorimetric determination of the sugar by means of carbazole. Hydrolysis of the cerebroside is brought about with 2 *N* hydrochloric acid for 2 hours. Glycerol and fatty acids must be removed before the reagent is added to determine galactose. This micromethod can be applied to amounts of cerebroside as low as 0.2 to 0.6 mg. Brückner⁴⁸⁰ described a method for the separate determination of the glucose- and galactose-containing cerebroside by the use of the orcinol test.

Smith and Mair⁴⁸¹ have employed direct quantitative determination. By this method a weighed amount of the dried brain is extracted in the Soxhlet apparatus with hot chloroform; the chloroform residue is heated with a methanolic baryta solution to saponify the phosphatides. After addition of acetic acid it is evaporated to dryness; the residue is extracted in a Soxhlet apparatus with hot acetone. The cerebroside separating on cooling is filtered and weighed.

9. Compounds Related to the Cerebroside

(1) *Gangliosides*

In 1927, Walz,³⁹⁷ working in Thierfelder's laboratory, isolated a cerebroside-like compound from beef brain and beef spleen which, however, differed from the known cerebroside. It possessed a high sensitivity toward

⁴⁷⁷ F. C. Brand and W. M. Sperry, *J. Biol. Chem.*, *141*, 545-553 (1941).

⁴⁷⁸ B. F. Miller and D. D. Van Slyke, *J. Biol. Chem.*, *114*, 583-595 (1936).

⁴⁷⁹ P. V. Edman, *J. Biol. Chem.*, *143*, 219-221 (1942).

⁴⁸⁰ J. Brückner, *Z. physiol. Chem.*, *275*, 73-79 (1942).

⁴⁸¹ J. L. Smith and W. Mair, *J. Path. Bact.*, *16*, 131-135 (1911); *17*, 123-126, 418-420 (1912).

acids and it gave a purple color when the Bial test was applied. On heating with 16% sulfuric acid, a black humin-like precipitate was formed. In the same year, Levene and Landsteiner⁴⁵² also reported the presence of a similar compound in the brain. Since these compounds contain a sugar but no phosphate, it is evident that they are more closely related to the cerebroside than to other conjugated lipids such as the phosphatides. Blix⁴⁵³ confirmed the observations of Walz, and noted that a similar compound occurred in the normal human brain. The sugar component was classified as a hexosamine, since it gave a positive reaction with Ehrlich dimethylaminobenzaldehyde reagent.

Klenk^{454,455} isolated a lipid, which he called "substance X," from the tissues of an individual suffering from Niemann-Pick's disease. Later he prepared the same compound from the tissues of an individual who had died of the so-called Tay-Sachs disease,⁴⁵⁵⁻⁴⁵⁷ where it had completely replaced the normally occurring cerebroside. It is now evident that this new lipid is closely allied to the cerebroside. Since it occurs predominantly in the ganglion cells of the nervous system, and because it possesses a glucoside structure, this substance has been named ganglioside.⁴⁵⁸ Under normal conditions the highest concentration is in the cerebral cortex, while the white matter contains negligible amounts.⁴⁵⁹ In the Tay-Sachs type of infantile amaurotic idiocy, this lipid makes up ten times the normal content in the cortex. A relatively high concentration likewise occurs in the whole brain in the so-called Niemann-Pick's disease, but this is probably largely to be ascribed to the more even distribution of the ganglioside throughout the brain, due to the decreased proportion of ganglioside-deficient white matter in this disease. Although gangliosides are present in the spinal cord, their concentration is so small that it is impossible to separate them from other components. Schuwirth⁴⁶⁰ points out that these differences may be related to the variations in structure of the two tissues. For this same reason, also, the sphingomyelin content of the spinal cord is considerably higher than that of the brain.⁴⁹⁰ Klenk and Rennkamp⁴⁷² have also isolated gangliosides from normal beef spleen.

The gangliosides are insoluble in ether, acetone, and ethyl acetate, and are only slightly soluble in alcohol. They readily dissolve in mixtures of chloroform or benzene and alcohol. They form clear colloidal solutions in water up to concentrations of 10%. Such solutions are non-dialyzable,

⁴⁵² P. A. Levene and K. Landsteiner, *J. Biol. Chem.*, **75**, 607-612 (1927).

⁴⁵³ G. Blix, *Skand. Arch. Physiol.*, **80**, 46-51 (1938).

⁴⁵⁴ E. Klenk, *Z. physiol. Chem.*, **235**, 24-36 (1935).

⁴⁵⁵ E. Klenk, *Ber. ges. Physiol., exptl. Pharmakol.*, **96**, 659-660 (1937).

⁴⁵⁶ E. Klenk, *Z. physiol. Chem.*, **262**, 128-143 (1939).

⁴⁵⁷ E. Klenk, *Z. physiol. Chem.*, **267**, 128-144 (1940).

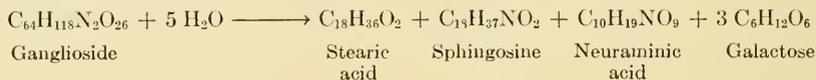
⁴⁵⁸ E. Klenk and H. Langerbeins, *Z. physiol. Chem.*, **270**, 185-198 (1941).

⁴⁵⁹ E. Klenk, *Z. physiol. Chem.*, **282**, 84-88 (1947).

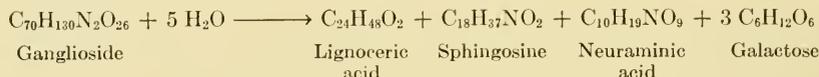
⁴⁹⁰ K. Schuwirth, *Z. physiol. Chem.*, **278**, 1-6 (1943).

and are apparently monobasic acids. They do not reduce Fehling solution, and no free amino nitrogen is present. They are levo-rotatory ($[\alpha]_D^{20}$ (pyridine) = -2.79°).

On hydrolysis, the ganglioside from *brain* yields the following products: fatty acids, 20%; sphingosine or sphingosine-like base, 13%; neuraminic acid, 21%; and sugar, which is galactose, 40–43%. Klenk⁴⁹¹ suggests the following equation for the hydrolysis:



Apparently more than one ganglioside exists, since differences in fatty acid composition are noted. While human brain ganglioside contains exclusively stearic acid, only neuraminic acid is found in that isolated from the *spleen*.⁴⁷² The hydrolysis of beef spleen ganglioside differs somewhat from the reactions observed with brain cerebroside:



a. Neuraminic Acid. Neuraminic acid was first obtained in crystalline form by Klenk⁴⁹² in 1941. This acid is presumably the group in the ganglioside molecule responsible for the characteristic color reactions of the gangliosides, as well as for their property of charring when heated with dilute mineral acid. It is a monobasic acid with an empirical formula of $\text{C}_{10}\text{H}_{19}\text{NO}_8$ or $\text{C}_{10}\text{H}_{19}\text{NO}_9$. It possesses a strong levo-rotation ($[\alpha]_D = -54.91^\circ$). It is easily soluble in water but only slightly soluble in methanol and completely insoluble in ethanol and diethyl ether.

Neuraminic acid has a primary amino group, and it gives a positive ninhydrin reaction. Although it decomposes on heating with acids, under which condition it yields a brown-colored solution, it is stable when heated in neutral or alkaline solution. It develops an intense red color with Bial reagent. When heated with Ehrlich reagent at 140°C ., it displays a strong red color which is positive in amounts as small as 60 μg .¹⁰ This acid does not reduce an alkaline copper solution. Whether neuraminic acid occurs in brain lipids other than in the gangliosides is not known. However, its presence in these latter compounds seems to be well substantiated.

(2) Cerebrosides Containing Two Sugar Molecules

In addition to the regular cerebrosides, which contain only one carbohydrate group, and the gangliosides, which have three carbohydrate molecules, Klenk and Remkamp⁴⁷² have isolated an intermediate compound from

⁴⁹¹ E. Klenk, *Z. physiol. Chem.*, 273, 76–86 (1942).

⁴⁹² E. Klenk, *Z. physiol. Chem.*, 268, 50–58 (1941).

normal beef spleen which has two carbohydrate residues. The ratio of fatty acid to sphingosine to sugar in this case was found to be 1:1:2. Both glucose and galactose were present in this cerebroside in a ratio of 2:3.

The cerebroside differs from a ganglioside in being insoluble in water, with which it forms an emulsion. It is phosphorus-free. The dicerebroside dissolves readily in pyridine; at room temperature, it is difficultly soluble in acetic acid, still less soluble in methanol, and insoluble in acetone. It is a white powder which melts at 240°C. The cerebroside is weakly levo-rotatory in pyridine ($[\alpha]_D^{20} = -6.81^\circ$). The orcin reaction for neuraminic acid is negative. The fatty acids consist of behenic acid ($\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$) and lignoceric acid ($\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$). The nature of the carbohydrates is not known, but it is believed that they exist as dihexosides.

CHAPTER VI

CAROTENOIDS AND RELATED COMPOUNDS

A. GENERAL CONSIDERATIONS ON THE RELATIONSHIP BETWEEN CAROTENES AND THE VITAMINS A

The carotenoids were recognized as chemical entities long before anything was known about the existence of vitamin A. In fact, Wachenroder¹ isolated carotene (*carotin*) from carrots as early as 1826, while the first concentrated preparation of vitamin A was not made until 105 years later by Karrer.^{2,3} Undoubtedly, the early recognition of the carotenoids is due to the fact that they are so widely distributed as coloring agents in vegetable and animal products. Moreover, they can be readily separated from the protein, carbohydrate, and salts of natural products because of their specific solubility in fat solvents; their preparation is especially simple where such foods have a low fat content. However, even in the presence of considerable amounts of fat, the carotenoids can readily be separated from the triglycerides, since they are components of the non-saponifiable fraction.

The first evidence of the existence of vitamin A was the result of the pioneer work of Hopkins⁴ in Great Britain in 1912. This worker demonstrated that normal growth did not occur in rats unless certain so-called "accessory food factors" were included in the diet. These supplements were necessary when the basal diet consisted of mixtures of purified proteins, fats, carbohydrates, and minerals, and was devoid of natural foods. One of the groups of these accessory substances was shown by Osborne and Mendel⁵⁻⁷ and by McCollum and Davis⁸⁻¹¹ to be a fraction, present in

¹ H. Wachenroder, *Über das Oleum radicum Dauci Aetherum, das Carotin, den Carotinzucker, und den officinellen Succus Dauci*, Dissertation de anthelminticis, Göttingen, *Geiger's Mag. Pharm.*, 33, 144-172 (1831). Cited by L. S. Palmer, *Carotinoids and Related Pigments*, Chemical Catalog Co., New York, 1922, p. 25.

² P. Karrer, R. Morf, and K. Schöpp, *Helv. Chim. Acta*, 14, 1036-1040 (1931).

³ P. Karrer, R. Morf, and K. Schöpp, *Helv. Chim. Acta*, 14, 1431-1436 (1931).

⁴ F. G. Hopkins, *J. Physiol.*, 44, 425-460 (1912).

⁵ T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, 15, 311-326 (1913).

⁶ T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, 16, 423-437 (1913).

⁷ T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, 17, 401-408 (1914).

⁸ E. V. McCollum and M. Davis, *J. Biol. Chem.*, 15, 167-175 (1913).

⁹ E. V. McCollum and M. Davis, *J. Biol. Chem.*, 19, 245-250 (1914).

¹⁰ E. V. McCollum and M. Davis, *J. Biol. Chem.*, 20, 641-658 (1915).

¹¹ E. V. McCollum and M. Davis, *J. Biol. Chem.*, 23, 231-246 (1915).

some fats, which they termed "fat-soluble accessory factor." Drummond later suggested that this somewhat cumbersome term be replaced by the expression vitamin A. This terminology has been employed almost exclusively since that time. In 1922, it was demonstrated that the fat-soluble A of Osborne and Mendel was in fact not a single vitamin but at least two different factors.¹² The designation vitamin A was retained for the growth-promoting, antixerophthalmic factor, while the name vitamin D was assigned to the antirachitic factor, *i.e.*, the compound which prevents rickets.

Because the early work on the qualitative identification of vitamin A and on its quantitative estimation was based largely upon the biological response, considerable confusion has existed as to its chemical nature, due to the fact that several widely varying substances appeared to have vitamin A potency. For example, Steenbock¹³ alone, and in collaboration with co-workers,¹⁴⁻¹⁷ postulated as early as 1919 that vitamin A was related to the yellow carotenoid pigments. Thus, butter, peas, and maize had a vitamin A potency which was to some extent proportional to their content of the yellow lipochromes.

However, a number of facts soon accumulated which argued against the identity of the carotenoid with vitamin A. Preparations which were colorless were shown in some cases to be extremely potent in vitamin A. Second, when vitamin A is treated with antimony trichloride, an intense blue color obtains which has an absorption maximum at 610-630 $m\mu$. On the other hand, the reaction between carotene and antimony trichloride gives rise to a greenish blue color where the absorption band occurs, with a maximum at 590 $m\mu$. Another marked difference is that vitamin A has a characteristic absorption band in the ultraviolet region of the spectrum with a maximum at 328 $m\mu$, while carotene does not exhibit specific absorption in this region.

The reason for the interrelationship in biological activity between carotene and vitamin A became evident shortly after Euler and associates¹⁸ demonstrated the effectiveness of pure crystalline carotene prepared from carrots in curing rats suffering from vitamin A deficiency. Quantities of 10 μ g. of carotene were found to be sufficient to alleviate the symptoms of avitaminosis and to restore growth promptly. Several years after von

¹² E. V. McCollum, N. Simmonds, J. E. Becker, and P. G. Shipley, *J. Biol. Chem.*, *53*, 293-312 (1922).

¹³ H. Steenbock, *Science*, *50*, 352-353 (1919).

¹⁴ H. Steenbock and P. W. Boutwell, *J. Biol. Chem.*, *41*, 81-96 (1920).

¹⁵ H. Steenbock, M. T. Sell, and M. V. Buell, *J. Biol. Chem.*, *47*, 89-101 (1921).

¹⁶ H. Steenbock, M. T. Sell, and P. W. Boutwell, *J. Biol. Chem.*, *47*, 303-308 (1921).

¹⁷ H. Steenbock and M. T. Sell, *J. Biol. Chem.*, *51*, 63-76 (1922).

¹⁸ H. von Euler, P. Karrer, and M. Rydholm, *Ber.*, *62*, 2445-2451 (1929).

Euler's work, Moore¹⁹⁻²¹ arrived at an entirely satisfactory explanation for the carotene-vitamin A interrelationship. This investigator found that, although the liver fat of a vitamin-A-depleted rat failed to respond to the antimony trichloride reaction for vitamin A, the test was strongly positive after the administration of either vitamin A or carotene. In either case, the product present in the liver was shown to be vitamin A, since it exhibited the characteristic absorption band at 328 m μ . Carotene thus assumes the role of a precursor of vitamin A or of a provitamin A, since it can be transformed into the active form of this vitamin in the animal body.

The relationship between the carotenoids and vitamin A is further elucidated by the discovery of their structure. β -Carotene was shown to have an empirical formula of C₄₀H₅₆, while vitamin A has exactly one-half this number of carbons, and has a composition corresponding to the formula, C₂₀H₂₉OH. Carotene contains two β -ionone rings, while vitamin A possesses one. Both molecules have conjugated double bonds in the hydrocarbon chains, there being 11 such linkages in β -carotene and 5 in vitamin A. Vitamin A is a primary alcohol, while β -carotene has no such group. There is now ample evidence that the β -carotene molecule is made up of two vitamin A molecules, joined together at the site of the terminal alcohol group, which is removed and replaced by an unsaturated linkage.

There are many excellent sources of specialized information on the carotenoids. The classic monographs in this field are undoubtedly those of L. S. Palmer,²² of L. Zechmeister,²³ and of Lederer,²⁴ and most recently of Karrer and Jucker.²⁵ A discussion of the most recently developed field in carotenoid chemistry, namely that of the carotenoid epoxides and their furanoid oxides, is given by Karrer.²⁶ An excellent summary of the role of carotenoids in animal metabolism was published by Zechmeister,²⁷ and a more recent one by Fox.²⁸ Wald²⁹ reviewed the subject of visual purple in its relationship to the carotenoids, while Heilbron, Jones, and Bacharach,³⁰

¹⁹ T. Moore, *Biochem. J.*, *23*, 803-811 (1929).

²⁰ T. Moore, *Biochem. J.*, *24*, 692-702 (1930).

²¹ T. Moore, *Biochem. J.*, *25*, 275-286 (1931).

²² L. S. Palmer, *Carotinoids and Related Pigments*, Chemical Catalog Co., New York, 1922.

²³ L. Zechmeister, *Carotinoide*, Springer, Berlin, 1934.

²⁴ E. Lederer, *Récherches sur les Caroténoïdes des Animaux inférieurs, et des Cryptogames*, Lons-de-Saunier, Paris, 1937. Cited by D. L. Fox, *Ann. Rev. Biochem.*, *16*, 454 (1947).

²⁵ P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948. Translated and revised by E. A. Braude, Elsevier, New York, 1950.

²⁶ P. Karrer, *Fortschr. Chem. organ. Naturstoffe*, *5*, 1-19 (1948).

²⁷ L. Zechmeister, *Ergeb. Physiol.*, *39*, 117-191 (1937).

²⁸ D. L. Fox, *Ann. Rev. Biochem.*, *16*, 443-470 (1947).

²⁹ G. Wald, *Vitamins and Hormones*, *1*, 197-227 (1943).

³⁰ I. M. Heilbron, W. E. Jones, and A. L. Bacharach, *Vitamins and Hormones*, *2*, 155-213 (1944).

and Heilbron³¹ presented the modern viewpoint on the chemistry and physiology of vitamin A. An over-all picture of the chemical and physiological relationships of the provitamins A and vitamins A is included in Rosenberg's treatise.³²

B. THE CAROTENOIDS

I. Introduction

The carotenoids represent a group of pigments which have an extremely wide distribution in nature. Not only do they occur in a wide variety of plants, ranging from the bacteria—which may be classed as the lowest forms of cryptogams—to the dicotyledons—which may be considered as the highest level of phanerogams—but they are found to be equally widely distributed in the animal kingdom. Thus, they are present in both invertebrates and vertebrates and, in fact, in most species from the protozoa to man.

Much confusion has arisen in naming the carotenoids. In many cases identical pigments have not been recognized when prepared from two sources, with the result that two names have been applied to the same substance. This is in part due to the failure to prepare pure pigments which were not admixed with other carotenoids. In some cases contamination with fat changed the properties. In other cases, the examination of the pigment in different solvents showed variations in properties, a fact which has not always been recognized. Finally, a considerable number of stereoisomers with markedly different properties can be prepared from the same parent carotenoid when it is subjected to relatively mild treatment. It has been possible to obtain a clean-cut separation of these latter isomers from each other only by the use of chromatography.

The first classification of carotenoids was proposed by Thudichum,³³ who suggested the name, *luteine* or *luteins*, for the yellow pigments found in animal and plant tissues. He included in this group not only the yellow pigment from the corpus luteum of mammals, from which the name was derived, but also that in the blood serum, adipose tissue, and butter, as well as the chromogenic material of egg-yolk. According to Thudichum, such pigments as that of yellow maize (*Zea mays*), of anatto seeds (*Bixa orellana*), of the carrot (*Daucus carota*), of such yellow leaves as the *Coleus*, and of the stamens and petals of many flowers, comprise the plant luteins.

³¹ I. M. Heilbron, *J. Chem. Soc.*, 1948, 386-393.

³² H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945.

³³ J. W. L. Thudichum, *Proc. Roy. Soc. London*, 17, 253-256 (1869). Cited by L. S. Palmer, *Carotinoids and Related Pigments*, Chemical Catalog Co., New York, 1922, pp. 14. 87.

The classification was based upon the similarity of such properties as solubility, the presence of three absorption bands in the blue, indigo, and violet region of the spectrum, the ability of these pigments to be crystallized in rhombic plates, and especially upon their ready combination with protein, from which they could be extracted only with difficulty. It is now recognized that the luteins, as defined by Thudichum, represent a number of different types of pigment. Since 1912, the name "lutein" has been specifically applied to one of the pigments which can be prepared in crystalline form from egg-yolk.³⁴

The term *lipochrome* was proposed by Krukenberg^{35,36} to cover a number of animal and plant pigments which had been known by such diverse names as luteins, carotin, zoonerythrin, tetronerythrin, chlorophane, xanthophane, and rhodophane. Although this designation was originally limited to pigments with yellow or reddish tints, by implication it obviously should include any fat-soluble pigment such as chlorophyll. However, the yellow pigments were frequently found to be free from appreciable amounts of fat, while the name would suggest their association with fat in the natural state. Krukenberg³⁶ used a number of properties as a basis for inclusion into the lipochrome group; these included solubility in a number of organic solvents, the development of a blue-green to blue color when the solid was treated with concentrated sulfuric or nitric acid, the presence of two or three absorption bands in the violet end of the spectrum, and a stability toward boiling alcoholic potash. Moreover, in the solid state, the lipochromes are greenish yellow, yellow, or orange to red, while their solutions are yellow; they are defined as being sensitive to light, and can be bleached to colorless compounds which appear to be similar to cholesterol. Krukenberg believed that all lipochromes contain oxygen as well as carbon and hydrogen, although he recognized that nitrogen was absent. He therefore failed to note the essential differences between the hydrocarbons with the empirical formula $C_{40}H_{56}$ and the group of carotenols and other oxyhydrocarbons which contain one or more oxygens.

Lipoxanthin is another designation which has been used to include all the animal and plant pigments. Schrötter-Kristelli³⁷ proposed that this term be used to include pigments previously referred to by the following names: eriolin, chlorophyll yellow, xanthin, anthoxanthin, phylloxanthin, phycoxanthin, lipoxanthin, lutein, xanthophyll, carotin, chrysophyll,

³⁴ R. Willstätter and H. H. Escher, *Z. physiol. Chem.*, *76*, 214–225 (1912).

³⁵ C. F. W. Krukenberg, *Zur Kenntnis der Verbreitung der Lipochrome im Tierreiche. Vergleichend-physiologische Studien*, [2], Part 3, 92–107 (1886). Cited by L. S. Palmer, *Carotinoids and Related Pigments*, Chemical Catalog Co., New York, 1922, pp. 16, 17.

³⁶ C. F. W. Krukenberg, *Grundzüge einer vergleichenden Physiologie der Farbstoffe und der Farben. Vergleichend-physiologische Vorträge*, Part 3, 85–104 (1886). Cited by L. S. Palmer, pp. 16, 17, ff.

³⁷ H. R. Schrötter-Kristelli, *Botan. Centr.*, *61*, 33–46 (1895).

erythrophyll, solanorubin, vitellorubin, hematochrome, chlororufin, bacteriopurpurin, hemolutein, and tetronerythrin. It was realized that these pigments formed a homologous group of substances even if they were not completely identical, and Schrötter-Kristelli believed that the term "lipoxanthin" was a satisfactory designation. The chief characteristics were defined as an affinity for fats, insolubility in water, a blue color reaction with sulfuric acid, absorption at the violet end of the spectrum, lack of fluorescence in solution, and finally their ready destruction by light and oxygen. Palmer²² states that the lipoxanthins thus comprise a more or less indefinite group of pigments which can be classified together under the heading of "lipochromes."

Czapek³⁸ has suggested the name *chromolipoids* as a more satisfactory designation for such pigments. This terminology is based upon the idea that lipochromes, at least in plants, should be classed as lipoids because of their fat-like behavior as regards solubility and their common distribution in the cell. Another property which is similar in the lipochromes and the phosphatides (as well as in unsaturated neutral fats) is the readiness with which they absorb oxygen. The word *chromolipoids* would automatically place these pigments as a sub-group of the lipids.

However, a more specific term was desirable to designate the fat-soluble yellow pigments than the general designation of *chromolipoids*. The term *carotinoids* was ultimately suggested³⁹ as the one to cover the yellow pigments which comprised a group of substances related genetically to carotene and to similar compounds. The term *carotin* was first assigned to the pigment now known as β -carotene, which Wachenroder¹ separated from the carrot (*Daucus carota*) in 1826. The term *carotinen* was used to cover the related pigments which were referred to as the *carotin* group. The present spelling, *i.e.*, *carotene*, was first proposed by Arnaud⁴⁰ in 1886, after it was demonstrated that the substance was a hydrocarbon. However, the earlier spelling, namely, *carotin*, was retained by many workers until quite recently⁴¹; the proposal of Arnaud has now been almost universally adopted. Zopf^{42,43} suggested that the carotene group be divided into true carotenes (*eucarotines*) and carotinines, which contain oxygen as well as carbon and hydrogen. The latter substances were believed to be acids, and did not include the real oxygen-containing members of the carotenoids, such as the alcohols and ketones.

³⁸ F. Czapek, *Biochemie der Pflanzen*, 2nd ed., Vol. I, Jena, 1913, p. 803.

³⁹ M. Tswett, *Ber. deut. botan. Ges.*, 29, 630-636 (1911).

⁴⁰ A. Arnaud, *Compt. rend.*, 102, 1119-1122 (1886).

⁴¹ The earlier spelling was used as recently as 1922 in the monograph of L. S. Palmer, *Carotinoids and Related Pigments*, Chemical Catalog Co., New York, 1922.

⁴² W. Zopf, *Beitr. Physiol. Morphol. niederer Organismen*, 3, 26-47 (1893). Cited by L. S. Palmer, p. 19.

⁴³ W. Zopf, *Biol. Zentr.*, 15, 417-427 (1895).

The present designation carotenoids (carotinoids) was originally proposed by Tswett³⁹ to include those chromolipoids which are chemically and generically related to carotin. A further subdivision of the carotenoids, suggested by Tswett, was into carotenes (the hydrocarbons) and xanthophylls (oxyhydrocarbons). The latter designation has now been replaced by *carotenols* for the oxycarotenoids containing an alcohol group, while no satisfactory designation to cover all of the oxygen-containing carotenoids is at present in general use.

It is now recognized that many classes of plant and animal pigments occur which do not belong to the carotenoid group. Some of these may have a yellow or orange color similar to that of the carotenoids. Examples of such compounds are the flavones, xanthonenes, anthoxanthonenes, and anthocyanins, in the plant kingdom, and the bile pigments in the animal products. These are in all cases distinguished from carotenoids by their solubility in water rather than in organic solvents.

2. Structure and Occurrence of Hydrocarbon Carotenoids of the C₄₀ Series

Prior to 1931, it was believed that carotene was a single entity. Some confusion existed, however, since carotenes which were isolated from different plant sources had variable melting points, although superficially they appeared to be the same compounds. Thus, Collison, Hume, Smedley-MacLean, and Smith⁴⁴ reported that the melting points of carotene preparations from cabbage, spinach, and carrots were 178°C., 163–164°C., and 164–170°C., respectively. Moore⁴⁵ noted that carotene from palm oil melted at 162°C. while that from carrots melted at 174°C.

Another discrepancy among various carotene preparations was noted in their optical activity. Although Kuhn and Lederer^{46–48} reported that the carotene preparations from winter spinach, grass, and stinging nettle (*Urtica dioica*) were uniformly inactive as far as optical rotation is concerned, they found that many carotene samples were dextro-rotatory after having been subjected to several crystallizations. It was found that the carotenes from palm oil,⁴⁹ carrots, *Sorbus aucuparia* (European mountain ash), and *Aesculus hippocastanum* (horse chestnut) all belong to the latter category. These workers also demonstrated that the optically active carotene preparations could be separated by chromatographic adsorption as well as by fractional precipitation with iodine into two isomers. The first

⁴⁴ D. L. Collison, E. M. Hume, I. Smedley-MacLean, and H. H. Smith, *Biochem. J.*, **23**, 634–647 (1929).

⁴⁵ T. Moore, *Biochem. J.*, **23**, 1267–1272 (1929).

⁴⁶ R. Kuhn and E. Lederer, *Naturwissenschaften*, **19**, 306 (1931).

⁴⁷ R. Kuhn and E. Lederer, *Ber.*, **64**, 1349–1357 (1931).

⁴⁸ R. Kuhn and E. Lederer, *Z. physiol. Chem.*, **200**, 246–254 (1931).

⁴⁹ R. Kuhn and H. Brockmann, *Z. physiol. Chem.*, **200**, 255–258 (1931).

of these, which they called α -carotene, was strongly dextro-rotatory ($[\alpha]_D^{20} = 34.0^\circ$ in benzene),⁴⁸ while the second preparation, which they designated as β -carotene, was found to be optically inactive. Similar results were obtained simultaneously and independently by Karrer *et al.*^{50,51} and by Rosenheim and Starling,⁵² who reported that crystalline carotene prepared from carrots contained an optically active, as well as an optically inactive, component. Further confirmation of the existence of α - and β -isomers of carotene was presented by van Stolk, Guilbert, and Péneau.⁵³

The presence of a third isomer, γ -carotene, was discovered by Winterstein and Ehrenberg,⁵⁴ and was confirmed somewhat later by Kuhn and Brockmann.^{55,56} Although this pigment was not described as early as the α - or β -isomers, inasmuch as it normally occurs to the extent of only 0.1% in carotene preparations from most sources, it may account for as much as 50% of the total carotenoids in some instances; this is the case with the pigments in the fruit peel of the Moluccan plant, *Gonocaryum pyriforme*.⁵⁷

(1) β -Carotene

a. Structure. Because of the fact that β -carotene is the carotenoid most widely distributed in nature and also the one most readily prepared in pure form, it was the first member of this group for which the structural relationships were understood. The structure was largely established through the pioneer work of Karrer and associates,^{2,3,58} Kuhn and von Euler, as well as of Zechmeister and co-workers.⁵⁹ Furthermore, it is the carotenoid which possesses the greatest provitamin A activity.

The empirical formula of the several carotenes has been shown repeatedly to agree with the relationship $C_{40}H_{56}$ as first postulated by Willstätter and Mieg.⁶⁰ Kuhn and Winterstein⁶¹ and also Karrer and Salomon⁶² established the fact that the carotenoids possess a series of conjugated double bonds. The total number of double bonds in the β -carotene molecule was

⁵⁰ P. Karrer, A. Helfenstein, H. Wehrli, B. Pieper, and R. Morf, *Helv. Chim. Acta*, **14**, 614-632 (1931).

⁵¹ P. Karrer, H. v. Euler, and H. Hellström, *Arkiv Kem. Mineral. Geol.*, **B10**, No. 15, 1-6 (1931).

⁵² O. Rosenheim and W. W. Starling, *Chemistry & Industry*, **50**, 443 (1931).

⁵³ D. van Stolk, J. Guilbert, and H. Péneau, *Chimie et industrie*, Special No., 550-553S (March, 1932); *Chem. Abst.*, **26**, 3826 (1932); *Compt. rend.*, **193**, 209-210 (1931). D. van Stolk, J. Guilbert, H. Péneau, and H. Simonnet, *Bull. soc. chim. biol.*, **13**, 616-635 (1931).

⁵⁴ A. Winterstein and U. Ehrenberg, *Z. physiol. Chem.*, **207**, 25-34 (1932).

⁵⁵ R. Kuhn and H. Brockmann, *Naturwissenschaften*, **21**, 44 (1933).

⁵⁶ R. Kuhn and H. Brockmann, *Ber.*, **66**, 407-410 (1933).

⁵⁷ A. Winterstein, *Z. physiol. Chem.*, **219**, 249-252 (1933).

⁵⁸ P. Karrer and R. Morf, *Helv. Chim. Acta*, **16**, 625-641 (1933).

⁵⁹ L. Zechmeister, L. v. Cholnoky, and V. Vrabély, *Ber.*, **61**, 566-568 (1928).

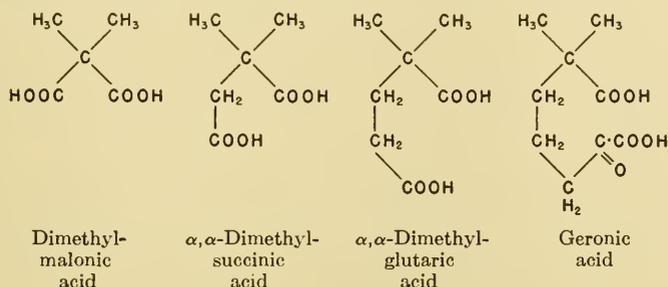
⁶⁰ R. Willstätter and W. Mieg, *Ann.*, **355**, 1-28 (1907).

⁶¹ R. Kuhn and A. Winterstein, *Helv. Chim. Acta*, **11**, 427-431 (1928).

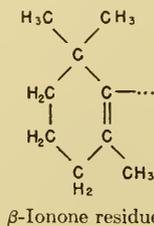
⁶² P. Karrer and H. Salomon, *Helv. Chim. Acta*, **11**, 513-525 (1928).

shown by Zechmeister *et al.*^{59,63,64} to be 11, on the basis of hydrogenation tests. Confirmation of this finding was given by Pummerer and Rebmann,⁶⁵ who proved that β -carotene is capable of absorbing a maximum of 11 molecules of iodine.

Since the completely saturated hydrocarbon perhydrocarotene has an empirical formula of C₄₀H₇₈, it was suggested that two ring structures occur in the β -carotene molecule.⁶⁵ The nature of such ring structures was indicated by the fact that geronic acid and other characteristic derivatives originate on oxidation of β -carotene with potassium permanganate or ozone.⁶⁶⁻⁶⁸ All of these products originate through the oxidation of β -



ionone and, in fact, their proportions are similar to those produced when β -carotene is oxidized. Since pure β -carotene gives rise to 16% of geronic acid on oxidation, calculated on the basis of two β -ionone rings, while β -ionone itself was shown to produce 19.4% under similar conditions, Karrer and Morf⁶⁶ concluded that β -carotene possesses two ionone residues.



Further proof of the structure of β -carotene is furnished by degradation tests with potassium permanganate. Under this treatment, four molecules of acetic acid originate.^{67,69} One acetic acid molecule is formed from the following group:

⁶³ L. Zechmeister and L. v. Cholnoky, *Ber.*, 61, 1534-1539 (1928).

⁶⁴ L. Zechmeister, L. v. Cholnoky, and V. Vrabély, *Ber.*, 66, 123-124 (1933).

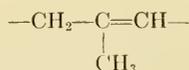
⁶⁵ R. Pummerer and L. Rebmann, *Ber.*, 61, 1099-1102 (1928).

⁶⁶ P. Karrer and R. Morf, *Helv. Chim. Acta*, 14, 1033-1036 (1931).

⁶⁷ P. Karrer and A. Helfenstein, *Helv. Chim. Acta*, 12, 1142-1144 (1929).

⁶⁸ P. Karrer, A. Helfenstein, H. Wehrli, and A. Wettstein, *Helv. Chim. Acta*, 13, 1084-1099 (1930).

⁶⁹ J. H. C. Smith and H. A. Spoehr, *J. Biol. Chem.*, 86, 755-760 (1930).



It was therefore concluded that a chain containing four isoprene groups separates the two ionone residues. On the other hand, when oxidation is carried out by the so-called "wet oxidation" method of Kuhn and L'Orsa,⁷⁰ which employs chromic acid,⁷¹⁻⁷³ six molecules of acetic acid are produced per molecule of β -carotene.^{68,71} The acetic acid which arises with permanganate treatment belongs only to the aliphatic chain, while the two additional acetic acid molecules which result from the wet oxidation method must originate from the two β -ionone rings at each end of the molecule. Since one acetic acid molecule is produced for each branched methyl group present, it is evident that four such groups exist in the central aliphatic part of the molecule, and one on each β -ionone ring at the two ends of the molecule.

The relative position of the methyl groups in the molecule was determined from the products of thermal decomposition. Thus, it was shown that toluene and *m*-xylene could be identified among these compounds,⁷⁴⁻⁷⁶ as well as 2,6-dimethylnaphthalene,⁷⁷ but not toluic acid, which was formed only by bixin and azafrin.^{78,79} Finally, the position of the methyl groups was conclusively established by the method of successive oxidative degradation, and also by the synthesis of the completely hydrogenated carotenoids and their hydrogenated fission products.

Karrer, Helfenstein, Wehrli, and Wettstein⁶⁸ in 1930, and Karrer and Morf⁶⁶ in 1931, postulated the structural formula for β -carotene now known to be correct. The later investigations of Pummerer *et al.*⁸⁰ and of Strain⁸¹ give added confirmation to the above structure, as do the subsequent data of Kuhn and Winterstein,⁷⁷ who stated that 2,6-dimethylnaphthalene is formed on thermal decomposition of β -carotene.

A further rigorous proof of the structure of β -carotene was carried out by Kuhn and Brockmann,⁸² who established the correctness of the Karrer formula. The demonstration of the conversion of β -carotene to β -carot-

⁷⁰ R. Kuhn and F. L'Orsa, *Z. angew. Chem.*, *44*, 847-853 (1931).

⁷¹ R. Kuhn and H. Brockmann, *Ber.*, *67*, 885-888 (1933).

⁷² R. Kuhn and L. Ehmann, *Helv. Chim. Acta*, *12*, 904-915 (1929).

⁷³ R. Kuhn and F. L'Orsa, *Ber.*, *64*, 1732-1736 (1931).

⁷⁴ L. Zechmeister and L. v. Cholnoky, *Ann.*, *478*, 95-111 (1930).

⁷⁵ R. Kuhn and A. Winterstein, *Ber.*, *66*, 1733-1741 (1933).

⁷⁶ R. Kuhn, A. Winterstein, and W. v. Wiegand, *Helv. Chim. Acta*, *11*, 716-724 (1928).

⁷⁷ R. Kuhn and A. Winterstein, *Ber.*, *66*, 429-432 (1933).

⁷⁸ R. Kuhn and A. Winterstein, *Ber.*, *65*, 1873-1880 (1932).

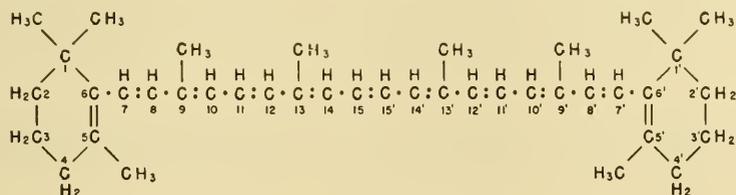
⁷⁹ R. Kuhn and A. Winterstein, *Ber.*, *65*, 646-651 (1932).

⁸⁰ R. Pummerer, L. Rebmann, and W. Reindel, *Ber.*, *64*, 492-502 (1931).

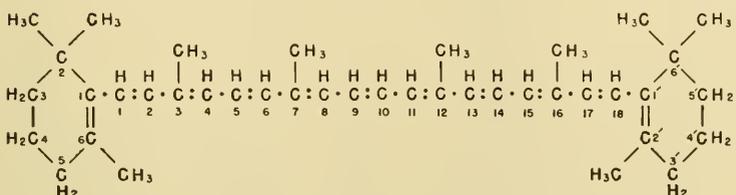
⁸¹ H. H. Strain, *J. Biol. Chem.*, *102*, 137-150 (1933).

⁸² R. Kuhn and H. Brockmann, *Ann.*, *516*, 95-143 (1935).

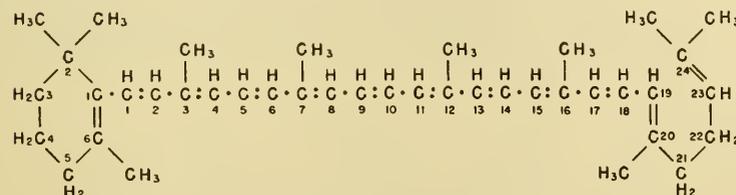
enone,⁸³⁻⁸⁶ which is converted to azafrin by means of a mild oxidation reaction,⁷¹ yielded further support for the Karrer structure. Kuhn,⁸⁷ in 1935, stated that the formula for β -carotene definitely agrees with that suggested earlier by Karrer.^{66,68,88}



β -Carotene (Karrer numbering)



β -Carotene (Kuhn numbering)



γ -Carotene (Heilbron numbering)

There are several methods for designating the carbon atoms in the carotenoid molecule. The most common one is that employed by Karrer, which is used throughout the present monograph. Alternative systems are those of Kuhn and of Heilbron, also illustrated here.⁸⁹

b. Occurrence. β -Carotene is the most widely distributed of any of the carotenoids. At the same time, it is the one which has the greatest provitamin A activity of any of the members of this group which have been investigated. It occurs in plants chiefly in connection with chlorophyll, which may effectively mask the yellow color. However, in many fruits, it

⁸³ R. Kuhn and H. Brockmann, *Ber.*, 65, 894-898 (1932).

⁸⁴ R. Kuhn and H. Brockmann, *Ber.*, 66, 1319-1326 (1935).

⁸⁵ R. Kuhn and H. Brockmann, *Z. physiol. Chem.*, 213, 1-7 (1932).

⁸⁶ R. Kuhn and H. Brockmann, *Ber.*, 67, 1408-1409 (1934).

⁸⁷ R. Kuhn, *Ann. Rev. Biochem.*, 4, 479-496 (1935).

⁸⁸ P. Karrer and A. Helfenstein, *Ann. Rev. Biochem.*, 1, 551-580 (1932).

⁸⁹ F. Mayer and A. H. Cook, *The Chemistry of Natural Coloring Matter*, Reinhold, New York, 1943.

TABLE I
 β- AND α-CAROTENE CONTENTS OF LEAVES OBTAINED FROM VARIOUS SOURCES^a

Family	Genus and species	Common name	Yield, mg./kg. fresh wt.	Melting point, °C.	α- Carotene content, %
Compositae	<i>Helianthus annuus</i> L.	Sunflower	14.2	180.2	0
	<i>Silybum marianum</i> Gaertner	St. Mary's milk-thistle	7.6	178.0	0
	<i>Lactuca sativa</i>	Lettuce	3.0	178.0	0
	<i>Taraxacum officinale</i> Weber	Dandelion	6.1	179.6	0
Cucurbitaceae	<i>Petasites palmata</i> Gray	Palmate butterbur	3.0	178.0	Tr.
	<i>Megarrhiza californica</i> (Echinocystis fabacea)				
Myltaceae	<i>Cucurbita maxima</i>	Wild cucumber (bigroot)	4.3	178.2	0
	<i>Eucalyptus globulus</i> Labillardiere	Hubbard squash (winter squash)	23.8	179.0	Tr.
Rubiaceae	<i>Coprosma baueri</i> Endlicher	Tasmanian blue-gum	9.0	179.1	0
	<i>Daucus carota</i> L.	Hedge cypripedium	4.0	175.1	5
Umbelliferae	<i>Petroselinum crispum</i> (hortense)	Carrot	7.4	176.0	10
	<i>Hetera helix</i> L.	Curly garden parsley	7.5	178.0	5
Araliaceae	<i>Quercus agrifolia</i> Nees von Esenbeck	English ivy	9.0	171.5	10-15
	<i>Aesculus californica</i> Nuttall	California live-oak	—	—	5
Sapindaceae	<i>Parthenocissus quinquefolia</i> Planchon	California buckeye	13.8	176.8	10
	<i>Prunus armeniaca</i>	Virginia creeper	3.0	175.7	5
Rosaceae	<i>Photinia arbutifolia</i> Lindley	Apricot	6.3	180.2	0
	<i>Medicago sativa</i> Linn.	Alfalfa (lucerne)	9.0	175	Tr.
Leguminosae	<i>Mentha arvensis</i> Linn.	Field mint	4.2	175.8	Tr.
	<i>Catalpa bignonioides</i> Walter	Southern catalpa	4.7	178.5	0
Borraginaceae	<i>Amsinckia douglasiana</i> De Candolle	Douglas fiddleneck	12.1	179.1	0
	<i>Cuscuta salina</i> Engelmann	Salt-marsh dodder	6.0	178.0	5
Convolvulaceae	<i>Solanum tuberosum</i>	Potato	12.5	177.0	5
	<i>S. nigrum</i> Linn.	Black nightshade	30.0	178	3
Solanaceae	<i>Lycopersicon esculentum</i> Miller	Tomato	7.4	176.8	Tr.
	<i>Nicotiana tabacum</i> Linn.	Virginia tobacco	22.2	180.2	3
Primulaceae	<i>Anagallis arvensis</i> Linn.	Scarlet pimpernel	13.4	180.7	Tr.
	<i>Plantago lanceolata</i> Linn.	Ribwort (buckhorn) plantain	5.0	179.1	0
Chenopodiaceae	<i>Chenopodium album</i> Linn.	Lambsquarters (goosefoot)	5.6	178.0	0
	<i>Stellaria media</i> Linn.	Chickweed	10.0	180.2	0
Linaceae	<i>Linum usitatissimum</i>	Flax (linseed)	3.7	177.0	Tr.
			2.0	181.5	0

Family	Genus and species	Common name	Yield, mg./kg. fresh wt.	Melting point, °C.	α - Carotene content, %
<i>Euphorbiaceae</i>	<i>Ricinus communis</i> L.	Castor bean	32.1	175.7	Tr.
<i>Rutaceae</i>	<i>Citrus grandis</i> (maxima)	Pummelo (shaddock)	3.0	171.5	5-10
<i>Urticaceae</i>	<i>Maltva parviflora</i> Linn.	Small-flowered mallow	18.0	179.1	3
<i>Urticaceae</i>	<i>Urtica urens</i> Linn.	Dwarf nettle (dog nettle)	8.0	178.5	8
<i>Moraceae</i>	<i>Ficus carica</i> L.	Common fig	—	—	5
<i>Theaceae</i> (Ternstroemiaceae)	<i>Thea</i> (<i>Camellia</i>) spp.	Tea	1.5	175.7	10
	<i>Camellia spectabilis</i>	Camellia (Jap.)	—	—	7
<i>Crassulaceae</i>	<i>Sedum acre</i> Linn.	Goldmoss, stonecrop	—	—	5
<i>Ranunculaceae</i>	<i>Ranunculus californicus</i> Bentham	California buttercup, crowfoot ^b	3.5	178.0	5
<i>Magnoliaceae</i>	<i>Magnolia grandiflora</i> Linn.	Southern magnolia	11.8	169.5	20
<i>Iridaceae</i>	<i>Iris germanica</i> Lindl.	Iris (purple queen)	9.1	174.6	Tr.
<i>Gramineae</i>	<i>Hordeum sativum</i> (vulgare)	Barley	—	—	0
	<i>Bambusa</i> spp.	Bamboo	—	—	+
<i>Cyperaceae</i>	<i>Zea mays</i>	Flint corn (Indian maize)	6.0	180.2	—
	<i>Elcocharis palustris</i> Schultes	Roemer and	—	—	—
	<i>Carex senta</i> Boott	Spike sedge	2.4	178.0	0
<i>Juncaceae</i>	<i>Juncus balticus</i> Willdenow	Rough sedge	22.8	179.1	0
	<i>Juncus tenuis</i> Willdenow	Baltic rush	8.3	177.0	Tr.
<i>Liliaceae</i>	<i>Dracaena draco</i> Linn.	Poverty rush	3.5	178.5	Tr.
<i>Araceae</i>	<i>Zantedeschia aethiopica</i> Sprengel	Dragon tree	5.0	177.5	5
<i>Palmaeae</i>	<i>Washingtonia filifera</i> Wendland	Calla lily	19.8	179.1	0
	<i>Phoenix canariensis</i>	North American Washington palm	12.0	174	5-10
<i>Coniferae</i>	<i>Pinus radiata</i> Don	Canary island date palm	12.2	178	7
	<i>Sequoia sempervirens</i> Engl.	Monterey pine	8.0	173	10
	<i>Libocedrus decurrens</i> Torrey	California redwood	8.5	175.1	10-15
<i>Pteridophyta</i>	<i>Pteridium aquilinum</i> (<i>Pteris aquilina</i>) Linn.	California incense cedar	8.0	159-160	35
		Fern (bracken)	9.8	179.1	0
<i>Bryophyta</i>	<i>Musci</i> spp.	Moss	3.0	—	10
<i>Thallophyta</i>	<i>Chlorella vulgaris</i>	Green fresh-water alga	28.0	176	5

^a Data from G. MacKinney, *J. Biol. Chem.*, **111**, 75-84 (1935).

^b Extremely low carotene content.

is present without chlorophyll, under which condition it may confer upon the fruit its yellow or orange color, as, in fact, occurs in the case of peaches (*Prunus persica*), apricots (*P. armeniaca*), squash (*Cucurbitaceae*), mango (*Mangifera indica* L.),⁹⁰ and like products. The wide distribution of β -carotene in the leaves of various plants has been shown by the studies of MacKinney,⁹¹ which are summarized in Table 1.

Thus, it was found that carotene is present as the major pigment in 59 different plant species distributed among 40 botanical families. In 40 cases, α -carotene was also present in amounts ranging from a trace to as high as 35%.

Strain⁹² carried out a study comparable to that of MacKinney with a series of leaves from trees and from many common plants. By the use of magnesium oxide columns, he determined whether the carotenoid was β - or α -carotene or a mixture of both. The following leaves were found to contain β -carotene but no α -carotene: *Ailanthus altissima* Swingle (tree of heaven); *Cynara scolymus* L. (artichoke); *Erodium cicutarium* L'Heritier de Brutelle (afileria pin-clover or heronsbill); *Grevillea robusta* Cunningham (silkoak); *Hordeum vulgare* L. (barley); *Lathyrus odoratus* L. (sweet pea); *Medicago hispida* Gaertner (burr clover); *Claytonia (Montia) perfoliata* Howell (miner's lettuce or winter purslane); *Phoradendron villosum* Nuttall (California mistletoe); *Rumex pulcher* L. (fiddleleaf dock); *Schinus molle* L. (California pepper tree); *Symphoricarpos albus* Blake (snowberry); *Tropaeolum majus* L. (nasturtium); *Zauschneria californica* Presl. (California fire-chalice); and *Cladophora* spp. (fresh-water alga). Another series of leaves were shown to contain principally β -carotene, although α -carotene was also present in small concentration. In this group, leaves from the following species are included: *Mahonia (Berberis) nervosa* Pursh (Cascades barberry or Oregon grape); *Brachychiton populneus* Brown (kurrajong bottle-tree, sterculia); *Calycanthus occidentalis* Hooker and Arnott (California sweet-shrub); *Cupressus macrocarpa* Hartweg (Monterey cypress); *Eriobotrya japonica* Lindley (loquat); *Hydrangea paniculata* Siebold (panicle hydrangea); *Phaseolus vulgaris* L. (kidney bean); and *Photinia serrulata* Lindley (Chinese photinia, shining shrub). Spinach and nettles contain β -carotene free from α -carotene,^{47,48} while chestnut leaves have a certain proportion of α -carotene associated with the β -carotene.⁴⁸ *Medicago sativa* (lucerne grass) was found to contain β -carotene and phytoanthin.⁹³

Strain⁹⁴ later reported that β -carotene is found in most higher plants, in a

⁹⁰ R. Yamamoto, Y. Osima, and T. Goma, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 19, 122-126 (1932).

⁹¹ G. MacKinney, *J. Biol. Chem.*, 111, 75-84 (1935).

⁹² H. H. Strain, *J. Biol. Chem.*, 111, 85-93 (1935).

⁹³ H. Willstaedt and T. K. With, *Z. physiol. Chem.*, 253, 40-46 (1938).

⁹⁴ H. H. Strain, *Ann. Rev. Biochem.*, 13, 591-610 (1944).

majority of the green algae, as well as in a large proportion of the following groups: *Euglenophyceae* (green fresh-water algae), brown algae, diatoms, dinoflagellates, yellow-green algae, red algae, and blue-green algae. Heilbron *et al.*⁹⁵ also reported the presence of β -carotene in the red marine alga (*Rhodomenia palmata*). α -Carotene is occasionally found in the higher plants and in the green algae, but it is not present in the other species. A green salt-water alga (*Ulva* spp.) has been found to contain both α - and β -carotene.⁹² The α - and β -carotenes isolated from the leaves were shown by Strain⁹² to be identical with those previously prepared from carrot root and palm oil.

In the case of flowers and fruits, β -carotene is the most widely distributed carotenoid, although a number of other members of this group are frequently present. For example, the petals of the California poppy (*Eschscholtzia californica* Chamisso) also contain α -carotene, a carotenoid less strongly adsorbed than β -carotene, and two types more strongly adsorbed than the β -isomer.⁹² Zechmeister and Polgár⁹⁶ were able to isolate as much as 150 mg. of crystalline β -carotene per kilogram of dried silk-oak flowers (*Grevillea robusta* Cunningham), while about 20% of the pigment was a complicated xanthophyll mixture. Carotenoids were absent from the red flowers of the *Zauschneria californica* Presl. (California fire-chalice); however, β -carotene was present in the leaves of the same species.⁹² Furthermore, β -carotene has been isolated from the bush monkeyflower (*Mimulus longiflorus* Grant, Scrophulariaceae), although the presence of γ -carotene in large amounts was of more interest in this case.⁹⁷ Drumm and O'Connor found that β -carotene is an important pigment in the yellow iris (*Iris pseudocorus*).⁹⁸ β -Carotene has likewise been found in a still wider variety of flowers such as the *Calendula officinalis* (pot-marigold),⁹⁹ *Ulex europaeus* (furze) and *Ulex galli* (Planchon's furze),¹⁰⁰ *Genista tridentata* (woadwaxen),¹⁰¹ *Gazania rigens* (red-brown treasure-flower gazania),^{102,103} *Calltha palustris* (marsh marigold),¹⁰⁴ *Ranunculus arvensis* (crow-foot or corn buttercup),¹⁰⁴ and *R. acris* (buttercup),¹⁰⁵ *Tragopogon pratensis* (subspecies *orientalis*) (goat's beard, salsify),^{104,105} *Cytisus* (*Sarothamnus*) *scoparius* (Scotch broom),¹⁰⁶ *Trollius europaeus* (globe-flower), *Laburnum*

⁹⁵ I. M. Heilbron, E. G. Parry, and R. F. Phipers, *Biochem. J.*, **29**, 1376-1381 (1935).

⁹⁶ L. Zechmeister and A. Polgár, *J. Biol. Chem.*, **140**, 1-3 (1941).

⁹⁷ L. Zechmeister and W. A. Schroeder, *Arch. Biochem.*, **1**, 231-238 (1942-1943).

⁹⁸ P. J. Drumm and W. F. O'Connor, *Biochem. J.*, **39**, 211-212 (1945).

⁹⁹ L. Zechmeister and L. v. Cholnoky, *Z. physiol. Chem.*, **208**, 26-32 (1932).

¹⁰⁰ K. Schön, *Biochem. J.*, **30**, 1960-1965 (1936).

¹⁰¹ K. Schön and B. Mesquita, *Biochem. J.*, **30**, 1966-1969 (1936).

¹⁰² K. Schön, *Biochem. J.*, **32**, 1566-1570 (1938).

¹⁰³ L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.*, **65**, 1535-1540 (1943).

¹⁰⁴ P. Karrer and A. Notthafft, *Helv. Chim. Acta*, **15**, 1195-1204 (1932).

¹⁰⁵ P. Karrer, E. Jucker, J. Rutschmann, and K. Steinlin, *Helv. Chim. Acta*, **28**, 1146-1156 (1945).

¹⁰⁶ P. Karrer and E. Jucker, *Helv. Chim. Acta*, **27**, 1585-1588 (1944).

anagyroides (golden chain) and *Kerria japonica* var. *pleniflora* (double Japanese kerria).¹⁰⁷ Petrie¹⁰⁸ reported the presence of β -carotene in the blossoms of four varieties of acacia, the Australian golden wattles: *Acacia decurrens* var. *mollis* (black-green wattle), *A. discolor* (sunshine wattle), *A. linifolia* (flax-leaf acacia), and *A. longifolia* (Sydney green wattle).

Many of the red berries contain large amounts of β -carotene. This is the case with those of the narrow-leaf firethorn (*Pyracantha angustifolia*), in which it occurs in conjunction with the unusual pigment pro- γ -carotene^{109,110} Karrer and Rutschmann¹¹¹ have also reported a large content of γ -carotene in another species of pyracantha, the scarlet firethorn (*Pyracantha coccinia*), along with two varieties of γ -carotene which were not more completely identified. These same workers failed to detect appreciable amounts of β -carotene in one species of cotoneaster (*Cotoneaster occidentalis*). In the ripe seeds of the *Euonymus europaeus* L., *Celastraceae* (spindle tree), the main finding is the unesterified zeaxanthin.^{112,113} In the *Euonymus fortunei* Rehd., which is commonly termed the "winter-creeper euonymus" (or evonymus), zeaxanthin is the principal polyene; however, prolycopene and pro- γ -carotene are also present, in addition to β -carotene.¹¹⁴ Le Rosen and Zechmeister¹¹⁵ reported that in the case of the red berries of *Celastrus scandens* L., commonly known as the "false bitter-sweet," β -carotene occurred to the extent of only 3% of the total carotenoid pigments, while an ester of zeaxanthin was found in the amount of 80% of the polyenes, and another unusual carotenol which was named celaxanthin accounted for an additional 15% of the pigment. Both α - and β -carotene have been isolated from mountain ash berries, or rowanberries (*Sorbus aucuparia*)^{47,48} and from the fruit of the rugosa rose (*Rosa rugosa* Thumb.),¹¹⁶ in association with γ -carotene, lycopene, and rubixanthin.

A number of fruits contain β -carotene. Some of these are listed in Table 2.

The paprika has been shown by Kuhn and Lederer⁴⁸ to contain no α -carotene but only β -carotene. In the case of another fruit—the watermelon—although β -carotene was found to make up 0.46 mg. of the 6.6 mg. of pigment isolated per kilogram of the fresh fruit pulp, lycopene was the

¹⁰⁷ P. Karrer and E. Jucker, *Helv. Chim. Acta*, *29*, 1539-1544 (1946).

¹⁰⁸ J. M. Petrie, *Biochem. J.*, *18*, 957-964 (1924).

¹⁰⁹ L. Zechmeister and W. A. Schroeder, *J. Biol. Chem.*, *144*, 315-320 (1942).

¹¹⁰ L. Zechmeister and J. H. Pinckard, *J. Am. Chem. Soc.*, *69*, 1930-1935 (1947).

¹¹¹ P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, *28*, 1528-1529 (1945).

¹¹² L. Zechmeister and K. Szilárd, *Z. physiol. Chem.*, *190*, 67-71 (1930).

¹¹³ L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *196*, 199-200 (1931).

¹¹⁴ L. Zechmeister and R. B. Escue, *J. Biol. Chem.*, *144*, 321-323 (1942).

¹¹⁵ A. L. Le Rosen and L. Zechmeister, *Arch. Biochem.*, *1*, 17-26 (1942-1943).

¹¹⁶ H. Willstaedt, *Svensk. Kem. Tid.*, *47*, 112-114 (1935); *Chem. Abst.*, *29*, 6276 (1935).

TABLE 2
 FRUITS FROM WHICH β -CAROTENE HAS BEEN PREPARED

Systematic name	Common name	Ref.
<i>Arbutus unedo</i>	Strawberry tree (madrone)	<i>a</i>
<i>Capsicum frutescens</i> jap. (skins)	Japanese bush red pepper (chillies)	<i>b</i>
<i>Capsicum frutescens</i> (annuum L.)	Bush red pepper (paprika)	<i>c</i>
<i>Citrullus vulgaris</i> Schrad.	Watermelon	<i>d</i>
<i>Citrus aurantium</i> Risso	Seville orange	<i>e</i>
<i>Citrus madurensis</i>	Mediterranean mandarine	<i>e</i>
<i>Citrus poonensis</i> hort.		<i>f</i>
<i>Convallaria majalis</i>	Lily-of-the-valley	<i>g</i>
<i>Cucurbita maxima</i> Duch. (fruit pulp)	Hubbard squash (giant)	<i>h, i</i>
<i>Diospyros costata</i> (fruit)	Persimmon	<i>a</i>
<i>Gonocaryum pyriforme</i> (shells)	Dutch East India fruit	<i>j</i>
<i>Mangifera indica</i> (fruit)	Mango	<i>k</i>
<i>Sorbus aucuparia</i> (berries)	Mountain ash (rowan)	<i>l</i>
<i>Prunus armeniaca</i>	Apricot	<i>m</i>
<i>Rosa canina</i> , <i>R. damascena</i> , <i>R. eglanteria</i> (<i>rubiginosa</i>) (hips)	Dog-rose, damask-rose, sweetbriar	<i>n</i>
<i>Lycopersicon esculentum</i>	Tomato	<i>o</i>
<i>Taxus baccata</i>	Yew	<i>p</i>

^a K. Schön, *Biochem. J.*, **29**, 1779-1785 (1935).

^b L. Zechmeister and L. v. Cholnoky, *Ann.*, **489**, 1-6 (1931).

^c L. Zechmeister and L. v. Cholnoky, *Ann.*, **455**, 70-81 (1927); **509**, 269-287 (1934).

^d L. Zechmeister and P. Tuzson, *Ber.*, **63**, 2881-2883 (1930).

^e L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, **221**, 278-280 (1933).

^f R. Yamamoto and S. Tin, *J. Agr. Chem. Soc. Japan*, **9**, 642-645 (1933); *Chem. Abst.*, **27**, 5097 (1933).

^g A. Winterstein and U. Ehrenberg, *Z. physiol. Chem.*, **207**, 25-34 (1932).

^h H. Suginone and K. Ueno, *Bull. Chem. Soc. Japan*, **6**, 221-228 (1931).

ⁱ L. Zechmeister and P. Tuzson, *Ber.*, **67**, 824-829 (1934).

^j A. Winterstein, *Z. physiol. Chem.*, **215**, 51-58 (1933); **219**, 249-252 (1933).

^k R. Yamamoto, Y. Oshima, and T. Goma, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **19**, 122-126 (1932).

^l R. Kuhn and E. Lederer, *Ber.*, **64**, 1349-1357 (1931).

^m H. Brockmann, *Z. physiol. Chem.*, **216**, 45-48 (1933).

ⁿ R. Kuhn and C. Grundmann, *Ber.*, **67**, 339-344 (1934).

^o R. Willstätter and H. H. Escher, *Z. physiol. Chem.*, **64**, 47-61 (1910).

^p R. Kuhn and H. Brockmann, *Ber.*, **66**, 828-841 (1933).

chief polyene, accounting for over 90% of the total carotenoids.^{117,118} Cryptoxanthin is present in corn, but carotenes are not generally present in the cereals. Although earlier workers^{119,120} had considered carotene to be the chief fat-soluble pigment of wheat flour, Zechmeister and Cholnoky¹²¹ demonstrated by the application of chromatography that less than 0.01 mg. of carotene was present per kilogram, "if, in fact, any at all was found." Definite quantities of xanthophyll (lutein) were isolated; the above authors succeeded in preparing 15 mg. of pure crystals from 60 kg. of flour.

¹¹⁷ L. Zechmeister and P. Tuzson, *Ber.*, **63**, 2881-2883 (1930).

¹¹⁸ L. Zechmeister and A. Polgár, *J. Biol. Chem.*, **139**, 193-198 (1941).

¹¹⁹ J. A. Wesener and G. L. Teller, *J. Ind. Eng. Chem.*, **3**, 912-919 (1911).

¹²⁰ G. W. Monier-Williams, Report of the Local Government Board on Public Health and Medical Subjects, N.S., No. 73, 1-10, London (1912).

¹²¹ L. Zechmeister and L. v. Cholnoky, *J. Biol. Chem.*, **135**, 31-36 (1940).

Other workers,^{122,123} likewise failed to detect any carotene in wheat flour, although the presence of xanthophyll was demonstrated.

It has long been known that β -carotene is the chief polyene in carrots, since this was the source from which it was originally isolated. However, Strain¹²⁴ proved that α -, γ -, and δ -carotene were also present in this vegetable, as well as a carotenoid similar to flavoxanthin. β -Carotene is known to be a component of yeast and of other fungi,¹²⁵⁻¹²⁷ as well as of a saprophytic sporangiferous fungus (*Pilobolus kleinii*).¹²⁸

Although preformed vitamin A is more frequently found in the animal tissues than are provitamins A, nevertheless there is a fairly wide distribution of carotenoids in the animal kingdom. β -Carotene is frequently found in the reproductive organs. Its presence has been noted in corpora lutea,^{48,129,130} and in the corpus rubrum of the cow,¹³¹ in human placenta,¹³¹ and in bull testes.¹³² It has also been found in the sex glands of the sea urchin (*Echinus esculentus*).¹³³ It has been reported in human depot fat¹³⁴ and blood serum,^{93,135} ox blood serum,¹³⁶ and human and cow milk.^{93,137,138} It would seem to be an important component of the adrenal glands of practically all mammals, where its presence has been noted by a number of workers.¹³⁹⁻¹⁴⁴ It occurs in the fatty tissues of those animals which do not convert it to vitamin A in the intestinal wall. Thus, it is not found in the rat, chicken, pig, and goat, but it is present in the fat of dogs, cows, and man.^{140,145-147} An accumulation of β -carotene likewise occurs in the livers

¹²² M. Malmberg and H. v. Euler, *Biochem. Z.*, *284*, 238-243 (1936).

¹²³ F. P. Bowden and T. Moore, *Nature*, *132*, 204-205 (1933).

¹²⁴ H. H. Strain, *J. Biol. Chem.*, *127*, 191-201 (1939).

¹²⁵ E. Lederer, *Les Caroténoïdes des Plantes*, Paris, 1934. Cited by R. J. Williams, *Vitamins and Hormones*, *1*, 229-247 (1943).

¹²⁶ A. Scheunert and J. Reschke, *Deut. med. Wochschr.*, *57*, 349-351 (1931).

¹²⁷ P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, *29*, 355-356 (1946).

¹²⁸ E. Bünning, *Planta*, *26*, 719-736 (1937).

¹²⁹ R. Kuhn and W. Schlientz, *Helv. Chim. Acta*, *17*, 7-8 (1934).

¹³⁰ H. H. Escher, *Z. physiol. Chem.*, *83*, 198-211 (1913).

¹³¹ R. Kuhn and H. Brockmann, *Z. physiol. Chem.*, *206*, 41-64 (1932).

¹³² R. Netter, *Bull. soc. chim. biol.*, *14*, 1555-1559 (1932).

¹³³ E. Lederer, *Compt. rend.*, *201*, 300-302 (1935).

¹³⁴ L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *231*, 259-264 (1935).

¹³⁵ H. Willstaedt and T. Lindquist, *Z. physiol. Chem.*, *240*, 10-18 (1936).

¹³⁶ B. v. Euler, H. v. Euler, and H. Hellström, *Biochem. Z.*, *203*, 370-384 (1928)

¹³⁷ L. S. Palmer and C. H. Eckles, *J. Biol. Chem.*, *17*, 245-249 (1914).

¹³⁸ A. E. Gillam and M. S. El Ridi, *Biochem. J.*, *31*, 251-253 (1937).

¹³⁹ O. Bailly and R. Netter, *Compt. rend.*, *193*, 961-963 (1931).

¹⁴⁰ H. van den Bergh, P. Muller, and J. Broekmeyer, *Biochem. Z.*, *108*, 279-303 (1920).

¹⁴¹ C. L. Connor, *J. Biol. Chem.*, *77*, 619-626 (1928).

¹⁴² C. L. Connor, *Am. J. Path.*, *4*, 293-308 (1928).

¹⁴³ H. v. Euler and E. Virgin, *Biochem. Z.*, *245*, 252-264 (1932).

¹⁴⁴ H. v. Euler, U. Gard, and H. Hellström, *Svensk. Kem. Tid.*, *44*, 191-198 (1932).

¹⁴⁵ O. Bailly, *Chem. Zentr.*, *1935*, *1*, 3806. Cited by P. Karrer and E. Jucker, *Carotin-oid*, Birkhäuser, Basle, 1948.

¹⁴⁶ L. S. Palmer and C. H. Eckles, *J. Biol. Chem.*, *17*, 211-221 (1914).

¹⁴⁷ L. Zechmeister and P. Tuzson, *Ber.*, *67*, 154-155 (1934).

of those animals in which it is not broken down in the intestinal wall.^{135,142} Gallstones from cattle have been reported to contain β -carotene.^{148,149} The feces of sheep and cows are other sources of this pigment.¹⁵⁰ Carotene has been found in calf thymus, and to a lesser extent in the spleen of cattle.¹⁴¹

The occurrence of β -carotene as a component of butter fat has long been recognized. Small amounts of α -carotene may also be present,¹²⁴ depending upon the α -carotene content of the rations fed the cow. Another animal product which has been reported to contain appreciable quantities of β -carotene is the hen egg.¹⁵¹⁻¹⁵⁵ Euler *et al.*¹⁵⁶ have demonstrated the occurrence of β -carotene in salmon muscle; this pigment has also been noted in the roe of many fishes.¹⁴⁴

The widespread importance of the carotenoids in marine life is indicated by the recent report of Fox *et al.*¹⁵⁷ on the distribution of these compounds in sediment from the ocean floor. β -Carotene is the polyene most frequently found in this deposit; the xanthophyllic type is present in much smaller amounts. This relationship is reversed in the marine plants, in which the β -carotene is the less prominent pigment. The high concentration of β -carotene in the sediment may be the result of the greater rate of autoxidation of the xanthophylls as compared with that of the carotenes in regions containing dissolved oxygen. A second suggestion is that there may be a selective absorption of the xanthophylls and a fecal rejection of the carotenes by a majority of the marine animals. A final possibility is that a reduction of the xanthophylls to polyene hydrocarbons may be brought about by some marine bacteria capable of acting under anaerobic conditions. β -Carotene has been found in the sponges, *Ficulina ficus*, (fig-shaped sponge), *Suberites domuncula*,¹⁵⁵ and *Hymeniacion sanguineum* (red sponge),¹⁵⁹ as well as in the red-brown tunicate *Botryllus schlosseri*.¹⁶⁰

c. Related Compounds. (a) β -Carotene Oxide, C₄₀H₅₆O. This was obtained by von Euler *et al.*¹⁶¹ by action of perbenzoic acid on β -carotene. It has the empirical formula C₄₀H₅₆O, and it is biologically active. Although

¹⁴⁸ H. Fischer and H. Röse, *Z. physiol. Chem.*, **88**, 331-333 (1913).

¹⁴⁹ H. Fischer and R. Hess, *Z. physiol. Chem.*, **187**, 133-136 (1930).

¹⁵⁰ P. Karrer and A. Helfenstein, *Helv. Chim. Acta*, **13**, 86-87 (1930).

¹⁵¹ H. v. Euler and E. Klussmann, *Z. physiol. Chem.*, **208**, 50-54 (1932).

¹⁵² S. M. Hauge, F. P. Zscheile, C. W. Carriek, and B. B. Bohren, *Ind. Eng. Chem.*, **36**, 1065-1068 (1944).

¹⁵³ T. B. Mann, *Analyst*, **68**, 233-238 (1943).

¹⁵⁴ C. R. Thompson, M. A. Ervan, S. M. Hauge, B. B. Bohren, and F. W. Quackenbush, *Ind. Eng. Chem., Anal. Ed.*, **18**, 113-115 (1946).

¹⁵⁵ F. Harms, *Chem. Zentr.*, **1941**, II, 3007.

¹⁵⁶ H. v. Euler, H. Hellström, and M. Mahnberg, *Svensk. Kem. Tid.*, **45**, 151-152 (1933); *Chem. Abst.*, **27**, 5563 (1933).

¹⁵⁷ D. L. Fox, D. M. Updegraff, and D. G. Novelli, *Arch. Biochem.*, **5**, 1-23 (1944).

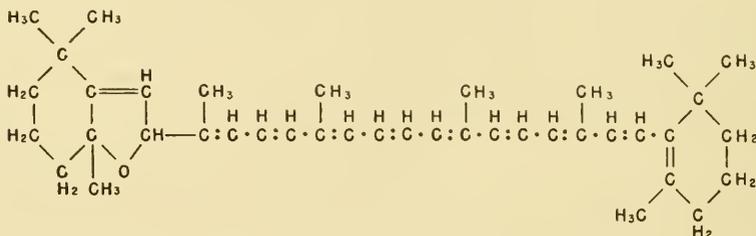
¹⁵⁸ E. Lederer, *Bull. soc. chim. biol.*, **20**, 567-610 (1938).

¹⁵⁹ P. J. Drumm, W. F. O'Connor, and L. P. Renouf, *Biochem. J.*, **39**, 208-210 (1945).

¹⁶⁰ E. Lederer, *Compt. rend. soc. biol.*, **117**, 1086-1088 (1934).

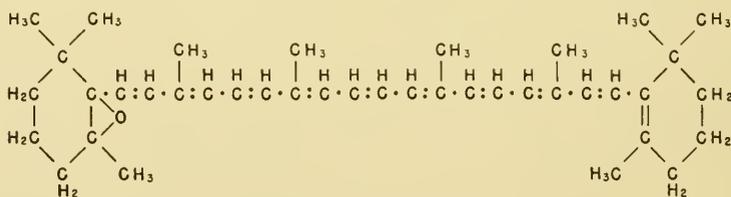
¹⁶¹ H. v. Euler, P. Karrer, and O. Walker, *Helv. Chim. Acta*, **15**, 1507-1510 (1932).

β -carotene oxide was first assumed to be an epoxide, the more recent work of Karrer and Jucker¹⁶² has demonstrated that it has a furanoid structure.



β -Carotene oxide (mutatochrome)

(b) β -Carotene Mono-epoxide, $C_{40}H_{56}O$. Karrer and his co-workers^{105,162-164} have recently made extensive studies of carotene oxide and related oxidation products. When β -carotene was oxidized with monopero-phthalic acid, Karrer and Jucker¹⁶² obtained both the furanoid compound and the epoxide. The epoxide readily crystallizes from a benzene-methanol or from a methanol-ether mixture, in orange platelets with beautiful glistening surfaces. The mono-epoxide melts at $160^{\circ}C$. When an ethereal solution is shaken with concentrated aqueous hydrochloric acid, it assumes a weak blue color which quickly disappears.



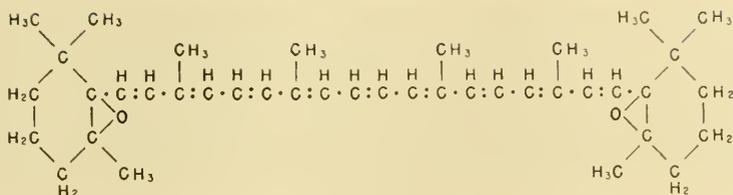
β -Carotene mono-epoxide

(c) β -Carotene Di-epoxide, $C_{40}H_{56}O_2$. When oxidation occurs on both β -ionone rings of β -carotene, the di-epoxide results. Thus an oxygen bridge is formed, not only between carbons 5 and 6 but also between carbons 5' and 6'. The di-epoxide can be crystallized from a benzene-methanol mixture in yellow platelets, melting at $184^{\circ}C$. β -Carotene epoxide shows true epiphasic behavior. For a description of epiphasic and hypophasic behavior, see page 619. When the ethereal solution of the di-epoxide is shaken with concentrated aqueous hydrochloric acid, the solution assumes a deep blue color which is permanent over a period of several days.

¹⁶² P. Karrer and E. Jucker, *Helv. Chim. Acta*, 28, 427-436 (1945).

¹⁶³ P. Karrer and E. Jucker, *Helv. Chim. Acta*, 28, 471-473 (1945).

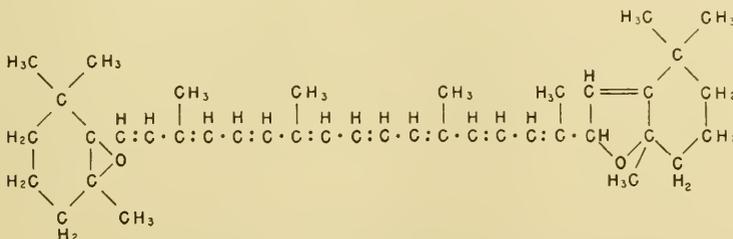
¹⁶⁴ P. Karrer, *Helv. Chim. Acta*, 28, 474-475 (1945).



β -Carotene di-epoxide

Karrer *et al.*¹⁰⁵ report that epoxides are naturally present in plant but not in animal tissues. It is suggested that they may be changed to the furanoid structure in the plant. The epoxides possess considerable biological activity and, in fact, they are to some extent convertible to carotenes in the test tube when treated with hydrochloric acid. Maximum vitamin A activity was produced by daily doses of 10 γ of β -carotene epoxide and 17 γ of β -carotene di-epoxide.¹⁰⁵

(d) *Luteochrome*, C₄₀H₅₅O₂. When β -carotene is oxidized with perchthalic acid, luteochrome originates in addition to β -carotene mono-epoxide and β -carotene di-epoxide. It crystallizes in thin yellow-orange platelets from a benzene-methanol mixture. It melts at 176°C. When it is treated with concentrated aqueous hydrochloric acid, its behavior is similar to that of β -carotene di-epoxide and aurochrome. When it is partitioned between methanol and petroleum ether, most of it goes into the upper layer.

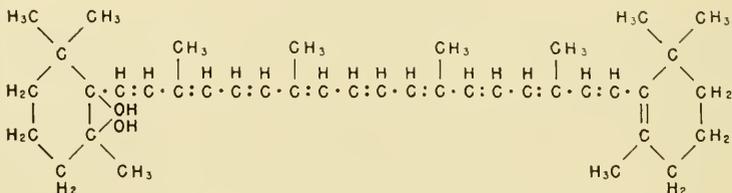


Luteochrome

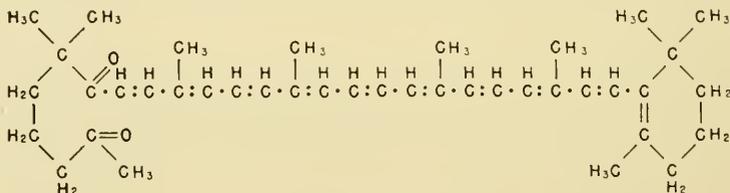
The β -carotene epoxides and the furanoid derivatives may be separated from each other by chromatographic analysis, using a column of calcium oxide, and petroleum ether as the solvent. These oxides have the same qualitative and quantitative solubility properties as does β -carotene. They dissolve well in carbon disulfide, chloroform, benzene, and ether, somewhat less readily in petroleum ether and with difficulty in methanol and ethanol.

(e) *Oxy- β -carotene*, C₄₀H₅₅O₂. This compound is closely related to β -carotene oxide, except that hydroxyl groups have been introduced at positions 5 and 6. These take the place of the single oxygen in the bridge structure between these carbons. Oxy- β -carotene originates through the oxidation of β -carotene with aqueous 0.1 N chromic acid. It crystallizes

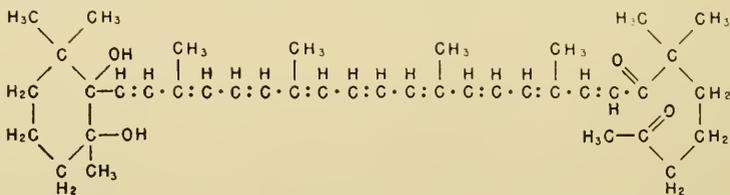
from a benzene-methanol mixture in orange-red needles which melt at 184°C .^{82,83,86} Oxy- β -carotene is readily adsorbed from hexane on aluminum oxide, but not on calcium carbonate. It dissolves easily in benzene, chloroform, and carbon disulfide, less readily in hexane, and it is insoluble in alcohol. When partitioned between petroleum ether and 90% methanol, it is found exclusively in the top layer. Oxy- β -carotene is biologically active.

Oxy- β -carotene

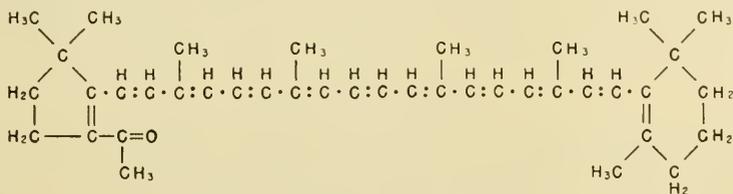
(f) *Semi- β -carotenone*, $\text{C}_{40}\text{H}_{56}\text{O}_2$. Kuhn and Brockmann⁸⁴ prepared this diketone by oxidation of β -carotene with 0.1 *N* chromic acid. An alternative procedure involves the treatment of oxy- β -carotene in benzene with lead tetraacetate in acetic acid.⁸² The compound crystallizes in four-sided, crimson platelets which melt at 118 – 119°C . It is easily soluble in hexane but dissolves more difficultly in alcohol. It shows an epiphasic reaction. It is partially active as a precursor of vitamin A.

Semi- β -carotenone

(g) *Oxy-semi- β -carotenone*, $\text{C}_{40}\text{H}_{58}\text{O}_4$. This compound has been prepared by Kuhn and Brockmann⁸² by oxidation of oxy- β -carotene with 0.1 *N* chromic acid. It gives dark red or bluish shiny prisms when crystallized from a benzene-petroleum ether mixture. These melt at 172°C . It dissolves readily in chloroform, more difficultly in benzene and ethanol, and only slightly in petroleum ether. It gives a hypophasic reaction.

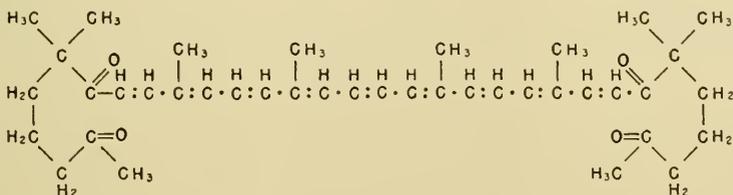
Oxy-semi- β -carotenone

(h) *Anhydro-semi-β-carotenone*, C₄₀H₅₄O. Kuhn and Brockmann⁵² prepared the anhydro compound by removal of a molecule of water from semi-β-carotenone by means of methanolic potash. The resulting product crystallizes in almost black prisms which have a greenish lustre. It melts at 177°C., is readily soluble in carbon disulfide, chloroform, and benzene, and dissolves with difficulty in hexane, petroleum ether, and absolute ethanol. It is almost entirely epiphasic.



Anhydro-semi-β-carotenone

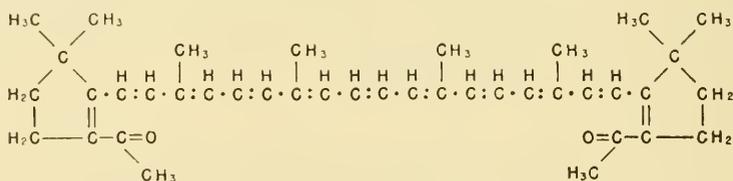
(i) *β-Carotenone*, C₄₀H₅₆O₄. This tetraketone, in which both β-ionone rings of β-carotene have been split, can be prepared through oxidation of the parent hydrocarbon with chromic acid.^{71,82,83} Semi-β-carotenone may likewise serve as the starting material in the chromic acid oxidation.⁸⁴ β-Carotenone separates from a benzene-ligroin mixture in crimson-red platelets. The crystals melt at 174–175°C. The tetraketone is adsorbed from a petroleum ether solution by aluminum oxide or calcium carbonate. β-Carotenone dissolves easily in chloroform, carbon disulfide, and benzene, less readily in cold methyl and ethyl alcohol, while it is very difficultly soluble in hexane and petroleum ether. When it is shaken with a petroleum ether-90% methanol mixture, it is partitioned almost entirely in the lower (methanol) layer. β-Carotenone is completely without provitamin A activity.



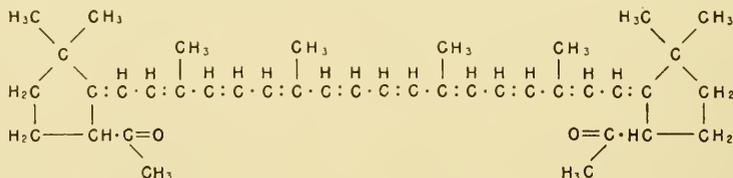
β-Carotenone

(j) *Bis-anhydro-β-carotenone*, C₄₀H₅₂O₂. β-Carotenone undergoes a condensation similar to that exhibited by semi-β-carotenone, to form the dianhydro compound. Under these conditions two molecules of water are removed and two five-membered carbon rings replace the two six-membered ionone rings present in the parent hydrocarbon. Kuhn and Brockmann⁸² reported the synthesis of this compound by treatment of β-carotenone with methanolic potash. It crystallizes from a benzene-methanol mixture in

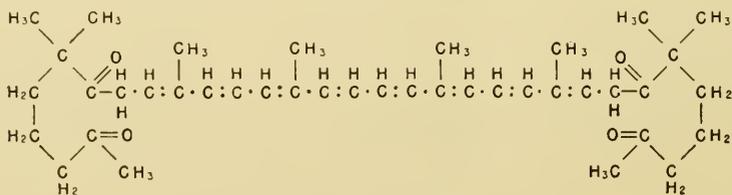
steel-blue prisms or platelets which melt at 209°C. It dissolves readily in chloroform, benzene, and carbon disulfide, but is only slightly soluble in hexane, petroleum ether, and methanol. It is almost completely epiphasic. The pigment is biologically inactive.

Bis-anhydro- β -carotenone

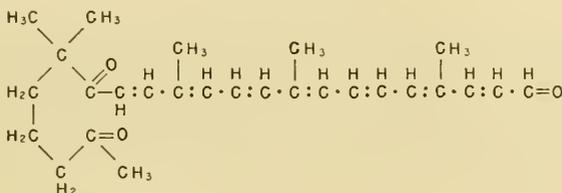
(k) *Bis-anhydro-dihydro- β -carotenone*, $C_{40}H_{54}O_2$. Kuhn and Broeckmann⁸² demonstrated the conversion of bis-anhydro- β -carotenone to its dihydro derivative when a pyridine solution was reduced on treatment with zinc dust and acetic acid. The pigment can be crystallized from pyridine-containing water in glistening red needles which melt at 217°C. This compound dissolves well in carbon disulfide, benzene, chloroform, and pyridine, but only poorly in hexane or petroleum ether. When an alkaline alcoholic solution is treated with oxygen, it rapidly reverts to bis-anhydro- β -carotenone.

Bis-anhydro-dihydro- β -carotenone

(l) *Dihydro- β -carotenone*, $C_{40}H_{58}O_4$. When β -carotenone in a pyridine solution is reduced with zinc dust and acetic acid, dihydro- β -carotenone is the resultant product.⁸⁴ The dihydro compound forms gold-yellow needle-like crystals from a benzene-hexane mixture. These melt at 130°C. They dissolve readily in pyridine, chloroform, and benzene, but only slightly in petroleum ether and alcohols. The compound is almost completely hypophasic. The dihydro- β -carotenone forms a dioxime, $C_{40}H_{60}O_4N_2$, which crystallizes in golden-yellow platelets from hot benzene, melting at 151°C. The dioxime is less soluble in benzene and hexane; it dissolves more readily in alcohol than does the parent dihydro compound.

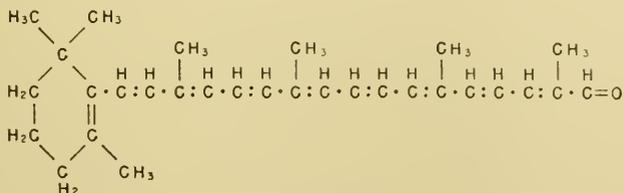
Dihydro- β -carotenone

(m) *β*-Carotenone Aldehyde, C₂₇H₃₆O₃. When *β*-carotene or *β*-carotenone is oxidized with chromic acid, a rupture of the polyene chain results giving an aldehyde of considerably shorter chain length.^{71,82} This is obtained from a benzene-hexane mixture in yellow-red needles, having a bluish cast, which melt at 146–147°C. It is quite soluble in chloroform, carbon disulfide, benzene, and hot methanol, and poorly soluble in cold hexane and petroleum ether. After prolonged treatment with an excess of hydroxylamine, the dioxime is formed, which melts at 183–184°C. When only one molecule of hydroxylamine and one molecule of *β*-carotenone are used, the resulting product is a mixture of the aldoxime and ketoxime.



β-Carotenone aldehyde

(n) *β*-Apo-2-carotenal, C₃₀H₄₀O. When *β*-carotene is oxidized with potassium permanganate at room temperature, the second *β*-ionone ring is completely destroyed and the hydrocarbon chain is ruptured between carbons 7' and 8'. Karrer and Solmssen¹⁶⁵ obtained as much as 60 mg. of the aldehyde from 700 mg. of *β*-carotene by isolating it on a chromatographic column. The name for this compound was suggested by Karrer *et al.*¹⁶⁶ to indicate where the rupture in the carbon chain had occurred. The apo-2-carotenal can be crystallized in violet plates from methanol. They melt at 139°C. The aldehyde forms a characteristic oxime (shiny violet platelets or prisms melting at 180°C.) and a semicarbazone (sinters at 205°C.). When the ethereal solution is treated with concentrated hydrochloric acid, a strong permanent blue coloration results. *β*-Apo-2-carotenal has strong provitamin A activity.



β-Apo-2-carotenal

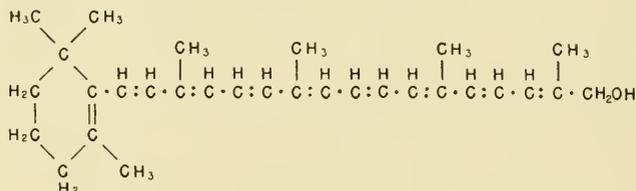
(o) *β*-Apo-2-carotenol, C₃₀H₄₂O. An alcohol corresponding to *β*-apo-2-carotenal is likewise well known. Euler and co-workers¹⁶⁷ obtained the

¹⁶⁵ P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 20, 682–690 (1937).

¹⁶⁶ P. Karrer, U. Solmssen, and W. Gugelmann, *Helv. Chim. Acta*, 20, 1020–1024 (1937).

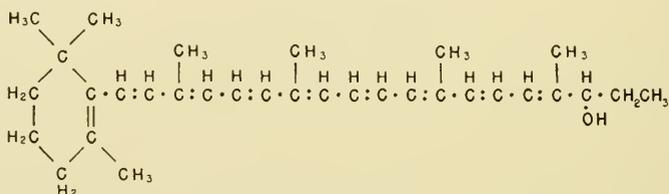
¹⁶⁷ H. v. Euler, P. Karrer, and U. Solmssen, *Helv. Chim. Acta*, 21, 211–222 (1938).

alcohol by reduction of the aldehyde by aluminum isopropoxide in isopropanol. It is a crystalline solid which gives a blue color with antimony trichloride. The β -apo-2-carotenol forms yellow platelets which melt at



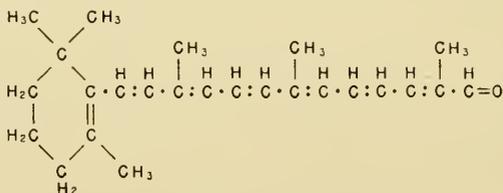
β -Apo-2-carotenol

145°C. A secondary alcohol with similar spectroscopic properties was subsequently prepared by Karrer *et al.*¹⁶⁸ by treatment of β -apo-2-carotenol with ethyl magnesium bromide. Both of these products have been found to be effective as sources of vitamin A in the animal body, as demonstrated by rat growth tests.¹⁶⁹



Secondary alcohol

(*p*) β -Apo-4-carotenol, $C_{25}H_{34}O$. The β -apo-4-aldehyde is obtained on permanganate oxidation of β -carotene, in addition to the corresponding apo-2-compound. Although the apo-4-aldehyde has not been obtained in crystalline form, a well-defined oxime (m.p., 165°C.) and a semicarbazone (red powder melting at 217°C.) are known. β -Apo-4-carotenol is biologically active.



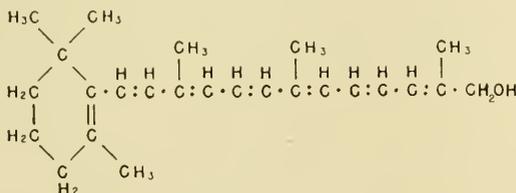
β -Apo-4-carotenol

(*q*) β -Apo-4-carotenol, $C_{25}H_{36}O$. The alcohol corresponding to the apo-4-aldehyde can be prepared by the reduction of the latter compound with

¹⁶⁸ P. Karrer, A. Rügger, and A. Geiger, *Helv. Chim. Acta*, *21*, 1171-1174 (1938).

¹⁶⁹ H. v. Euler, G. Günther, M. Malmberg, and P. Karrer, *Helv. Chim. Acta*, *21*, 1619-1621 (1938).

aluminum isopropoxide in isopropanol. Up to now it has been obtained only as an oil.



β -Apo-4-carotenol

(r) *Dihydrocarotenes*. Polgár and Zechmeister¹⁷⁰ prepared a new compound by treating β -carotene in petroleum-ether solution with a commercial concentrated hydrogen iodide without the application of heat. This was isolated on the chromatographic column and was shown to be 5,6-dihydro- β -carotene. Other double bonds were not affected, as was indicated by the fact that these were still able to undergo *trans-cis* rearrangement when heated, on melting, or by acid or iodine catalysis.

d. Properties. Such properties as solubility, absorption maxima, melting point, and stereoisomerism of β -carotene and its derivatives are discussed in Section 6 of this chapter.

(2) α -Carotene

a. Structure. Although α -carotene was discovered almost simultaneously, in 1931, by Kuhn and Lederer⁴⁶⁻⁴⁸ and Karrer and associates,^{50,51} it was not until several years later that it was prepared in pure form. Karrer and Walker¹⁷¹ succeeded in accomplishing this purification by the use of new adsorbents—calcium oxide and calcium carbonate—which they demonstrated to be effective in the separation of the epiphasic polyene pigments. It was at first thought that α -carotene possessed neither a β - nor an α -ionone ring, since on ozonization neither geronic nor isogeronic acid could be detected.¹⁷² However, it was later shown by Karrer, Morf, and Walker¹⁷³ that geronic and, to a slightly lesser extent, isogeronic acids, did originate when α -carotene was treated with ozone. Presumably the geronic acid is formed from the β -ionone residue which is present at one end of the molecule, while the isogeronic acid results from the degradation of an α -ionone structure which occurs on the opposite end of the aliphatic chain. α -Carotene also contains a series of conjugated double bonds. On catalytic

¹⁷⁰ A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, **65**, 1528-1534 (1943).

¹⁷¹ P. Karrer and O. Walker, *Helv. Chim. Acta*, **16**, 641-643 (1933).

¹⁷² P. Karrer, R. Morf, E. von Krauss, and A. Zubrys, *Helv. Chim. Acta*, **15**, 490-493 (1932).

¹⁷³ P. Karrer, R. Morf, and O. Walker, *Helv. Chim. Acta*, **16**, 975-977 (1933).

proportion of α -carotene found in the leaves of 59 species covering 40 different genera occurs in the white incense cedar (*Libocedrus decurrens* Torrey) in which it amounts to 35%⁹¹ (Table 1). Large proportions of α -carotene were also noted in magnolia leaves (*Magnolia grandiflora* L.) and in the leaves of the English ivy (*Hedera helix* L.) in which the proportions recorded were 20 and 15%, respectively. Strain⁹² reported that leaves of many common plants contain β -carotene but no α -carotene; however, a considerable number of leaves were shown to include small amounts of α -carotene in addition to the β -carotene. Considerable quantities of α -carotene have been isolated from carrots,^{48,49,53,182} from palm oil,⁴⁹ from the

TABLE 3
SOURCES FROM WHICH α -CAROTENE HAS BEEN ISOLATED^a

Systematic name	Common name	Part of organism	Ref.
Plant Tissues			
<i>Arbutus unedo</i>	Arbutus (strawberry tree)	Fruit	<i>b</i>
<i>Cantharellus cibarius</i>	Chanterelle (edible mushroom)	Cap	<i>c</i>
<i>Capsicum</i> spp.	Paprika	Fruit skins	<i>d</i>
<i>Citrullus vulgaris</i> Schrad.	Watermelon	Fruit	<i>e</i>
<i>Colcosporium senecionis</i>	Rust fungus	Uredospores on groundsel leaf	<i>f</i>
<i>Convallaria majalis</i>	Lily-of-the-valley	Berries	<i>g</i>
<i>Crocus sativus</i>	Saffron crocus	Stigma	<i>h</i>
<i>Cucurbita maxima</i>	Hubbard squash (giant)	Pulp	<i>i</i>
<i>Diospyros costata</i>	Persimmon	Fruit	<i>b</i>
<i>Euglena heliorubescens</i>	Red euglene, rain-water alga	Leaves	<i>j</i>
<i>Genista tridentata</i>	Woadwaxen	Flowers	<i>k</i>
<i>Gonocaryum obovatum</i>	Dutch East India fruit	Fruit husk	<i>l</i>
<i>Gonocaryum pyriforme</i>	Dutch East India fruit	Fruit husk	<i>l</i>
<i>Haematococcus pluvialis</i>	Fresh-water alga	Red spores	<i>m</i>
<i>Ipomoea batatas</i>	Sweet potato (ubi)	Pulp	<i>n</i>
<i>Oedogonium</i>	Fresh-water alga	Whole plant	<i>o</i>
<i>Cytisus scoparius</i>	Scotch broom	Flowers	<i>p</i>
<i>Secale cereale</i>	Rye germ oil	Seed germ	<i>q</i>
<i>Glycine soja</i>	Soybean oil	Seed	<i>r</i>
<i>Sorbus aucuparia</i>	Mountain ash (rowan)	Berries	<i>s</i>
<i>Thea</i> spp.	Formosa tea (oolong, touchang)	Leaves	<i>t</i>
<i>Ulex galli</i>	Planchon's furze	Leaves	<i>u</i>
<i>Zea mays</i>	Yellow corn	Kernels	<i>v</i>
Animal Tissues			
<i>Bos</i> spp.	Cow fat	Renal depot fat	<i>w</i>
<i>Cyclopterus lumpus</i>	Lump-sucker	Oil (liver)	<i>x</i>
<i>Styelopsis (Dendrodoa) grossularia</i>	"Red currant squirter" (solitary fixed ascidian)	Shell	<i>y</i>
<i>Echinus esculentus</i>	Sea urchin	Sex glands	<i>z</i>
<i>Hymeniacidon sanguineum</i>	Red rock sponge	Sponge tissue	<i>aa</i>
<i>Mytilus californianus</i>	Plankton-feeding mussel	Sea and deep-sea slime (micro- plankton) in feces	<i>ab</i>
<i>Orthogoriscus mola</i>	Sunfish	Oil (liver)	<i>ac</i>
<i>Regalecus glesne</i>	Oarfish	Oil (liver)	<i>x</i>

(footnotes to Table 3 on page 536)

¹⁸² P. Karrer, K. Schöpp, and R. Morf, *Helv. Chim. Acta*, 15, 1158-1165 (1932).

berries of the mountain ash⁴⁵ and chestnut,⁴⁸ from the mango,⁹⁰ and from spinach and nettles.^{47,48} A list of other sources of α -carotene in which the pigment has been definitely identified is given in Table 3.

c. Related Compounds. Only a relatively few of the comparable derivatives described for β -carotene have been reported in the case of α -carotene.

(a) α -Carotene Mono-epoxide, $C_{40}H_{56}O$. α -Carotene mono-epoxide originates on oxidation of α -carotene with perphthalic acid.¹⁶² It separates in golden-yellow crystals (m.p., 175°C.) from a benzene-methanol mixture. On treatment of an ether solution of the epoxide with aqueous concentrated hydrochloric acid, the acid layer assumes a weak ephemeral blue color. Only the mono-epoxide is formed in the case of α -carotene¹⁶²; this oxygen bridge occurs on the β -ionone portion of the molecule. The fact that the formation of the furanoid structure is related to epoxide synthesis and can occur only with the β -ionone residue explains the failure to form an α -carotene di-epoxide. According to Karrer *et al.*,¹⁰⁵ α -carotene mono-epoxide is found naturally in such different flowers as *Tragopogon pratensis* (goat's

^a Data adapted from P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948. Translated and revised by E. A. Braude, Elsevier, New York, 1950.

^b K. Schön, *Biochem. J.*, **29**, 1779-1785 (1935).

^c H. Willstaedt, *Chem. Zentr.*, **1938**, *II*, 2272; *Svensk Kem. Tid.*, **49**, 318-323 (1937); *Chem. Abst.*, **32**, 2090 (1938).

^d L. Zechmeister and L. v. Cholnoky, *Ann.*, **509**, 269-287 (1934).

^e L. Zechmeister and A. Polgár, *J. Biol. Chem.*, **139**, 193-198 (1941).

^f E. Lederer, *Compt. rend. soc. biol.*, **117**, 1083-1085 (1934); *Bull. soc. chim. biol.*, **20**, 611-634 (1938).

^g A. Winterstein and U. Ehrenberg, *Z. physiol. Chem.*, **207**, 25-34 (1932).

^h R. Kuhn and A. Winterstein, *Ber.*, **67**, 344-357 (1934).

ⁱ L. Zechmeister and P. Tuzson, *Ber.*, **67**, 824-829 (1934).

^j J. Tischer, *Z. physiol. Chem.*, **259**, 163-170 (1939).

^k K. Schön and G. Mesquita, *Biochem. J.*, **30**, 1966-1969 (1936).

^l A. Winterstein, *Z. physiol. Chem.*, **215**, 51-58 (1933).

^m J. Tischer, *Z. physiol. Chem.*, **252**, 225-233 (1938).

ⁿ J. C. Lanzing and A. G. van Veen, *Geneeskund. Tijdschr. Nederland. Indië*, **77**, 2074-2094 (1937); *Chem. Abst.*, **31**, 8625 (1937).

^o I. M. Heilbron, E. G. Parry and R. F. Phipers, *Biochem. J.*, **29**, 1376-1381 (1935).

^p P. Karrer and E. Jucker, *Helv. Chim. Acta*, **27**, 1585-1588 (1944).

^q H. A. Schuette and R. C. Palmer, *Oil & Soap*, **14**, 295-297 (1937); *Chem. Abst.*, **32**, 377 (1938).

^r W. C. Sherman, *Food Research*, **5**, 13-22 (1940).

^s R. Kuhn and E. Lederer, *Ber.*, **64**, 1349-1357 (1931).

^t R. Yamamoto and T. Muraoka, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **19**, 127-133 (1932).

^u K. Schön, *Biochem. J.*, **30**, 1960-1965 (1936).

^v G. S. Fraps and A. R. Kemmerer, *Ind. Eng. Chem., Anal. Ed.*, **13**, 806-809 (1941).

^w L. Zechmeister and P. Tuzson, *Ber.*, **67**, 154-155 (1934).

^x N. A. Sørensen, *Chem. Zentr.*, **1934**, *I*, 3817. Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948. Translated and revised by E. A. Braude, Elsevier, New York, 1950.

^y E. Lederer, *Compt. rend. soc. biol.*, **117**, 1086-1088 (1934).

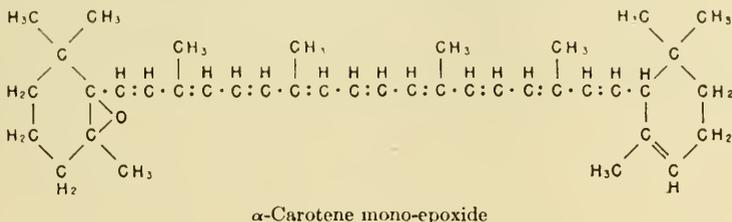
^z E. Lederer, *Compt. rend.*, **201**, 300-302 (1935).

^{aa} P. J. Drumm and W. F. O'Connor, *Nature*, **145**, 425 (1940).

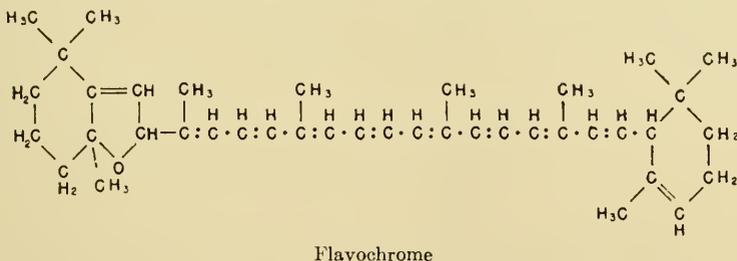
^{ab} D. L. Fox, *Proc. Natl. Acad. Sci.*, **23**, 295-301 (1937).

^{ac} N. A. Sørensen, *Kgl. Norske Videnskab. Selskabs Forh.*, **6**, No. 40, 154-157 (1933); *Chem. Abst.*, **28**, 4758 (1934).

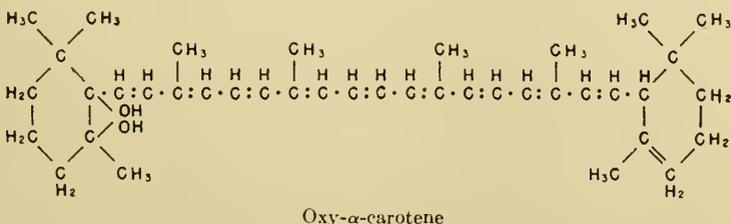
beard) and *Ranunculus acris* (buttercup or crowsfoot). Karrer and Jucker²⁵ state that the epoxide possesses vitamin A activity.



(b) *Flavochrome*, C₄₀H₅₆O. When the mono-epoxide is treated with a dilute acid, *i.e.*, chloroform-containing hydrochloric acid, thin yellow platelets melting at 189°C. and having a strong luster precipitate from a benzene-methanol mixture. These have been shown to be flavochrome, in which the epoxide structure has been transformed to a furanoid ring arrangement. The new pigment gives the same reaction with hydrochloric acid as does the mono-epoxide. Flavochrome is not a provitamin A. However, it does occur naturally in the *Ranunculus acris* and *Tragopogon pratensis*, just as the mono-epoxide does. α-Carotene mono-epoxide and flavochrome both exhibit an epiphasic reaction.



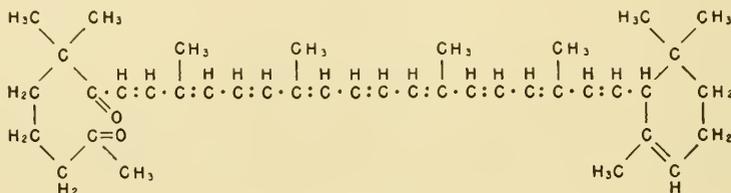
(c) *Oxy-α-carotene*, C₄₀H₅₈O₂. When α-carotene is oxidized with chromic acid, oxy-α-carotene is formed in addition to α-semi-carotene and α-carotone, C₄₀H₅₆O₅.^{183,184} Oxy-α-carotene crystallizes from a methanol-petroleum ether mixture in needles which melt at 183°C. This compound is slightly soluble in petroleum ether. It is of especial interest since it is optically active. It is not effective as a source of vitamin A.¹⁸³ The following formula has been postulated for this compound:¹⁸³



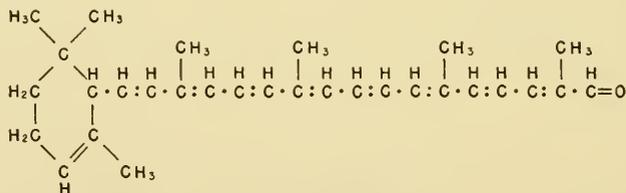
¹⁸³ P. Karrer, H. v. Euler, and U. Solmssen, *Helv. Chim. Acta*, 17, 1169-1172 (1934).

¹⁸⁴ P. Karrer, U. Solmssen, and O. Walker, *Helv. Chim. Acta*, 17, 417-419 (1934).

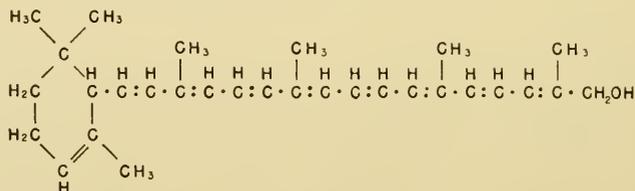
(d) *Semi- α -carotenone*, $C_{40}H_{56}O_2$. When α -carotene is oxidized with chromic acid, this diketone is one of the products formed. *Semi- α -carotenone* crystallizes from ethanol in needles which melt at 135°C . It possesses no biological activity as a provitamin A. It forms a monoxime, $C_{40}H_{57}O_2N$, which is a red crystalline product melting at 132°C .¹⁸⁵ According to Karrer and co-workers,¹⁸³ *semi- α -carotenone* has the following formula:

Semi- α -carotenone

(e) *α -Apo-2-carotenal*, $C_{30}H_{40}O$. On permanganate oxidation of α -carotene, an aldehyde having 10 less carbons than the original hydrocarbon is formed. The rupture in the hydrocarbon chain takes place between carbons 7 and 8. The β -ionone residue is destroyed and the α -ionone ring is attached to the polyene chain. *α -Apo-2-carotenal* crystallizes from petroleum ether in bright red prisms which melt at 158°C . In general, this compound is less soluble than the corresponding β -isomer.

 α -Apo-2-carotenal

(f) *α -Apo-2-carotenol*, $C_{30}H_{42}O$. The alcohol, corresponding to the apo-aldehyde, is formed when the latter substance is treated with aluminum isopropoxide in isopropanol. It yields spherical concavities lined with golden-yellow crystals which melt at 157°C .

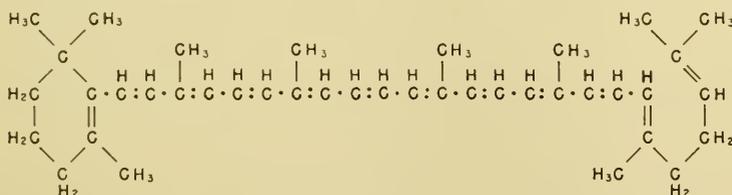
 α -Apo-2-carotenol

¹⁸⁵ P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 18, 25-27 (1935).

(g) 5,6-Dihydro- α -carotene, C₄₀H₅₈. When a petroleum ether solution of α -carotene is treated with concentrated hydriodic acid in the cold state, a number of chromatographically separable products are formed. Polgár and Zechmeister¹⁷⁰ succeeded in separating one of these products in crystalline form. On the basis of elementary analysis and of other considerations the structure of 5,6-dihydro- α -carotene was assigned to it. It melts at 202–203°C., and has no optical rotation. The crystals obtained from a benzene-methanol mixture resemble those of α -carotene.

(3) γ -Carotene

The presence of a third member of the carotene series was first suggested by the work of Winterstein and Ehrenberg.⁵⁴ Kuhn and Brockmann⁵⁵ were able to separate the new pigment by the use of chromatographic analysis. When it was oxidized with ozone, only one equivalent of geronic acid was formed per molecule of pigment. This result was interpreted to mean that this carotenoid possesses only one β -ionone ring. On ozonization, 0.85 mole of acetone was produced, presumably arising from the breakdown of the end of the aliphatic chain. This obviously represents an open-chain structure.⁵⁵ On catalytic reduction, γ -carotene was shown to have 12 double bonds,⁵⁶ 11 of which were conjugated. This result is in contradistinction to the structure of α - and β -carotenes, which have only 11 double bonds.⁶³ The formula suggested by Kuhn and Brockmann⁵⁶ is still the generally accepted one.



γ -Carotene

Although rigorous proof has not been brought forward to establish the β -ionone structure, the fact that γ -carotene is biologically active as a precursor of vitamin A would seem to be adequate evidence of the presence of this structure.

There are a number of reasons to make one doubt the homogeneity of the various samples of all-*trans*- γ -carotene described in the literature. In the first place, variations in the melting point of different samples of the pigment have been demonstrated.⁹⁷ Secondly, discrepancies in the biological potency have likewise been indicated. Although a number of workers have reported provitamin A activities for γ -carotene which are approximately

one-half of that of β -carotene,^{178,180,186,187} the potency of that prepared from *Mimulus longiflorus*¹⁸⁸ (monkey flower) and *Pyracantha angustifolia* Schneid. (narrow-leaf firethorn)¹⁸⁹ has been shown to be only approximately 26% of that of β -carotene. More recently, an all-*trans*- γ -carotene, prepared by isomerization of a natural poly-*cis* compound, namely pro- γ -carotene, has been found to have a definitely higher biological value, 42%.¹⁹⁰ γ -Carotene is sometimes referred to as β -lyco- β' -carotene, since the open ring has the same configuration as lycopene.

γ -Carotene is quite widely distributed in nature, but it is usually present in exceedingly small amounts. It has been found in commercial carotene, especially that obtained from palm oil,¹⁹¹ to the extent of 0.1%.⁵⁶ It also constitutes about the same proportion in the carotene prepared from carrots,⁵⁶ and makes up a considerable percentage of the pigment in the apricot (*Prunus armeniaca*).¹⁹² It has also been shown to be a component of the Dutch East Indian fruit *Gonocaryum pyriforme*,^{57,186} where it may account for over 50% of the total carotenoids. Willstaedt¹¹⁶ reported the preparation of γ -carotene from the fruit of the *Rosa rugosa* Thumb. (red rugosa single rose). According to Kuhn and Grundmann,¹⁹³ this carotenoid is present in the rose hips of *Rosa eglanteria* L. (sweetbriar). Other interesting sources of γ -carotene are from the berries of the lily-of-the-valley (*Convallaria majalis*)⁵⁴ and from the fruit of two varieties of pyracantha (firethorn). It has been isolated in large amounts from the berries of *Pyracantha angustifolia* Schneid.,¹⁹⁴ while it is present in only small amounts in the case of the berries of *Pyracantha coccinia*.¹¹¹ MacKinney¹⁹⁵ found that two parasitic plants commonly known as dodder, *i.e.*, *Cuscuta subinclusa* and *C. salina* (salt-marsh dodder), are excellent sources of this pigment. As much as 25 mg. of γ -carotene could be isolated per kilogram of fresh material. Zechmeister and Schroeder⁹⁷ have also demonstrated the presence of γ -carotene in the chaparral dodder, *Cuscuta californica*.

In some cases, flowers may also be an excellent source of γ -carotene. This is true of the petals of the monkey flower (*Mimulus longiflorus* Grant, *Scrophulariaceae*) from which it has been prepared in amounts of 45 to 75

¹⁸⁶ A. Winterstein, *Z. physiol. Chem.*, **215**, 51-58 (1933).

¹⁸⁷ R. Kuhn and H. Brockmann, *Klin. Wochschr.*, **12**, 972-973 (1933).

¹⁸⁸ H. J. Deuel, Jr., C. Johnston, E. Sumner, A. Polgár, W. A. Schroeder, and L. Zechmeister, *Arch. Biochem.*, **5**, 365-371 (1944).

¹⁸⁹ H. J. Deuel, Jr., C. Hendrick, E. Straub, A. Sandoval, J. H. Pinckard, and L. Zechmeister, *Arch. Biochem.*, **14**, 97-103 (1947).

¹⁹⁰ I. Zechmeister, J. H. Pinckard, S. M. Greenberg, E. Straub, T. Fukui, and H. J. Deuel, Jr., *Arch. Biochem.*, **23**, 242-245 (1949).

¹⁹¹ R. F. Hunter and A. D. Scott, *Biochem. J.*, **35**, 31-38 (1941).

¹⁹² H. Brockmann, *Z. physiol. Chem.*, **216**, 45-48 (1933).

¹⁹³ R. Kuhn and C. Grundmann, *Ber.*, **67**, 339-344 (1934).

¹⁹⁴ L. Zechmeister and W. A. Schroeder, *Science*, **94**, 609-610 (1941).

¹⁹⁵ G. MacKinney, *J. Biol. Chem.*, **112**, 421-424 (1935).

mg. per kilogram of dried flowers.^{97,196} Other flowers which contain γ -carotene include those of *Gazania rigens* (yellow treasure-flower gazania)^{102,197} and of *Crocus sativus* (saffron crocus).¹⁹⁵

γ -Carotene has been reported as a component of a number of bacteria, including the "timothy bacillus" (*Mycobacterium phlei*),^{199,200} and the acid-resistant *Bacillus Lombardo Pellegrini* and *B. Grassberger*.²⁰¹ The wide distribution of this pigment is also evident in view of its isolation from the uredospores of the *Puccinia coronifera* (crown cereal rust)²⁰² as well as from *Rhodotorula sanniei*,²⁰³ both of which are yeasts.

Fox and Emerson²⁰⁴ have also observed the presence of γ -carotene in the *Allomyces* (water mold) which belongs to the *Phycomycetes*. In this fungus, it is found as the coloring material in the gametangia of the male plants, while the female gametangia are colorless. This is in line with the suggestion of Dodge²⁰⁵ that coloring is linked with sex in the *Neurospora tetrasperma* (*Monilia*, bakery molds), in which case the races of one sex bear orange conidia while those of the other sex are pinkish.

a. Pro- γ -Carotene and Stereoisomeric Forms. Although samples of γ -carotene found in nature are usually the all-*trans* form, Zechmeister and Schroeder^{109,194} have demonstrated the presence of a pro- γ -carotene as one of the naturally occurring pigments of the *Pyracantha angustifolia* Schneid. However, Karrer and Rutschmann¹¹¹ failed to detect this isomer in the *coccinia* variety of pyracantha. Pro- γ -carotene is 3,5,7,9,11-penta-*cis*- γ -carotene. This poly-*cis*-carotene has been isolated in the amount of 0.3 mg. per kilogram from the fruit of the Brazilian butia palm (*Butia capitata* Becc.)²⁰⁶ The presence of the isomer was likewise demonstrated in another species of *Palmae*, the gum tree or woolly butia palm (*Butia eriospatha* Becc.),¹⁹⁴ where it was found in the fruit pulp. Other sources are the seeds of the winter creeper (*Euonymus fortunei* L.)¹¹⁴ and the petals of the bush monkey flower (*Mimulus longiflorus* Grant),⁹⁷ in which case it occurs in a much larger proportion than the all-*trans*- γ -carotene. Schroeder¹⁹⁶ found that higher concentrations of the poly-*cis*-isomer were present in the paler immature flowers than in those with the deeper shade which had developed naturally. The same was found to be the case with polycopene. It was suggested that pro- γ -carotene and polycopene may be precursors

¹⁹⁶ W. A. Schroeder, *J. Am. Chem. Soc.*, **64**, 2510-2511 (1942).

¹⁹⁷ L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.*, **65**, 1535-1540 (1943).

¹⁹⁸ R. Kuhn and A. Winterstein, *Ber.*, **67**, 344-357 (1934).

¹⁹⁹ E. Chargaff, *Ann. inst. Pasteur*, **52**, 415-423 (1934).

²⁰⁰ Y. Takeda and T. Ohta, *Z. physiol. Chem.*, **265**, 233-236 (1940).

²⁰¹ E. Chargaff and E. Lederer, *Ann. inst. Pasteur*, **54**, 383-388 (1935).

²⁰² E. Lederer, *Compt. rend. soc. biol.*, **117**, 411-413, 1083-1085 (1934).

²⁰³ C. Fromageot and J. L. Tehang, *Arch. Mikrobiol.*, **9**, 424-433 (1938).

²⁰⁴ D. L. Fox and R. Emerson, *Proc. Roy. Soc. London*, **B128**, 275-293 (1940).

²⁰⁵ B. O. Dodge, *Science*, **90**, 379-385 (1939).

²⁰⁶ L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.*, **64**, 1173-1177 (1942).

of the corresponding all-*trans* forms. Another neo- γ -carotene, namely, neo- γ -carotene P, has been reported in the firethorn (*Pyracantha angustifolia* Schneid.).¹¹⁰ It probably differs from the neo- γ -carotene in watermelon (*Citrullus vulgaris* Schrad.) reported earlier by Zechmeister and Polgár.¹¹⁸ Although γ -carotene or its isomers have been found in many types of plants, there are only two instances in which it has been noted in an animal form. Thus, Lederer²⁰⁷ isolated the pigment from the skin of the small green water tortoise (*Chrysemys elegans*), and Drumm and O'Connor²⁰⁸ were able to separate it in crystalline form from the red rock-sponge (*Hymeniacion sanguineum* Grant).

(4) Other Carotenes

In addition to α , β , and γ -carotene, several other members of this group have been reported, but their structure has not been exactly defined. These include δ -carotene, the presence of which in the bark of the *Gonocaryum pyriforme* was originally described by Winterstein.⁵⁷ Strain¹²⁴ later reported δ -carotene in carrots, but later renamed the pigment ζ -carotene²⁰⁹ and indicated that it was characterized by absorption spectra at 400 and 425 $m\mu$ and by the absence of absorption in the region of 450 $m\mu$. White *et al.*²¹⁰ confirmed the earlier work of Strain. More recently, Nash and Zscheile²¹¹ separated ζ -carotene from special tomato strains and carrots. These authors also state that ζ -carotene is present in butter, as well as in yellow beef fat and eggs. ζ -Carotene has been found to be inactive as a precursor of vitamin A.²¹² The empirical formula is believed to be $C_{40}H_{64}$, and its most probable structure is 5,6,7,8,5',6',7',8'-octahydrolycopene.²¹³ Like lycopene, the chain is open at both ends.

(5) Lycopene

Lycopene is one of the carotenoids which has been known for a long time. As early as 1873, Hartsen²¹⁴ isolated a dark red, crystalline pigment from the red berries of *Tamus communis* (black bryony) which was probably lycopene. It was also prepared in a somewhat impure state several years

²⁰⁷ E. Lederer, *Bull. soc. chim. biol.*, **20**, 554-566 (1938).

²⁰⁸ P. J. Drumm and W. F. O'Connor, *Nature*, **145**, 425 (1940).

²⁰⁹ H. H. Strain and W. M. Manning, *J. Am. Chem. Soc.*, **65**, 2258-2259 (1948).

²¹⁰ J. W. White, F. P. Zscheile, and A. M. Brunson, *J. Am. Chem. Soc.*, **64**, 2603-2606 (1942).

²¹¹ H. A. Nash and F. P. Zscheile, *Arch. Biochem.*, **7**, 305-311 (1945).

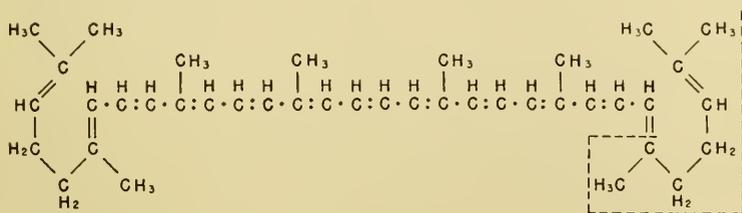
²¹² J. W. Porter, H. A. Nash, F. P. Zscheile, and F. W. Quackenbush, *Arch. Biochem.*, **10**, 261-265 (1946).

²¹³ H. A. Nash, F. W. Quackenbush, and F. P. Zscheile, *J. Am. Chem. Soc.*, **70**, 3613-3615 (1948).

²¹⁴ Hartsen, *Compt. rend.*, **76**, 385 (1873); *Chem. Zentr.*, **1873**, 204. Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 113.

later from the tomato by Millardet.²¹⁵ The latter worker called it "solano-rubin." Schunck²¹⁶ recognized that the tomato pigment, which he called lycopene, differed in absorption spectrum from carotene. The empirical formula of C₄₀H₅₆ was established by Willstätter and Escher²¹⁷ who recognized that lycopene is an isomer of the carotenes.

a. Structure. The structure of lycopene was largely worked out by Karrer and his co-workers. Karrer and Widmer²¹⁸ demonstrated that, on catalytic hydrogenation, lycopene takes up 13 molecules of hydrogen to form perhydrolycopene, which has the formula C₄₀H₈₂. This latter compound is a paraffin in the form of a colorless, optically inactive oil boiling at 238–240°C. at 0.03 mm. These results indicate not only that lycopene differs from β -carotene in having 13 instead of 11 double bonds, but also in the fact that the molecule contains no cyclic structures. The ends of the molecule were shown to be identical, since on ozonization it yields 1.6 molecules of acetone.²¹⁹ This would indicate that both terminal groups are (CH₃)₂CH=. The formation of succinic acid on oxidation with potassium permanganate suggests the presence of the group =CH·CH₂·CH₂·CH=; on treatment with chromic acid, 6 molecules of acetic acid are produced. Karrer *et al.*^{68,219} first suggested the structure which appears to explain the properties and reactions of this pigment. The Karrer formula has been further substantiated by the demonstration by Kuhn and Grundmann²²⁰ that, on partial oxidation of lycopene with chromic acid, methylheptenone and an aldehyde, lycopenal, are formed. On further oxidation in benzene, lycopenal disappears and bixin dialdehyde is produced, together with an additional molecule of methylheptenone.²²¹



Lycopene

²¹⁵ A. Millardet, *Bull. soc. Nancy* [2], 1, No. III, 21 (1875). Cited by P. Karrer and E. Jucker, *Carotinoide*, p. 113.

²¹⁶ C. A. Schunck, *Proc. Roy. Soc. London*, 72, 165–176 (1903).

²¹⁷ R. Willstätter and H. H. Escher, *Z. physiol. Chem.*, 64, 47–61 (1910).

²¹⁸ P. Karrer and R. Widmer, *Helv. Chim. Acta*, 11, 751–752, 752–754 (1928).

²¹⁹ P. Karrer, A. Helfenstein, B. Pieper, and A. Wettstein, *Helv. Chim. Acta*, 14, 435–438 (1931).

²²⁰ R. Kuhn and C. Grundmann, *Ber.*, 65, 898–902 (1932).

²²¹ R. Kuhn and C. Grundmann, *Ber.*, 65, 1880–1889 (1932).

and solitaire palm (*Ptycosperma elegans*),²²⁸ and the seeds of the blue passion flower (*Passiflora caerulea*).²²⁹

Many flowers owe a part or all of their color to lycopene, although this pigment is less common than are the carotenes or xanthophylls in this part of the plant. It occurs along with carotene in the yellowish red pigment in the pot-marigold (*Calendula officinalis*)⁹⁹ and in the coloring matter of the yellow iris (*Iris pseudacorus*). In the case of the monkey flower (*Mimulus longiflorus* Grant), it is associated with γ - and pro- γ -carotenes.⁹⁷ Lycopene likewise comprises a considerable portion of the pigment of the flowers of the cape marigold (*Dimorphotheca aurantiaca*),¹⁰⁴ of the purple-blue sweet pea (*Lathyrus odoratus*),¹⁰⁴ of the saffron crocus (*Crocus sativus*),¹⁹⁸ and of the treasure-flower gazania (*Gazania rigens*).¹⁹⁷ Other plant sources include the dodders (*Cuscuta subinclusa* and *C. salina*).¹⁹⁵ The presence of lycopene has likewise been reported in a variety of bacteria, including the air- and water-borne coccus, *Sarcina aurantiaca*,²³⁰ *Bacillus Lombardo Pellegrini* and *B. Grassberger*,²⁰¹ as well as in a *Thiocystis* species (sulfur bacteria).^{185,231}

Although lycopene is largely confined to the vegetable kingdom, it has been found in a number of animal products. Zechmeister and Tuzson^{134,232} were able to prepare small amounts of this pigment from human fat as well as from human liver.²³³ The latter finding has been confirmed by Willstaedt and Lindquist.¹³⁵ Several investigators^{234,235} have reported the presence of lycopene in human sera. As might be expected from the fact that it has been isolated from butter,²³⁶ it is also found in milk. The occurrence of lycopene in animal tissues is in all probability adventitious, and is related only to the animal's consumption of this carotenoid in the food. Since it is not readily broken down, it is stored for an interval in the various tissues.

Lubimenko²³⁷ has made a monumental study of the distribution of lycopene in the plant kingdom. The large quantity of this pigment found in palm oil^{191,238,239} is evidence of its fat solubility and of its tendency to

²²⁸ J. Zimmermann, *Rec. trav. chim.*, *51*, 1001-1003 (1932).

²²⁹ P. Karrer, F. Rübel, and F. M. Armstrong, *Helv. Chim. Acta*, *19*, 28-29 (1936).

²³⁰ V. Reader, *Biochem. J.*, *19*, 1039-1046 (1926).

²³¹ P. Karrer and U. Solmsen, *Helv. Chim. Acta*, *19*, 1019-1024 (1936).

²³² L. Zechmeister and P. Tuzson, *Bull. soc. chim. biol.*, *17*, 1110-1118 (1935).

²³³ L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *234*, 241-244 (1935).

²³⁴ E. V. Dániel and G. J. Scheff, *Proc. Soc. Exptl. Biol. Med.*, *33*, 26-30 (1935).

²³⁵ E. V. Dániel and T. Béres, *Z. physiol. Chem.*, *238*, 160-162 (1936).

²³⁶ A. E. Gillam and I. M. Heilbron, *Biochem. J.*, *29*, 834-836 (1935).

²³⁷ V. N. Lubimenko, *Rev. gen. botan.*, *25*, 475-493 (1914); *Compt. rend.*, *158*, 510-513 (1914).

²³⁸ P. Karrer, H. v. Euler, and H. Hellström, *Chem. Zentr.*, *1932*, *I*, 1800. Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 116.

²³⁹ R. F. Hunter and R. M. Krakenberger, *Biochem. J.*, *40*, 492-493 (1946).

accumulate in lipid material where possible. A summary of other sources of lycopene is given by Karrer and Jucker.²⁵

c. Related Compounds. There are several important derivatives in which the carbon chain has remained intact. A number of oxidation products are also well known in which varying numbers of carbon atoms have been split from the chain.

(a) *Prolycopene*, $C_{40}H_{56}$. The most interesting of the lycopene derivatives is probably prolycopene, which is a poly-*cis* compound discovered by Zechmeister *et al.*^{240,241} in the tangerine tomato (*Lycopersicon esculentum*). It is one of the rare examples of carotenoid pigments occurring naturally which does not have the all-*trans* structure. The new pigment was shown to have a wide distribution since it occurred in the palm fruits (*Butia capitata*)¹⁹⁴ and (*Butia criospatha* Becc.),²⁵ in the narrow-leaf firethorn (*Pyracantha angustifolia*),¹⁰⁹ in the euonymus (*Euonymus fortunei* Rehd.),¹¹⁴ and in the monkey flower (*Mimulus longiflorus* Grant).⁹⁷

Prolycopene has been shown to be 1,3,5,7,9,11-*cis*-lycopene.²⁴² On iodine catalysis, a complicated mixture results, from which ordinary all-*trans*-lycopene can be separated. Prolycopene crystallizes from petroleum ether and ethanol in plates which melt at 111°C. It is more readily soluble than is lycopene in various organic solvents. Zechmeister and Pinckard¹¹⁰ have reported the presence of at least 6 different poly-*cis*-lycopenes in the extract from the ripe berries of the *Pyracantha angustifolia* Schneid.

(b) *Perhydrolycopene*, $C_{40}H_{82}$. When lycopene is hydrogenated catalytically, it adds 13 molecules of hydrogen to form the saturated hydrocarbon.²¹⁸ The same compound has been synthesized from dihydrophytol by Karrer and his collaborators.²⁴³ It is a colorless oil boiling at 238–240°C. at 0.3 mm., having a density at 18° of 0.822 and a refractive index of 1.4560.

(c) *Dehydrolycopene*, $C_{40}H_{52}$. Karrer and Rutschmann²⁴⁴ prepared this compound by removing 4 hydrogen atoms from lycopene by the use of bromosuccinimide. The new product has 15 conjugated double bonds. It is only slightly soluble in most solvents, but it dissolves sufficiently in pyridine to enable one to crystallize it. The crystals appear dark violet to black. They are gradually decomposed at 200°C.

(d) *Apo-2-lycopenal*, $C_{32}H_{42}O$. The older designation for this compound was lycopenal. However, because several closely related substances are now known, the new terminology is preferable for differentiation.

²⁴⁰ L. Zechmeister, A. L. Le Rosen, F. W. Went, and L. Pauling, *Proc. Natl. Acad. Sci.*, **27**, 468–474 (1941).

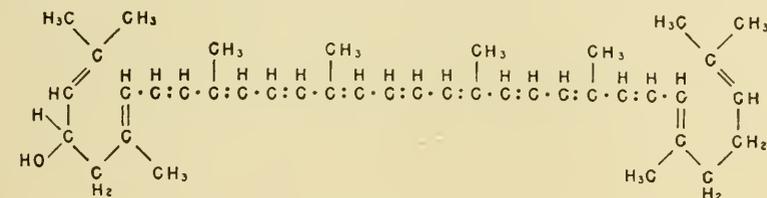
²⁴¹ A. L. Le Rosen and L. Zechmeister, *J. Am. Chem. Soc.*, **64**, 1075–1079 (1942).

²⁴² L. Zechmeister, A. L. Le Rosen, W. A. Schroeder, A. Polgár, and L. Pauling, *J. Am. Chem. Soc.*, **65**, 1940–1951 (1943).

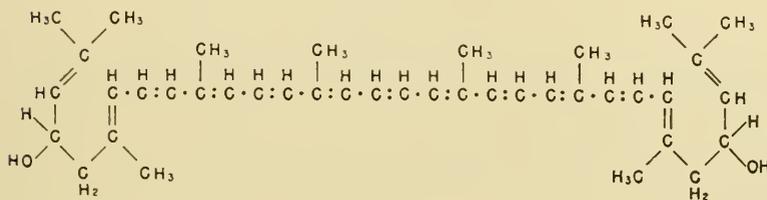
²⁴³ P. Karrer, A. Helfenstein, and R. Widmer, *Helv. Chim. Acta*, **11**, 1201–1209 (1928).

²⁴⁴ P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, **28**, 793–795 (1945).

Lycoxanthin forms a mono-acetate when treated with acetyl chloride in pyridine (m.p., 137°C.)



Lycoxanthin



Lycophyll

(2) Cryptoxanthin

Cryptoxanthin, C₄₀H₅₆O, is the xanthophyll with the smallest proportion of oxygen; hence, of all the carotenols, it most nearly approaches β -carotene in properties. It was first isolated from *Carica papaya* (papaya) in 1933 by Yamamoto and Tin,²⁵⁰ who gave it the name "caricaxanthin" and assigned it an empirical formula of C₄₀H₅₆O₂. The name "cryptoxanthin" was suggested by Kuhn and Grundmann,²⁵¹ who prepared the new carotenol from the red calyx and berries of the *Physalis alkekengi* (strawberry or winter ground-cherry) and likewise from the berries of *Physalis francheti* (Chinese lantern plant). The latter workers also gave the correct empirical formula. The identity of caricaxanthin and cryptoxanthin was established the following year.²⁵²

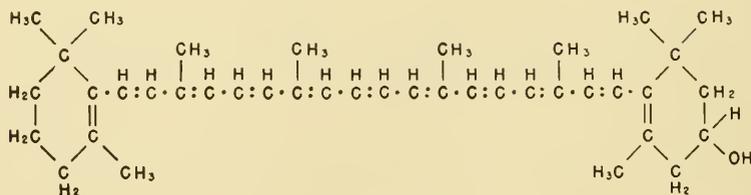
a. Structure. On catalytic hydrogenation, cryptoxanthin takes up 22 hydrogen atoms, which indicates the presence of 11 double bonds. It contains one active hydrogen. The oxygen is present in a hydroxyl group, as is proven by the acetylation which occurs in pyridine when the pigment is treated with acetic anhydride. Cryptoxanthin acetate, C₄₀H₅₅O·OCCH₃, can be obtained in garnet-red leaflet-like crystals which melt at 117–118°C. Kuhn and Grundmann²⁵¹ first proposed the formula for cryptoxanthin now known to be correct. This is based upon its provitamin A activity, which predicates a β -carotene structure on one side, while the

²⁵⁰ R. Yamamoto and S. Tin, *Bull. Inst. Phys. Chem. Research*, 12, No. 3, 354–359 (1933); abstract in *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 20, No. 411–413, p. 22 (1933).

²⁵¹ R. Kuhn and C. Grundmann, *Ber.*, 66, 1746–1750 (1933).

²⁵² P. Karrer and W. Schlientz, *Helv. Chim. Acta*, 17, 55–57 (1934).

other half of the molecule corresponds to that of a similar portion of a zeaxanthin molecule.



Cryptoxanthin

b. Occurrence. Because of the presence of the alcohol group in the molecule, cryptoxanthin generally occurs as the ester. This is true not only in the case of *Physalis alkekengi* and *P. franchetti*²⁵¹ but also in the papaya (*Carica papaya*).²⁵⁰ Cryptoxanthin occurs in a number of fruits, including those of *Arbutus unedo* (strawberry tree),²⁵³ *Diospyros costata* (persimmon),²⁵³ *Citrus poonensis*,²⁵⁴ and in orange peels.^{255,256} The carotenol is also reported in Mediterranean mandarines (*Citrus madurensis*).²⁵⁷ Other sources are the red berries of the false bitter-sweet (*Celastrus scandens*),¹¹⁵ paprika husks (*Capsicum frutescens*),²⁵⁸ yellow corn (*Zea mays*),²⁵⁹ and the flowers of *Cucurbita pepo* (pumpkin).²⁶⁰

Cryptoxanthin is a component of the flowers of the silk oak (*Grevillea robusta* Cunningham),⁹⁶ the monkey flower (*Mimulus longiflorus* Grant),⁹⁷ and perhaps of the sunflower (*Helianthus annuus*).²⁶¹ It has been reported as a pigment in the pennate marine diatom, *Nitzschia closterium*,²⁶² and in the timothy bacillus (*Mycobacterium phlei*).²⁶³

The presence of cryptoxanthin in animal products may probably be explained in the same way as in the case of lycopene, *i.e.*, that it is obtained in the food. Thus, it has been found in butter,²³⁶ eggs,²⁶⁴ and in the blood serum of cattle.²⁶⁵ Cryptoxanthin has been shown to act as a provitamin A

²⁵³ K. Schön, *Biochem. J.*, **29**, 1779-1785 (1935).

²⁵⁴ R. Yamamoto and S. Tim, *Bull. Inst. Phys. Chem. Research*, **12**, No. 5, 437-440 (1933); abstract in *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **21**, No. 422-425, abstr. p. 25 (1933).

²⁵⁵ L. Zechmeister and P. Tuzson, *Ber.*, **69**, 1878-1884 (1936).

²⁵⁶ P. Karrer and E. Jucker, *Helv. Chim. Acta*, **27**, 1695-1696 (1944).

²⁵⁷ L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, **240**, 191-194 (1936).

²⁵⁸ L. Zechmeister and L. v. Cholnoky, *Ann.*, **509**, 269-287 (1934).

²⁵⁹ R. Kuhn and C. Grundmann, *Ber.*, **67**, 593-595 (1934).

²⁶⁰ L. Zechmeister, T. Béres, and E. Ujhelyi, *Ber.*, **68**, 1321-1323 (1935).

²⁶¹ L. Zechmeister and P. Tuzson, *Ber.*, **67**, 170-173 (1934).

²⁶² N. Pace, *J. Biol. Chem.*, **140**, 483-489 (1941).

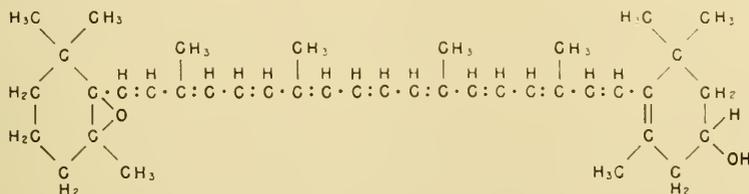
²⁶³ M. A. Ingraham and H. Steenbock, *Biochem. J.*, **29**, 2553-2562 (1935).

²⁶⁴ A. E. Gillam and I. M. Heilbron, *Biochem. J.*, **29**, 1064-1067 (1935).

²⁶⁵ A. E. Gillam and M. S. El Ridi, *Biochem. J.*, **29**, 2465-2468 (1935).

in rats.^{259,266} Recent studies have indicated that the biological potency somewhat exceeds 50% of that of β -carotene (54, 59, and 60%).^{267,268}

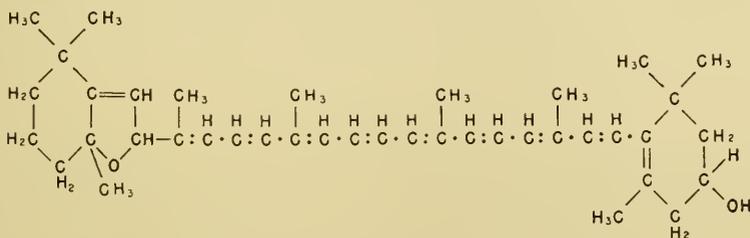
c. **Related Compounds.** (a) *Cryptoxanthin Mono-epoxide*, C₄₀H₅₆O₂. This mono-epoxide is prepared²⁶⁹ by the action of perphthalic acid on cryptoxanthin acetate. Since its derivative cryptoflavin is entirely inactive as a provitamin A, even in large doses,²⁷⁰ it is believed that both ionone rings are substituted.



Cryptoxanthin mono-epoxide

The solubility pattern of the mono-epoxide is similar to that of cryptoxanthin. When crystallized from a mixture of benzene and methanol, it forms needles or platelets which melt at 154°C. When an ethereal solution of the mono-epoxide is shaken with concentrated aqueous hydrochloric acid, a transitory blue color formation takes place.

(b) *Cryptoflavin*, C₄₀H₅₆O₂. When this mono-epoxide is treated with mineral acid, it is changed to the furanoid derivative cryptoflavin.²⁶⁹ This compound is biologically inactive.²⁷⁰ When crystallized from a mixture of petroleum ether and benzene, cryptoflavin forms leaflets having a strong surface gloss. These melt at 171°C. Cryptoflavin gives the same color reaction with hydrochloric acids as does the mono-epoxide.



Cryptoflavin

²⁶⁶ G. S. Fraps and A. R. Kemmerer, *Ind. Eng. Chem., Anal. Ed.*, **13**, 806-809 (1941).

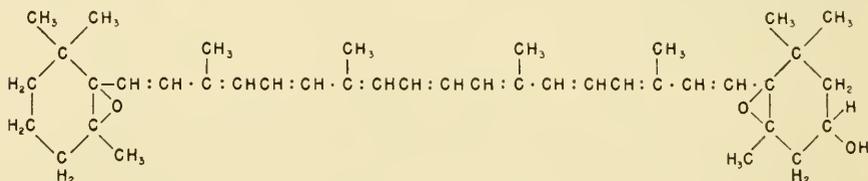
²⁶⁷ H. J. Deuel, Jr., E. R. Meserve, C. H. Johnston, A. Polgár, and L. Zechmeister, *Arch. Biochem.*, **7**, 447-450 (1945).

²⁶⁸ H. J. Deuel, Jr., S. M. Greenberg, E. Straub, T. Fukui, A. Chatterjee, and L. Zechmeister, *Arch. Biochem.*, **23**, 239-241 (1949).

²⁶⁹ P. Karrer and E. Jucker, *Helv. Chim. Acta*, **29**, 229-233 (1946).

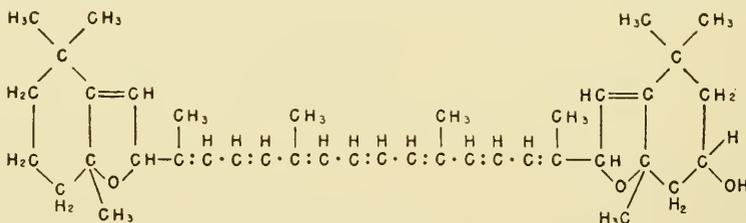
²⁷⁰ H. v. Euler, P. Karrer, and E. Jucker, *Helv. Chim. Acta*, **30**, 1159-1160 (1947).

(c) *Cryptoxanthin Di-epoxide*, $C_{40}H_{56}O_3$. In addition to the mono-epoxide, the di-epoxide is likewise formed on the oxidation of cryptoxanthin acetate with perphthallic acid.²⁶⁹ It can be crystallized from a mixture of benzene and petroleum ether (m.p., $194^{\circ}C.$). When treated with concentrated hydrochloric acid, the di-epoxide gives a deep blue coloration which is permanent over a long period.



Cryptoxanthin di-epoxide

(d) *Cryptochrome*, $C_{40}H_{56}O_3$. When cryptoxanthin di-epoxide is treated with chloroform containing hydrochloric acid, the difuranoid compound cryptochrome is formed. This compound has not been crystallized.



Cryptochrome

(e) *Cis-Trans Isomers*. Zechmeister and Lemmon²⁷¹ have described a number of *cis* isomers of cryptoxanthin which are designated as neocryptoxanthin U, neocryptoxanthin A, and neocryptoxanthin B. The absorption peaks are at somewhat shorter wave lengths than the corresponding ones of all-*trans*-cryptoxanthin.

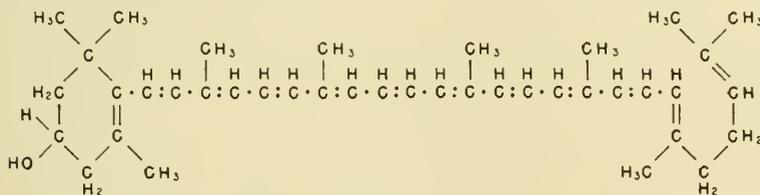
(3) *Rubixanthin*

Kuhn and Grundmann^{193,224} were the first to report the presence of an isomer of cryptoxanthin in the rose hips of *Rosa canina* (dog rose), *R. eglanteria* (sweetbriar rose), and *R. damascena* (damask rose).

Rubixanthin has an empirical formula of $C_{40}H_{56}O$. In contradistinction to other xanthophylls, rubixanthin absorbs 12 molecules of hydrogen on catalytic hydrogenation. It also yields one mole of acetone on ozonization. These two reactions are in harmony with those given by γ -carotene, and indicate that the compound possesses only a single β -ionone ring. The oxygen is present in a hydroxyl group. Since the carotenol is biologically

²⁷¹ L. Zechmeister and R. M. Lemmon, *J. Am. Chem. Soc.*, 66, 317-322 (1944).

inactive, it may be concluded that the hydroxyl is joined to the cyclic portion of the molecule rather than to the acyclic chain.



Rubixanthin

In addition to the roses listed above, *Rosa rugosa* has also been found to contain rubixanthin, according to Willstaedt.¹¹⁶ It has been found in the treasure-flower gazania (*Gazania rigens*),^{102,197} in pot-marigold (*Calendula officinalis*),¹⁹³ in the dodders (*Cuscuta subinclusa* and *C. salina*),¹⁹⁵ and in the yellow cloudberry (*Rubus chamaemorus*).²⁷²

(4) Lutein (Xanthophyll)

Lutein has long been known to be an invariable constituent of green leaves. As early as 1837, Berzelius²⁷³ coined the term "xanthophyll" for the yellow pigment appearing in the leaves in the fall foliage. It was first prepared by Willstätter and Mieg²⁷³ in 1907. Shortly thereafter Willstätter and Escher³⁴ isolated a beautifully crystalline carotenoid from the yolk of hen's egg, which they named *lutein*, and which possessed properties strikingly similar to those of leaf xanthophyll. The two products had a similar empirical composition, C₄₀H₅₆O₂; other properties also closely corresponded, with the exception of the melting point, which was higher in the case of the egg-yolk lutein, and the optical rotation, which was lower in the egg-yolk preparation.¹⁵¹ By the application of chromatography, Kuhn, Winterstein, and Lederer²⁷⁴ were able to demonstrate that the egg-yolk lutein consisted of two fractions. The first of these, which comprised about two-thirds of the total pigment, was identical with the leaf xanthophyll of Willstätter and Mieg, while the second fraction was apparently similar to a closely related isomer—zeaxanthin. Considerable confusion has since existed in the literature as regards terminology.¹⁴⁷ Although the Karrer school has preferred to employ the term xanthophyll or leaf xanthophyll^{104,275} for the single carotenol, Kuhn considered that the xanthophylls

²⁷² H. Willstaedt, *Skand. Arch. Physiol.*, 75, 155-165 (1936). Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 174.

²⁷³ R. Willstätter and W. Mieg, *Ann.*, 355, 1-28 (1907); cf. also Berzelius, *Ann.*, 21, 257-262, 263-267 (1837).

²⁷⁴ R. Kuhn, A. Winterstein, and E. Lederer, *Z. physiol. Chem.*, 197, 141-160 (1931).

²⁷⁵ P. Karrer and H. Wehrli, *Nova Acta Leopoldina (Halle-am-Saale)*, 1, 175-275 (1933).

represent a group in which lutein is a specific type. Karrer and Notthafft¹⁰⁴ retained the historic term for the chief component of leaf xanthophylls, and have coined the expression *phytoxanthin* for the polyene alcohols of the C₄₀ series. At the present time, the tendency is to employ the term *lutein* for the polyene alcohol from green leaves and from egg-yolk, since these are apparently identical. There seems to be little doubt that the egg-yolk lutein is derived from the xanthophyll in the diet.

a. Structure. The empirical formula for lutein is C₄₀H₅₆O₂. Since it is known to contain two functional alcohol groups, this may better be written as C₄₀H₅₄(OH)₂. The similarity in structure between the carotenes and lutein has long been suspected on the basis of the correspondence of their general properties. Zeaxanthin and lutein will add 11 molecules of hydrogen, just as is the case with β - or α -carotene; this indicates that the polyene alcohols must contain two ring systems.

The nature of the oxygen union was proved by Karrer, Helfenstein, and Wehrli,²⁷⁶ who showed that lutein has two active hydrogen atoms, according to the Zerewitinoff reaction, and that these must be present as components of hydroxyl groups. Further evidence that the oxygen atoms exist in alcohol groups is also afforded by the demonstration that lutein may occur naturally as a fatty acid ester.²⁷⁷⁻²⁸¹ The final evidence that the alcohol-like reactions are not the result of enolization but depend upon a true alcohol structure is obtained through oxidation of the perhydroxanthophyll to a diketone. According to Karrer *et al.*²⁸² this demonstrates that the hydroxyls are present as secondary alcohol groups.

The nature of the ring system and the position of the hydroxyl group have been established by oxidative degradation. Lutein and zeaxanthin both yield dimethyl malonic acid, HOOC·C(CH₃)₂·COOH, and α,α -dimethylsuccinic acid, HOOC·C(CH₃)₂·CH₂·COOH, while no α,α -dimethylglutaric acid, HOOC·C(CH₃)₂·CH₂·CH₂·COOH, or geronic acid, CH₃·CO·CH₂·CH₂·CH₂·C(CH₃)₂·COOH, is produced. These data indicate that the ionone ring must be substituted, which prevents the formation of the last-named products.^{50,283,284} Since this is the case, the hydroxyl must be in the *para* position to the carbon where the side chain is attached. For this reason, the hydroxyl groups are assigned to positions 3 and 3'.

²⁷⁶ P. Karrer, A. Helfenstein, and H. Wehrli, *Helv. Chim. Acta*, **13**, 87-89 (1930).

²⁷⁷ R. Kuhn and A. Winterstein, *Naturwissenschaften*, **18**, 754 (1930).

²⁷⁸ R. Kuhn, A. Winterstein, and W. Kaufmann, *Naturwissenschaften*, **18**, 418 (1930).

²⁷⁹ R. Kuhn, A. Winterstein, and W. Kaufmann, *Ber.*, **63**, 1489-1497 (1930).

²⁸⁰ L. Zechmeister and L. v. Cholnoky, *Z. physiol. Chem.*, **189**, 159-161 (1930).

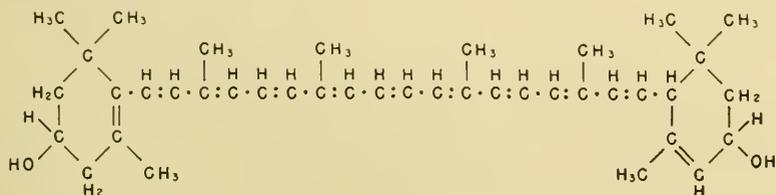
²⁸¹ L. Zechmeister and L. v. Cholnoky, *Ann.*, **481**, 42-56 (1930).

²⁸² P. Karrer, A. Zubrys, and R. Morf, *Helv. Chim. Acta*, **16**, 977-979 (1933).

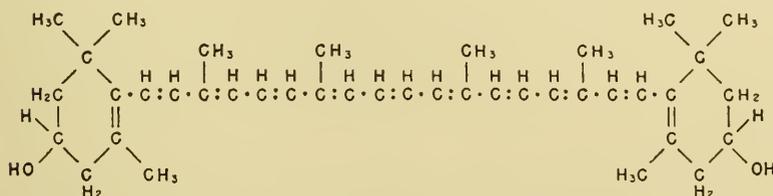
²⁸³ P. Karrer, H. Wehrli, and A. Helfenstein, *Helv. Chim. Acta*, **13**, 268-273 (1930).

²⁸⁴ R. Nilsson and P. Karrer, *Helv. Chim. Acta*, **14**, 843-845 (1931).

The differences between the two components of the egg-yolk lutein, lutein (xanthophyll) and zeaxanthin, seem to be similar to the variations between α - and β -carotenes. Both lutein and zeaxanthin are isomeric dihydroxy-carotenes with the hydroxyl substituted at 3 and 3'. Lutein is weakly dextro-rotatory, while zeaxanthin is optically inactive. It would appear, therefore, that lutein is a dihydroxy- α -carotene (3,3'-dihydroxy- β , α' -carotene), while zeaxanthin is a dihydroxy- β -carotene (3,3'-dihydroxy- β , β' -carotene). For these reasons, Karrer, Zubrys, and Morf²⁸² have proposed the following formula for lutein (xanthophyll), while Karrer²⁸⁵ has suggested that zeaxanthin possesses the structure indicated here. The close structural relationship between lutein and zeaxanthin is illustrated by the fact that the former can be changed to zeaxanthin by heating in an evacuated tube at 100–110°C. for 30 hours with benzene, sodium, and absolute alcohol.²⁸⁶ α -Carotene is partially changed to β -carotene by similar treatment.



Lutein



Zeaxanthin

b. Occurrence. Lutein is especially widely distributed in nature. In common with chlorophylls a and b and carotene, it is present throughout the plant kingdom.²⁴ Willstätter and Stoll²⁸⁷ were able to isolate 0.68 to 1.25 g. of lutein from one kilogram of dried leaves. It was shown that 1.5 to 2.0 molecules of this carotenol were present for each molecule of carotene, giving a carotene to lutein ratio which averaged 0.6. This is illustrated by the results of Willstätter and Stoll²⁸⁷ given in Table 4.

²⁸⁵ P. Karrer, *Arch. sci. biol. Italy*, 18, 30–39 (1933).

²⁸⁶ P. Karrer and E. Jucker, *Helv. Chim. Acta*, 30, 266–267 (1947).

²⁸⁷ R. Willstätter and A. Stoll, *Untersuchungen über Chlorophyll, Methoden und Ergebnisse*, Springer, Berlin, 1913. Cited by L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, pp. 72, 74, 169.

TABLE 4
 CHLOROPHYLL AND CAROTENOID CONTENT OF LEAVES^a

Plant	Exposed to light or shade	Amounts, g./kg. dried leaves			
		Total chl. (a + b)	Carotene	Lutein	Sum of carotenoids
<i>Sambucus nigra</i> (European elder)	Light	7.98	0.52	0.95	1.47
	Shaded	11.79	0.38	1.18	1.56
<i>Aesculus hippocastanum</i> (Horsechestnut)	Light	9.58	0.82	1.25	2.07
	Shaded	11.66	0.37	1.11	1.48
<i>Platanus acerifolia</i> (London plane tree, buttonwood)	Light	6.82	0.33	0.73	1.06
	Shaded	11.15	0.51	1.25	1.76

^a Table from L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, p. 115. Cited from R. Willstätter and A. Stoll, *Untersuchungen über Chlorophyll, Methoden und Ergebnisse*, Springer, Berlin, 1913.

Lutein has been found in a wide variety of green leaves.^{274,288} Fresh and fermented tea leaves both contain this carotenol, along with the carotenes.^{289,290} The green leaves of the stinging nettle (*Urtica dioica*)^{274,277,291} and the dwarf nettle (*Urtica urens* Heracleum)^{273,287} are both sources of this pigment. Other green leaves in which lutein has been shown to comprise an important part of the carotenoids are the following²⁷⁴: *Aesculus hippocastanum* (horsechestnut), *Trifolium pratense* (red meadow clover), *Zea mays* (yellow corn), *Medicago sativa* (Lucerne grass, alfalfa), *Spinacia oleracea inermis (glabra)* (round-seeded spinach), and grass. Karrer and Krause-Voigt²⁸⁸ have noted the presence of lutein in the Scotch broom plant (*Cytisus scoparius*), along with the carotenes and chrysanthemaxanthin. Strain⁹⁴ states that lutein occurs in green and red algae,⁹⁵ but that it is absent from brown and yellow-green algae, as well as from diatoms and dinoflagellates.

The yellow petals of a number of flowers contain lutein.^{274,277} These include *Tagetes grandiflora* (giant marigold), *T. erecta* (Aztec marigold), *T. patula* (French marigold), *T. nana* (dwarf marigold), *Helenium autumnale* (sneezeweed), *Rudbeckia speciosa Newmanni* (the showy coneflower), and *Helianthus annuus* (sunflower).^{261,274,292} Lutein has been proved by a number of different workers^{261,293-295} to be a component of the pigment of the common dandelion. The hawkbit or Fall dandelion (*Leontodon*

²⁸⁸ P. Karrer and E. Krause-Voigt, *Helv. Chim. Acta*, 30, 1158-1159 (1947).

²⁸⁹ R. Yamamoto and T. Muraoka, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 19, 127-133 (1932).

²⁹⁰ M. Tsujimura, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 18, 13-21 (1932).

²⁹¹ L. Zechmeister and P. Tuzson, *Ber.*, 61, 2003-2009 (1928); 62, 2226-2232 (1929).

²⁹² L. Zechmeister and P. Tuzson, *Ber.*, 63, 3203-3207 (1930).

²⁹³ P. Karrer and H. Salomon, *Helv. Chim. Acta*, 13, 1063-1067 (1930).

²⁹⁴ R. Kuhn and E. Lederer, *Z. physiol. Chem.*, 200, 108-114 (1931).

²⁹⁵ P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, 25, 1144-1149 (1942).

autumnalis)²⁹⁶ and the buttercup (*Ranunculus acris*)^{105,216,297} are common flowers which also contain lutein. Other closely related species likewise have this carotenol: *Ranunculus arvensis* (crowfoot or corn buttercup)¹⁰⁴ and *R. acris (steverni)* (tall buttercup).²²³ The flowers of a number of types of acacia are well-known sources, including *Acacia decurrens* var. *mollis* (black-green wattle),²⁷⁴ *A. discolor* (sunshine wattle), *A. linifolia* (flax-leaf acacia), and *A. longifolia* (Sydney green acacia).¹⁰⁸ Other familiar flowers which have the carotenol as an important component are the calendula (*Calendula officinalis*),⁹⁹ the treasure-flower gazania (*Gazania rigens*),^{102,197} the genista or woadwaxen (*Genista tridentata*),¹⁰¹ the wild pansy (*Viola tricolor*),^{298,299} and the red and yellow winter aster.³⁰⁰ Schunck²¹⁶ has likewise reported lutein in the musk-plant (*Mimulus moschatus*), the golden-chain laburnum (*Laburnum anagyroides*), and the coltsfoot (*Tussilago farfara*). Lutein in the unchanged form is also present in the flowers of the *Crepis aurea* (orange hawksbeard),³⁰¹ along with α - and β -carotene and violaxanthin. With the exception of α -carotene, these same pigments were found in the *Iris pseudacorus* (yellow iris).

Lutein frequently occurs in flowers in the esterified form. Thus, lutein ester is present in the dandelion in conjunction with taraxanthin. The ester form has also been reported by Karrer and Notthafft¹⁰⁴ in *Caltha palustris* (marsh marigold), *Trollius europaeus* (common globe-flower), *Ranunculus arvensis* (crowfoot), and *Tragopogon pratensis* (salsify or goat's beard). In the last instance, violaxanthin is likewise present. However, the most readily available source of esterified lutein is the pod of the paprika.²⁵⁸ Both free and esterified luteins have been found in the mango (*Mangifera indica* L.).⁹⁰ The berries of the lily-of-the-valley (*Convallaria majalis*) are another source of the esterified lutein.⁵⁴

Lutein is widely distributed in fruits. The so-called cucurbitaxanthin, obtained from *Cucurbita maxima* (giant winter squash), has been shown to be lutein; in this case the pigment occurs in conjunction with β -carotene and violaxanthin.^{302,303} Other fruit sources are the following: *Momordica balsamina* (balsam apple),^{304,305} *Citrus madurensis* (Mediterranean mandarine),^{257,306} *Citrus aurantium* (Seville orange),²⁵⁵ *Arbutus unedo* (straw-

²⁹⁶ R. Kuhn and E. Lederer, *Z. physiol. Chem.*, **213**, 188-191 (1932).

²⁹⁷ R. Kuhn and H. Brockmann, *Z. physiol. Chem.*, **213**, 192-198 (1932).

²⁹⁸ R. Kuhn and A. Winterstein, *Ber.*, **64**, 326-332 (1931).

²⁹⁹ P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, **27**, 1684-1690 (1944).

³⁰⁰ P. Karrer and E. Jucker, *Helv. Chim. Acta*, **26**, 626-630 (1943).

³⁰¹ P. Karrer, E. Jucker, and E. Krause-Voith, *Helv. Chim. Acta*, **30**, 537-538 (1947).

³⁰² L. Zechmeister and P. Tuzson, *Ber.*, **67**, 824-829 (1934).

³⁰³ H. Sugimoto and K. Ueno, *Bull. Chem. Soc. Japan*, **6**, 221-228 (1931).

³⁰⁴ G. Tobler and F. Tobler, *Ber. deut. botan. Ges.*, **28**, 365-375, 496-504 (1910).

³⁰⁵ B. M. Duggar, *Washington Univ. Studies Sci. and Tech.*, **1**, 22-45 (1913). Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 203.

³⁰⁶ L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, **221**, 278-280 (1933).

berry tree),²⁵³ *Ananas comosus (sativus)* (pineapple),³⁰⁷ *Vaccinium vitis idaea* (cowberry)³⁰⁸ *Prunus persica* (peach),³⁰⁹ and rose hips of *Rosa eglanteria* (red rose sweetbriar).¹⁹³ The occurrence of this pigment in seeds is indicated by its isolation from wheat germ oil (*Triticum* spp.).³¹⁰⁻³¹²

Animal products are a source of lutein, but in this case its presence is adventitious and is related to its occurrence in the diet. As noted earlier, the pigment originally designated as egg-yolk "lutein" was later found to be a mixture of lutein and zeaxanthin. Although none of the carotenol is present in cow or horse fat, it has been reported in chicken fat, as well as in human fat.^{25,134,313} Zechmeister and Tuzson³¹³ were able to isolate 0.57 mg. from one kilogram of the latter fat. In another study, these investigators²³² reported lipochromes in fats obtained from a variety of patients. The highest value for lutein was found in abdominal fat obtained from a woman suffering from jaundice. Considerable quantities of this carotenol were also found in the livers of normal men¹³⁵ and of patients who had died from a variety of diseases.²³³ Lutein has been shown to be a component of human skin, especially after the consumption of large quantities of winter squash (*Cucurbita maxima*) over a prolonged period.³⁰³

Despite the absence of lutein from cow depot fat, it is a constant component of cow milk and of butter.³¹⁴ The highest values were found in November butter, in which 0.7 mg. were present per 100 grams of water-free butter fat. However, the amount dropped to low values later in the winter; increased quantities were immediately evident as soon as the cow again had green fodder. As might be expected, lutein is also found in the serum of cattle.²⁶⁵

In the case of birds, lutein has been observed not only in the egg-yolk²⁶⁴ and fat of hens,³¹³ but also in the feathers of the wild Madeira canary (*Serinus canarius*) and of the yellow hammer (*Emberiza citrinella*).³¹⁵ It is not particularly surprising to find it in skin appendages of birds, such as feathers, in view of its deposition in human skin. Lutein has been isolated from the green water frog (*Rana esculenta*).³¹⁶ The pigment is found in many lower forms, such as the silkworm (*Bombyx mori*)²⁵ and the red-brown

³⁰⁷ O. C. Magistad, *Plant Physiol.*, *10*, 187-191 (1935).

³⁰⁸ H. Willstaedt, *Svensk Kem. Tid.*, *48*, 212-213 (1936); *Chem. Abst.*, *31*, 1900 (1937).

³⁰⁹ G. MacKinney, *Plant Physiol.*, *12*, 216-218 (1937).

³¹⁰ J. C. Drummond, E. Singer, and R. J. MacWalter, *Biochem. J.*, *29*, 456-471 (1935).

³¹¹ H. H. Strain, *Leaf Xanthophylls*, Carnegie Institution of Washington Publication No. 490, Washington, 1938.

³¹² B. Sullivan and C. H. Bailey, *J. Am. Chem. Soc.*, *58*, 390-393 (1936).

³¹³ L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *225*, 189-195 (1934).

³¹⁴ A. E. Gillam, I. M. Heilbron, R. A. Morton, G. Bishop, and J. C. Drummond, *Biochem. J.*, *27*, 878-888 (1933).

³¹⁵ H. Brockmann and O. Völker, *Z. physiol. Chem.*, *224*, 193-215 (1934).

³¹⁶ L. Zechmeister and P. Tuzson, *Z. physiol. Chem.*, *238*, 197-203 (1936).

tunicate (*Botryllus schlosseri*).¹⁶⁰ The carotenol has been reported in several bacteria, including *Mycobacterium phlei* (timothy bacillus),²⁶³ in the fresh-water alga (*Haematococcus pluvialis*),³¹⁷ and in the olive-green, branched fresh-water alga (*Cladophora sauteri*).⁹⁵

Lutein does not occur only in the free and the ester form; it has recently been demonstrated by Karrer and his collaborators³⁰¹ that it may likewise be present as the epoxide. Moreover, certain well-known carotenoids such as flavoxanthin have recently been proved to be closely related to the lutein epoxides and to differ only in having a furmanoid structure.

c. Related Compounds. (a) *Helenien*, C₇₂H₁₁₆O₄. Kuhn and Winterstein²⁷⁴ proved this to be the dipalmitic acid ester of lutein, which may be written as C₁₅H₃₁CO·OC₄₀H₅₄O·OCC₁₅H₃₁. On saponification, helenien yields two molecules of palmitic acid and one of lutein. It is optically active. Helenien occurs with another isomer, *physalien*, in the following flowers²⁷⁷: *Arnica montana* (mountain arnica), *Cheiranthus senoneri* (Greek wallflower), *Doronicum pardalianches* (goldbunch leopard bane), *Helenium autumnale* (sneezeweed), *H. nudiflorum* var. *grandicephalum striatum* (large-flowered purple-head sneezeweed), *Heliopsis scabra major* (giant rough heliopsis), *H. scabra zinniaeflora* (double rough heliopsis), *Narcissus pseudonarcissus* (common daffodil), *Silphium perfoliatum* (Indian cup, rosinweed), *Tagetes aurea* (golden marigold), *T. patula* (French marigold), *T. nana* Ehrenkreuz (dwarf marigold), and *Tropaeolum majus* (climbing nasturtium).

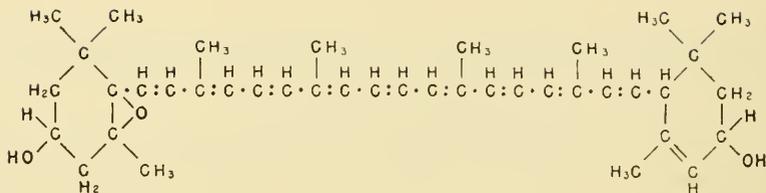
Karrer and Ishikawa³¹⁸ have synthesized helenien, as well as a series of the diesters of the lower fatty acids. The synthesis is readily accomplished by mixing the free lutein in pyridine with the appropriate acid anhydride or acid chloride. The esters are only slightly soluble in alcohol, but they dissolve readily in petroleum ether and in benzene. The following diesters of lutein have been prepared and characterized²⁵: lutein diacetate, C₄₄H₆₀O₄ (m.p., 170°C.); dipropionate, C₄₆H₆₄O₄ (yellow-red crystals melting at 138°C.); dibutyrate, C₄₈H₆₈O₄ (red platelets melting at 156°C.); di-*n*-valerate, C₅₀H₇₂O₄ (red-yellow platelets melting at 128°C.); di-*n*-caproate, C₅₂H₇₆O₄ (yellow-red platelets melting at 117°C.); diheptylate, C₅₄H₈₀O₄ (yellow-red platelets melting at 111°C.); dicaprylate, C₅₆H₈₄O₄ (yellow-red platelets melting at 108°C.); and dipalmitate, C₇₂H₁₁₆O₄ (red needles melting at 92°C.).

(b) *Lutein Epoxide*, C₄₀H₅₆O₃. Karrer and Jucker³¹⁹ have found that lutein is included among the carotenoids which may exist as epoxides. It may be obtained synthetically by oxidation of lutein diacetate with monoperphthalic acid.

³¹⁷ J. Tischer, *Z. physiol. Chem.*, **250**, 147-154 (1937).

³¹⁸ P. Karrer and S. Ishikawa, *Helv. Chim. Acta*, **13**, 709-713, 1099-1102 (1930).

³¹⁹ P. Karrer and E. Jucker, *Helv. Chim. Acta*, **28**, 300-315 (1945).



Lutein epoxide

The presence of an oxygen atom on the double bond of the β -ionone ring is indicated by several facts. In the first place, there is a shift in the absorption maximum of the epoxide of only 7 $m\mu$ from that of xanthophyll. If oxidation had occurred on one of the double bonds of the aliphatic side chain, an interference in the conjugation would have resulted, and a considerably greater alteration in absorption maximum in the ultraviolet might have been expected. Second, a dioxy- α -carotene, prepared earlier by Karrer *et al.*,¹⁸³ in which oxidation occurred on the 5,6 position of the β -ionone ring, had an absorption band at a wave length identical with that of lutein epoxide. The latter finding would seem to place the additional oxygen on the β - rather than on the α -ionone ring.

Lutein epoxide was found to separate in reddish yellow crystals (m.p., 192°C.) which are difficultly soluble in alcohol, but which dissolve easily in benzene. It possesses two active hydrogens as determined by the Zerewitinoff reaction; it absorbs 11 molecules of hydrogen on catalytic hydrogenation in glacial acetic acid. The maximum absorption in carbon disulfide was found³¹⁹ to be at 501.5 and 472 $m\mu$.

One of the most interesting properties of this epoxide is exhibited when it is treated with dilute acids. When hydrochloric acid is added to a methanol solution of lutein epoxide to make a 1% solution, an immediate shift of absorption maxima to shorter wave lengths occurs (479 and 450 $m\mu$). However, the new products cannot be crystallized from methanol. On the other hand, a similar shift of absorption maxima was also observed when the lutein epoxide was dissolved in chloroform; this change in position of the maxima was much greater than the usual difference of 7 to 8 $m\mu$ observed in chloroform. Karrer and Jucker³¹⁹ were able to crystallize two new compounds responsible for the lower absorption values from the chloroform solution; these had empirical formulas of C₄₀H₆₆O₃. They were identified as flavoxanthin and chrysanthemaxanthin; the latter appear to be optical isomers with different configurations on carbon atom 3 of the β -ionone ring. Their formation in chloroform is to be attributed to the small amount of hydrochloric acid present in this solvent, which brings about what is practically a catalytic conversion of lutein epoxide to these new compounds. The reaction is believed to be similar to that occurring in methanol when hydrochloric acid is added.

pigment was identical with xanthophyll. Karrer *et al.* succeeded in separating the pigment in pure form and in characterizing and naming it.^{233,324} The latter workers differentiated zeaxanthin from egg-yolk lutein. The formula for zeaxanthin has been given earlier (see page 555), where the justification for the structure assigned to it was discussed.

a. Occurrence. Zeaxanthin is largely confined to the plant kingdom, where it occurs in both free and esterified forms. It is present in the free state in yellow corn (*Zea mays*),³²⁵ as well as in the seeds of the *Euonymus europaeus* L. (spindle tree).^{112,113} This is also true in the case of rose hips (*Rosa canina*, *R. eglanteria*, and *R. damascena*),^{193,224} and in the flowers of *Senecio doricum* (leopard bane groundsel),¹⁰⁴ of *Crocus sativus* (saffron crocus),¹⁹⁸ and of *Viola tricolor* (pansy).²⁹⁹ Zeaxanthin has likewise been reported in the coneflower (*Rudbeckia speciosa Newmanni*).^{25,104}

Zeaxanthin is found in many fruits such as the *Solanum hendersoni* (Henderson Jerusalem cherry),⁵⁴ *Lycopersicon esculentum* (tomato),²²¹ *Diospyros costata* (persimmon),²⁵³ *Cucurbita pepo* L. (pumpkin),²⁶⁰ *Citrus aurantium* (Seville orange),²⁵⁵ and *Prunus persica* (peach).³⁰⁹

The esterified forms of zeaxanthin are found in the calices and berries of the *Physalis alkekengi* (winter ground-cherry) and *P. franchetti* (Chinese lantern),²⁷⁹ as well as in *Lycium halimifolium* (boxthorn berries),²⁸¹ where it is identified as physalien. It is also esterified in *Hippophaë rhamnoides* (sea buckthorn),³⁰¹ and in the pimienta or Japanese red pepper (*Capsicum frutescens japonicum*).³²⁶ Karrer *et al.*¹⁷² have likewise reported the esterified form of zeaxanthin in the berry of the red kaki or Japanese persimmon (*Diospyros kaki*).

It is not known whether the zeaxanthin present in the liver of the green edible water-frog (*Rana esculenta*)³¹⁶ is in the free form or appears as an ester. Chargaff³²⁷ has noted the presence of this carotenol in several types of microorganisms, *i.e.*, the cocci, *Sarcina aurantiaca* and *Staphylococcus pyogenes aureus*. Karrer and his co-workers²²⁹ found zeaxanthin as a constituent of the brown marine alga, *Halyseric (Dictyopteris) polypodioides*.

The presence of zeaxanthin in animal products must be regarded as adventitious, since it must originate in the ingested carotenol, at least as far as the higher animals are concerned. It has been demonstrated to be a component of human fat²⁵ and human liver.¹³⁵ It has been proved to be a component of the yolk of hen egg.²⁷⁴ Strain³²⁵ as well as Euler and Gard³²⁹

³²⁴ P. Karrer, H. Salomon, and H. Wehrli, *Helv. Chim. Acta*, **12**, 790-792 (1929).

³²⁵ J. C. Sadana and B. Ahmad, *Indian J. Med. Research*, **34**, 59-68 (1946); *Chem. Abst.*, **41**, 6602 (1947).

³²⁶ L. Zechmeister and L. v. Cholnoky, *Ann.*, **489**, 1-6 (1931).

³²⁷ E. Chargaff, *Compt. rend.*, **197**, 946-948 (1933).

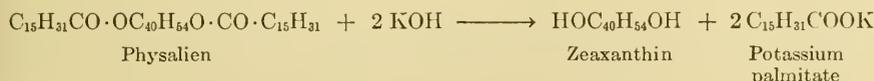
³²⁸ H. H. Strain, *Chromatographic Adsorption Analysis*, Interscience, New York, 1942.

³²⁹ H. v. Euler and U. Gard, *Arkiv Kemi Mineral. Geol.*, **B10**, No. 19, 1-6 (1931).

have prepared chromatographically pure zeaxanthin from the above source. This carotenol likewise occurs along with lutein in the feathers of *Serinus canarius* (wild canary).³¹⁵

b. Related Compounds. (a) *Physalien*, C₇₂H₁₁₆O₄. Physalien was first reported³³⁰ in the winter cherry (*Physalis alkekengi*) and also in the Chinese lantern plant (*Physalis francheti*). It was shown to be a dipalmitate of zeaxanthin.

The proof of the ester structure of physalien is based upon several facts. In the first place the molecular weight is about double that of carotene, and approaches 1000. Second, the melting point is about 100° higher than that of the leaf carotenoids. Finally, physalien does not follow the classical rule of solvent distribution between petroleum ether and dilute methanol for the oxygen-containing carotenoids, but rather follows the pattern of the polyene hydrocarbons, carotene and lycopene. On saponification, physalien gives rise to two molecules of palmitic acid and one of zeaxanthin²⁷⁸⁻²⁸¹ as follows:



Studies on the chemical nature of the carotenoid portion of physalien have shown it to be identical with zeaxanthin. Like zeaxanthin, it absorbs 11 molecules of hydrogen to give *perhydrophysalien* or perhydrozeaxanthin dipalmitic acid ester. All color is lost after 8 molecules of hydrogen have been absorbed.

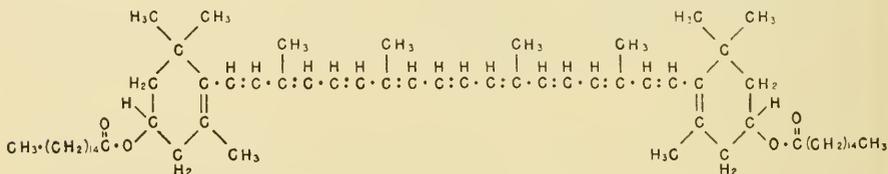
Besides being present in the berries and calices of the *Physalis alkekengi* and *P. francheti*, and *Lycium halimifolium* (box thorn, matrimony vine),²⁸¹ physalien has been reported by Winterstein and Ehrenberg⁵⁴ as present in the berries of *Lycium barbarum* (Barbary wolfberry), of *Solanum hendersoni* (Jerusalem cherry), and of *Asparagus officinalis* (asparagus berries). It has also been found as a component of the berry pigments of *Hippophaë rhamnoides* (sea buckthorn).³³¹

Physalien can be partially synthesized from zeaxanthin and palmitic acid.²⁷⁹ It can be prepared from the calices of the *Physalis*, in which the pigment usually comprises 0.9 to 1.8% of the dry weight. It crystallizes from a benzene-methanol mixture in long flat rods cut off obliquely at the ends, or in fine, undulating needles. When crystallized from a cyclohexane-ethanol solution, it forms long, flat, deep-red prisms. The ester melts at 98.5-99.5°C. It has an excellent solubility in carbon disulfide, benzene, chloroform, and carbon tetrachloride; it dissolves fairly well in ether, petroleum ether, hexane, and pyridine. It is soluble to a small extent in

³³⁰ R. Kuhn and W. v. Wiegand, *Helv. Chim. Acta*, 12, 499-506 (1929).

³³¹ P. Karrer and H. Wehrli, *Helv. Chim. Acta*, 13, 1104-1105 (1930).

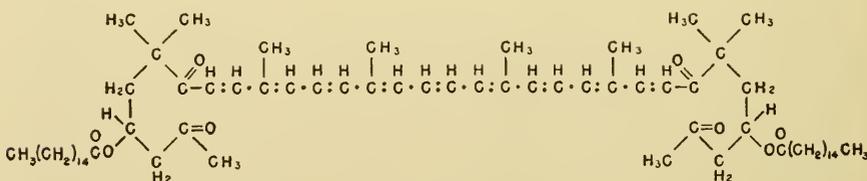
cyclohexane, acetic acid, and acetic anhydride, but only when these solvents are hot. Physalien is almost completely insoluble in ethanol and acetone. When dissolved in concentrated sulfuric acid, it develops a deep blue color.



Physalien (zeaxanthin dipalmitate)

In addition to the synthesis of physalien, a number of the di-esters and some mono-esters have been prepared by Karrer and several collaborators. The synthesis was accomplished by mixing the free zeaxanthin in pyridine with the appropriate acid anhydride or acid chloride; this was employed earlier by Karrer and Ishikawa³¹⁸ for the preparation of the lutein esters. The following zeaxanthin esters have been synthesized and characterized: zeaxanthin diacetate, $C_{44}H_{60}O_4$ (m.p., 154–155°C.)³³²; dipropionate, $C_{46}H_{64}O_4$ (m.p., 142°C.)¹⁰⁴; dibutyrate, $C_{48}H_{68}O_4$ (m.p., 132°C.)¹⁰⁴; di-*n*-valerate, $C_{50}H_{72}O_4$ (m.p., 125°C.)¹⁰⁴; di-*n*-caproate, $C_{52}H_{76}O_4$ (m.p., 117–118°C.)¹⁰⁴; di-*n*-caprylate, $C_{56}H_{84}O_4$ (m.p., 107°C.)¹⁰⁴; dilaurate, $C_{64}H_{100}O_4$ (m.p., 104°C.)²⁷⁹; and distearate, $C_{76}H_{124}O_4$ (m.p., 95°C.)²⁵ The monopalmitic ester of zeaxanthin ($C_{56}H_{86}O_4$) was prepared by partial saponification of physalien.²⁵² When crystallized from a benzene-ethanol mixture, it forms platelets which melt at 148°C. In addition to the several esters, zeaxanthin forms mono- and di-ethers. Dimethyl zeaxanthin ether ($C_{40}H_{54}(OCH_3)_2$) has been prepared³³³ as a crystalline product which melts at 175–176°C.

(b) *Physalienone*, $C_{72}H_{116}O_8$. When physalien is oxidized with chromic acid,¹⁸⁴ a tetraketone is formed which was found to have the structure³³⁴ given below. Physalienone forms fine, bushy needles. The melting point is 144–145°C.



Physalienone

(c) *Zeaxanthin Mono-epoxide*, *Antheraxanthin*, $C_{40}H_{56}O_3$. When zeaxanthin is oxidized with perphthalic acid, two oxidation products result,

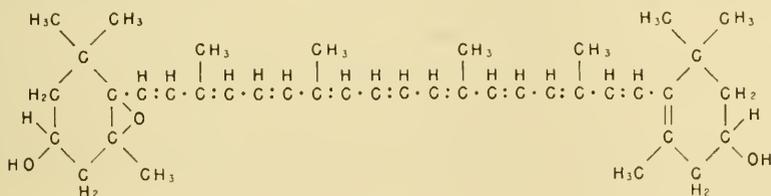
³³² P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 18, 477–480 (1935).

³³³ P. Karrer and T. Takahashi, *Helv. Chim. Acta*, 16, 1163–1165 (1933).

³³⁴ P. Karrer and W. Gugelmann, *Helv. Chim. Acta*, 20, 405–406 (1937).

zeaxanthin mono-epoxide and zeaxanthin di-epoxide, depending upon whether one or both β-ionone rings are attacked.³¹⁹

Zeaxanthin mono-epoxide was found by Karrer *et al.*³¹⁹ to be identical with *antheraxanthin*; the latter compound is a carotenoid which was isolated by Karrer and Oswald³³⁵ from the stamens of the tiger lily (*Lilium tigrinum*).



Antheraxanthin (zeaxanthin mono-epoxide)

(d) *Mutatoxanthin*, C₄₀H₅₆O₃. In the presence of halogen acids or chloroform, zeaxanthin mono-epoxide undergoes transformation similar to that of the lutein epoxides. Thus, the epoxide is converted to a product with an

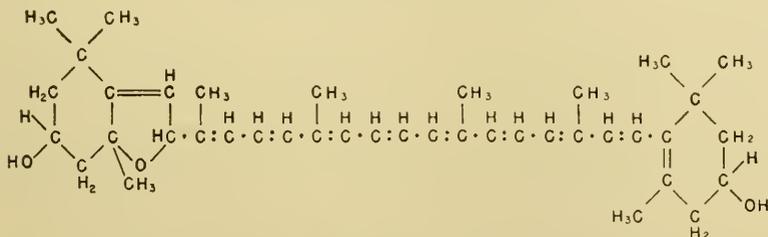
TABLE 5

COMPARISON OF PROPERTIES OF ANTHERAXANTHIN (ZEAXANTHIN MONO-EPOXIDE) AND MUTATOXANTHIN^a

Property	Antheraxanthin	Mutatoxanthin
Empirical formula.....	C ₄₀ H ₅₆ O ₃	C ₄₀ H ₅₆ O ₃
Number of double bonds.....	10	10
Number of conjugated double bonds.....	10	9
Number of hydroxyl groups.....	2	2
Number of ether-bound oxygens.....	1	1
Melting point, °C.....	205°	177°
Position in chromatogram.....	Upper	Lower
Absorption maxima (CS ₂), mμ.....	510, 475	488, 459, 431
Absorption maxima (C ₂ H ₅ OH), mμ.....	479, 449	457, 427
Reaction with conc. aq. HCl.....	Blue, fading rapidly	Blue, fading rapidly

^a P. Karrer and E. Jucker, *Helv. Chim. Acta*, 28, 300-315 (1945), p. 310.

absorption maximum 23 mμ lower. The compound so prepared was demonstrated by Karrer and Jucker³¹⁹ to be identical with the so-called *mutatoxanthin* which had been prepared earlier from violaxanthin.²⁹⁹



Mutatoxanthin

³³⁵ P. Karrer and A. Oswald, *Helv. Chim. Acta*, 18, 1303-1305 (1935).

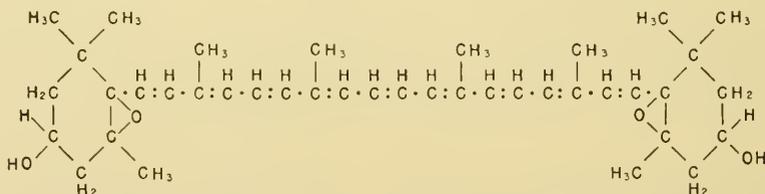
A comparison of the properties of antheraxanthin and mutatoxanthin is given in Table 5. Although mutatoxanthin might be expected to be an intermediate in the metabolism of zeaxanthin epoxide in plants, it has not as yet been identified among such pigments.

(e) *Zeaxanthin Di-epoxide, Violaxanthin, C₄₀H₅₆O₄*. Zeaxanthin di-epoxide can also be prepared by the oxidation of zeaxanthin with perchthalic acid. This compound has oxygens combined with both β -ionone rings in positions 5,6 and 5',6', respectively. Karrer and Jucker³¹⁹ found this compound to be identical with violaxanthin, which had been isolated frequently from many natural sources. The structure and properties of zeaxanthin di-epoxide are therefore discussed in the section on violaxanthin (see Section (6) below).

(6) *Violaxanthin*

Violaxanthin was first obtained from the petals of the yellow wild pansy (*Viola tricolor*).²⁹⁸ Obviously, it derives its name from this origin.

a. Structure. Violaxanthin has been known to have the empirical formula C₄₀H₅₆O₄, but it differs from taraxanthin, which has the same empirical formula, in giving a deep-blue color reaction with hydrochloric acid. Other oxygen-containing polyenes such as lutein, fucoxanthin, capsanthin, capsorubin, and azafrin give completely negative results with hydrochloric acid, while flavoxanthin responds with only a transitory reaction. The recent demonstration by Karrer and Jucker³¹⁹ that violaxanthin is actually zeaxanthin di-epoxide fits in with the earlier isolated facts about the nature of the molecule.



Violaxanthin (zeaxanthin di-epoxide)

Violaxanthin has only 9 double bonds.³³⁶ It is believed that two oxygens must be in the same position as in lutein (3 and 3'), which explains the formation of esters with fatty acid. Kuhn and Winterstein,²⁹⁸ on the basis of the Zerewitinoff reaction, believe that all four oxygens are associated with hydroxyl groups, although Karrer and Morf³³⁷ could detect only three active hydrogens. When violaxanthin is treated with lead

³³⁶ P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 19, 1024-1025 (1936).

³³⁷ P. Karrer and R. Morf, *Helv. Chim. Acta*, 14, 1044-1046 (1931).

tetraacetate by the Criegee method, it is not attacked; this indicates that the hydroxyls are not on adjoining carbon atoms.²⁸² Since on decomposition with permanganate violaxanthin yields α,α -dimethylsuccinic acid, just as do lutein and zeaxanthin, it is believed that it has a corresponding ring system.

Like other epoxides, violaxanthin is extremely susceptible to acid. In the presence of a low concentration of hydrochloric acid, or when dissolved in chloroform, it is converted to the difuranoid compound *auroxanthin*. For a discussion of this compound as well as for a comparison of the properties of violaxanthin with those of auroxanthin, see the next section (Section (7)). The conversion of violaxanthin to mutatoxanthin, which has also been observed, must involve a preliminary removal of one of the epoxide groups.

b. Occurrence. In addition to the yellow pansy, a number of other flowers contain violaxanthin, as well as lutein. These include *Tragopogon pratensis* (goat's beard), *Laburnum anagyroides* (golden chain), *Brassica Kaber* (*Sinapis officinalis*) (field mustard),¹⁰⁴ *Arbutus unedo* (strawberry tree),²⁵³ *Iris pseudacorus* (yellow iris),⁹⁵ and probably also *Ranunculus acris* (buttercup).²⁹⁷ Other flowers listed as sources of violaxanthin are the *Tulipa* spp. (yellow tulip),²¹⁶ *Ulex europaeus* (furze),²¹⁶ *Calendula officinalis* (calendula),⁹⁹ *Tagetes grandiflora* (giant marigold),²⁷⁴ *Tussilago farfara* (coltsfoot).³³³ Violaxanthin has also been found with taraxanthin in the dandelion (*Taraxacum officinale*).²⁹⁴ The presence of violaxanthin in the flowers of the *Crepis aurea* (orange hawk'sbeard) and of the birdsfoot trefoil, *Lotus corniculatus* (yellow cat's clover) has recently been noted.³⁰¹ Kuhn and co-workers²⁷⁴ have demonstrated the presence of violaxanthin in the green leaves of *Aesculus hippocastanum* (horsechestnut).

Fruits, likewise, sometimes contain violaxanthin. This is true of the fruit pulp of the giant squash (gourd) (*Cucurbita maxima*)³⁰² as well as of the *Citrus poonensis* and the papaya or paw paw (*Carica papaya*).^{250,254} Other fruit sources include the Seville orange (*Citrus aurantium*)^{339,340} and the persimmon (*Diospyros costata*).²⁵³

(7) *Auroxanthin*

Karrer and Rutschmann³⁴¹ observed a new pigment on chromatographic separation of the carotenoids in the wild pansy (*Viola tricolor*). They called this substance "auroxanthin" because of its golden yellow crystals. Although it was first found in the pansy, it has been observed in most instances where violaxanthin occurs.

The nature of auroxanthin became evident when Karrer and Jucker³¹⁹ proved it to be the difuranoid derivative of violaxanthin. As indicated

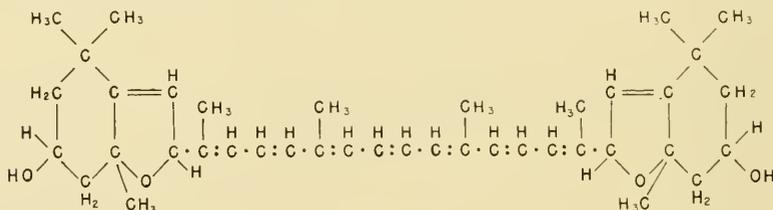
³³³ P. Karrer and R. Morf, *Helv. Chim. Acta*, 15, 863-864 (1932).

³³⁹ L. Zechmeister and P. Tuzson, *Naturwissenschaften*, 19, 307 (1931).

³⁴⁰ P. G. F. Vermast, *Naturwissenschaften*, 19, 442-443 (1931).

³⁴¹ P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, 25, 1624-1627 (1942).

earlier, when violaxanthin comes in contact with concentrated hydrochloric acid, or when it is dissolved in chloroform, the properties of the substance are immediately changed and auroxanthin is formed. All the



Auroxanthin

properties of auroxanthin correspond with those expected for a compound having two furanoid rings. The correctness of this structure is indicated by the fact that natural and partially synthesized auroxanthin have been shown to be identical.³⁴²

A comparison of the properties of violaxanthin and auroxanthin is given in Table 6.

TABLE 6
COMPARISON OF PROPERTIES OF VIOLAXANTHIN AND AUROXANTHIN^a

Property	Violaxanthin	Auroxanthin
Empirical formula.....	C ₄₀ H ₅₆ O ₄	C ₄₀ H ₅₆ O ₄
Number of double bonds.....	9	9
Number of conjugated double bonds...	9	7
Hydroxyl groups.....	2	2
Ether-bound oxygen.....	2	2
Melting point, °C.....	200°	203°
Position in chromatogram.....	Lower	Upper
Absorption maxima (CS ₂), mμ.....	502, 469, 440	454, 423
Absorption maxima (C ₂ H ₅ OH), mμ....	471.5, 442.5, 417	428, 403, 382
Reaction with 20% aq. HCl.....	Deep-blue (stable)	Deep-blue (stable)

^a P. Karrer and E. Jucker, *Helv. Chim. Acta*, 28, 300-315 (1945).

(8) Flavoxanthin and Chrysanthemaxanthin

Flavoxanthin is the lightest yellow carotenoid pigment known. It was discovered by Kuhn and Brockmann,²⁹⁷ who prepared it from buttercup petals (*Ranunculus acris*). It is apparently also present in the Spring groundsel (*Senecio vernalis*), but it has not been reported to be widely distributed in nature. However, it has been prepared from green leaves by Strain.^{323,343} Another carotenoid of similar nature which is absent from green leaves has been isolated by Strain¹²⁴ from carrot roots. The so-called

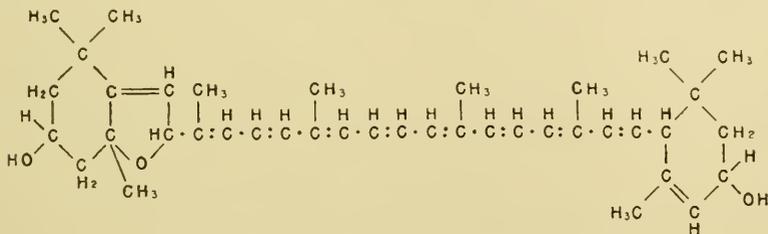
³⁴² P. Karrer, E. Jucker, and J. Rutschmann, *Helv. Chim. Acta*, 28, 1156-1157 (1945).

³⁴³ H. A. Spoehr, J. H. C. Smith, H. H. Strain, and H. W. Milner, *Carnegie Inst. Wash. Yearbook*, No. 35, 198-208 (1936).

loxanthin,¹ isolated from the leaves of *Anacharis canadensis* (flowering Canada waterweed),³²¹ is apparently isomeric with flavoxanthin.³⁴⁴ Of the mixed pigments from the buttercup, the principal component is lutein, while flavoxanthin, taraxanthin, possibly violaxanthin, and another unknown pigment comprise the rest of the mixture. About one-half of the pigment is combined in ester linkage as a colored fat.

Chrysanthemaxanthin was first isolated from the winter aster by Karrer and Jucker³⁰⁰ in 1943. The new phytoanthin was later isolated by these investigators¹⁰⁶ from *Cytisus scoparius* (Scotch broom) for further study. The following year it was partially synthesized and its structure was elucidated as a result of this investigation.³¹⁹

The structure of flavoxanthin, also, has recently been elucidated as a result of its synthesis from lutein epoxide by Karrer and Jucker.³¹⁹ This transformation was brought about in chloroform. It was found by chromatographic separation that two isomeric products were present—flavoxanthin and chrysanthemaxanthin. Both possess the same empirical formula, C₄₀H₅₆O₃, which is the same as that of lutein epoxide. The structural formula which is considered most probable for these compounds is given here. Strain³⁴⁵ found that violaxanthin from leaves of 50 plants rang-



Flavoxanthin or chrysanthemaxanthin

ing from ferns to angiosperms, and from the pansy (*Viola tricolor*), yielded flavoxanthin and auroxanthin on treatment with acids.

The properties of flavoxanthin and chrysanthemaxanthin are summarized in Table 7.

The identity of the synthetic products with the corresponding pigments isolated from natural sources is indicated by their specific rotations. Thus, the partially synthetic flavoxanthin was found³⁴² to have a specific rotation of 180–190°C., which agrees with a value of 190°C. reported by Kuhn and Brockmann²⁹⁷ for the natural product. The latter workers isolated 110 mg. of crude flavoxanthin from one kilogram of dried petals of the buttercup (*Ranunculus acris*), from which 40 mg. of pure pigment were prepared.²⁹⁷ The partially synthetic and the natural chrysanthemaxanthin had similar optical rotations which could not be differentiated from that of flavoxanthin.³⁴²

³⁴⁴ G. MacKinney, *Ann. Rev. Biochem.*, 9, 459–490 (1940).

³⁴⁵ H. H. Strain, *J. Am. Chem. Soc.*, 70, 1672 (1948).

TABLE 7
COMPARISON OF PROPERTIES OF FLAVOXANTHIN AND CHRYSANTHEMAXANTHIN^a

Property	Flavoxanthin	Chrysanthemaxanthin
Empirical formula.....	C ₄₀ H ₅₆ O ₃	C ₄₀ H ₅₆ O ₃
Number of double bonds.....	10	10
Number of hydroxyl groups.....	2	2
Number of ether-bound oxygens.....	1	1
Melting point, °C.....	180°	184-185°
Position in chromatogram.....	Upper	Lower
Absorption maxima (CS ₂), mμ.....	479, 449	479, 449
Absorption maxima (C ₂ H ₅ OH), mμ....	450, 421	450, 421
Reaction with conc. aq. HCl.....	Blue (unstable)	Colorless

^a P. Karrer and E. Jucker, *Helv. Chim. Acta*, 28, 300-315 (1945), p. 305.

(9) *Taraxanthin*

Taraxanthin, which is an isomer of violaxanthin, was first discovered in dandelion flowers (*Taraxacum officinale*) by Kuhn and Lederer.²⁹⁴ In harmony with the polyenes containing two ring structures, taraxanthin absorbs 11 molecules of hydrogen on catalytic hydrogenation. The Zerewitinoff reaction shows the presence of three to four active hydrogens, which must be attached in hydroxyl groups. Taraxanthin and violaxanthin differ in absorption spectra from lutein and zeaxanthin; this is interpreted to mean that the chromophore system of conjugated double bonds must be different. The close similarity in spectra between taraxanthin and violaxanthin must indicate an identical arrangement of the double bonds; the only variation, which is shown by differences in the reaction with hydrochloric acid and in optical activity, must be ascribed to the differences in the positions of the hydroxyl groups. The empirical formula for taraxanthin is C₄₀H₅₆O₄.

Taraxanthin occurs free in dandelion flowers; it is also present in the esterified form in the flowers of the coltsfoot (*Tussilago farfara*),³³⁸ as well as in those of the Fall hawkbit (*Leontodon autumnalis*),²⁹⁶ the buttercup (*Ranunculus acris*),²⁹⁹ and the balsam, touch-me-not, or yellow snapweed (*Impatiens noli me tangere*).²⁹⁶ In the last case, the pigment consists almost entirely of taraxanthin. According to Zechmeister and Tuzson,²⁶¹ a fraction of the pigment of the sunflower (*Helianthus annuus*) consists of the same oxygen-containing polyene. Taraxanthin occurs in the rose hips of *Rosa canina*, *R. eglanteria*, and *R. damascena* Mill.,^{193,224} and likewise in the flowers of *Ulex europaeus* (furze, gorse).¹⁰⁰ Strain³⁴⁵ found that the leaves of 11 species of cycads contained taraxanthin which was identical with that obtained from dandelions. The presence of this pigment has also been

demonstrated in a few species of red marine algae,^{95,346} including *Ceramium rubrum* and *Dilsea edulis*. However, Carter *et al.*³⁴⁷ doubt the presence of taraxanthin in these algae, and suggest that the pigment found was neolutein A or B. Taraxanthin has been observed in green algae.⁹⁵ Sørensen³⁴⁸ has reported the presence of this carotenol in the liver of the sea devil fish or angler (*Lophius piscatorius*).

(10) Fucoxanthin

As early as 1867, Rosanoff³⁴⁹ postulated that brown algae contain a yellow pigment in addition to chlorophyll. This coloring matter was first observed by Kraus and Millardet,³⁵⁰ who named it "phycoxanthin." Sorby³⁵¹ was able to prove that olive-brown algae contained three different pigments, which he called orange xanthophyll, lichnoxanthin, and fucoxanthin. These pigments were further characterized chromatographically by Tswett as carotene, fucoxanthophyll, and fucoxanthin.³⁵² Willstätter and Page³⁵³ were the first to isolate fucoxanthin in crystalline form, and to pursue investigations of the pure pigment. The structure of this polyene has only recently been established with reasonable certainty. It is chiefly characterized by its high oxygen content.

a. Structure. It has been shown by Heilbron and Phipers³⁵⁴ that fucoxanthin has an empirical formula of C₄₀H₆₀O₆ instead of C₄₀H₅₆O₆, as originally postulated by Karrer *et al.*⁵⁰ The latter workers also proved that it is optically inactive, in contradistinction to the earlier view that it possesses optical activity. The brown algae pigments take up nine molecules of hydrogen on catalytic hydrogenation, proving that fucoxanthin contains two less double bonds than does carotene. The Zerewitinoff value indicates between four and five active hydrogens; there are at least four hydroxyl groups, while the combinations of the remaining oxygen atoms are probably as ketones. Esters containing only two fatty acids have been prepared. On decomposition with alkaline permanganate, dimethylmalonic acid, HOOC·C(CH₃)₂·COOH, alone has been isolated. Karrer *et al.*⁵⁰ consider that the unusually high number of hydroxyl groups of fucoxanthin prevents the formation of dicarboxylic acids higher than

³⁴⁶ H. Kylin, *Kgl. Fysiograf. Sällskap. Lund. Förrh.*, 9, 213-231 (1939).

³⁴⁷ P. W. Carter, I. M. Heilbron, and B. Lythgoe, *Proc. Roy. Soc. London*, B128, 82-109 (1939).

³⁴⁸ N. A. Sørensen, *Chem. Zentr.*, 1934, II, 682. Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 330.

³⁴⁹ S. Rosanoff, *Mem. Soc. sci. nat. Cherbourg*, 13, 195 (1867). Cited by P. Karrer and E. Jucker, *Carotinoide*, p. 318.

³⁵⁰ G. Kraus and A. Millardet, *Compt. rend.*, 66, 505-508 (1868); 68, 462-466 (1869).

³⁵¹ H. C. Sorby, *Proc. Roy. Soc. London*, 21, 442-483 (1873).

³⁵² M. Tswett, *Ber. deut. botan. Ges.*, 24, 235-244 (1906).

³⁵³ R. Willstätter and H. J. Page, *Ann.*, 404, 237-271 (1914).

³⁵⁴ I. M. Heilbron and R. F. Phipers, *Biochem. J.*, 29, 1369-1375 (1935).

TABLE 8. THE ALGAE CAROTENOIDS DETERMINED BY THE CAPILLARY ANALYTICAL METHOD^a

Carotenoid type	Species of algae					
	Green algae	Rhodophyceae	Cyanophyceae	Phaeophyceae	Diatomaceae	Peridineeae
Carotene	+	+	+	+	+	+
Calorhodin	-	-	+	-	-	-
Phylloredin	+	+	-	-	-	-
Myxorhodin α	-	-	+	-	-	-
Myxorhodin β	-	-	+	-	-	-
Phylloxanthin	+	-	-	+	+	+
Fucoxanthin α	-	-	-	+	+	-
Fucoxanthin β	-	-	-	+	+	-
Xanthophyll	+	+	-	+	+	-
Peridinin	-	-	-	-	-	+

^a Table from L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, p. 211. Cited from H. Kylin, *Z. physiol. Chem.*, 166, 39-77 (1927), p. 75.

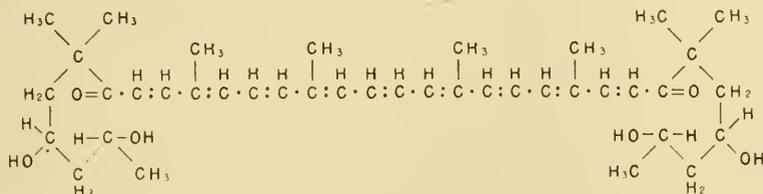
TABLE 9. THE CAROTENOID AND CHLOROPHYLL CONTENTS OF ALGAE^{a,b}

Genus	Per kg. fresh algae				Per kg. dried algae				Molecular ratios	
	1	2	3	4	1	2	3	4	Chlorophyll: carotene	Carotene: lutein: fucoxanthin
<i>Fucus</i> (rockweed)	0.503	0.169	0.089	0.087	1.765	0.593	0.312	0.305	0.95:1	1.08:1:1.75
<i>Dictyotaceae</i> (brown algae)	0.840	0.250	0.057	0.063	—	—	—	—	1.20:1	0.77:1:3.60
<i>Laminaria</i> (bladekelp)	0.185	0.081	0.006	0.038	1.202	0.528	0.038	0.247	1.07:1	0.16:1:1.92

^a Data of R. Willstätter and H. J. Page, *Ann.*, 404, 237-271 (1914).

^b 1, chlorophyll; 2, fucoxanthin; 3, carotene; 4, xanthophyll.

malonic acid. On energetic oxidation with permanganate or chromic acid, 4.5 to 7 molecules of acetic acid are formed. On exhaustive hydrogenation, fucoxanthin loses four oxygen atoms and gives a perhydrocompound³⁵⁴ with the composition C₄₀H₇₈O₂. The following structural formula has been proposed by Heilbron and Phipers,³⁵⁴ but Smith³⁵⁵ does not believe that the evidence is sufficient to prove the case.



Fucoxanthin (?)

b. Occurrence. As the name implies, the chief sources of fucoxanthin are the algae. According to Kylin,³⁵⁶ fucoxanthin does not occur in all algae (Table 8). The brown algae were shown by Willstätter and Page³⁵³ to have a considerably higher proportion of fucoxanthin than of carotene or of lutein (Table 9).

While the chlorophyll of the higher plants consists of both the a and b types, it is associated almost exclusively with carotene and lutein. In the case of the brown algae chlorophyll a is the only form of this pigment present, and the chief carotenoid is fucoxanthin. Moreover, the ratio of green to yellow pigments is about 1:1 in the brown algae, instead of 3:5 as in the higher plants.

11. Rhodoxanthin

According to Karrer and Jucker,²⁵ Montéverdé noted a new pigment in the red-brown leaves of *Potamogeton natans* (floating-leaf pondweed), in 1893, which was later found in various conifers by Tswett,³⁵⁷ and which was designated as "thujorhodin." In 1912-1913, the pigment was isolated by Montéverdé and Lýubimenko^{358,359} in a crystalline state. After studies by Prát³⁶⁰ and Lippmaa,^{361,362} Kuhn and Brockmann³⁶³ isolated 200 mg. of the

³⁵⁵ J. H. C. Smith, *Ann. Rev. Biochem.*, **6**, 489-512 (1937).

³⁵⁶ H. Kylin, *Z. physiol. Chem.*, **166**, 39-77 (1927).

³⁵⁷ M. Tswett, *Compt. rend.*, **152**, 788-789 (1911).

³⁵⁸ N. A. Montéverdé and V. N. Lýubimenko, *Bull. acad. imp. sci. St. Petersburg*, **VI**, 609-630 (1912); *Chem. Abst.*, **6**, 2092-2093 (1912). Cited by L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, p. 222.

³⁵⁹ N. A. Montéverdé and V. N. Lýubimenko, *Bull. acad. imp. sci. St. Petersburg*, **VII**, 7, 1105-1124 (1913); *Chem. Abst.*, **8**, 913 (1914). Cited by L. Zechmeister, *Die Carotinoide*, p. 222, and by P. Karrer and E. Jucker, *Carotinoide*, p. 246.

³⁶⁰ S. Prát, *Biochem. Z.*, **152**, 495-497 (1924).

³⁶¹ T. Lippmaa, *Compt. rend.*, **182**, 867-868 (1926).

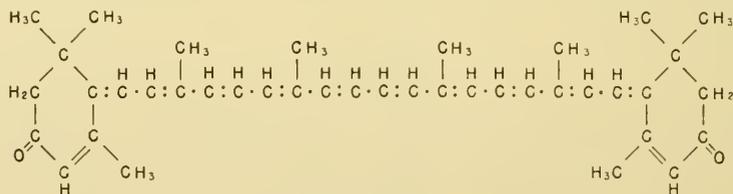
³⁶² T. Lippmaa, *Compt. rend.*, **182**, 1040-1042 (1926).

³⁶³ R. Kuhn and H. Brockmann, *Ber.*, **66**, 828-841 (1933).

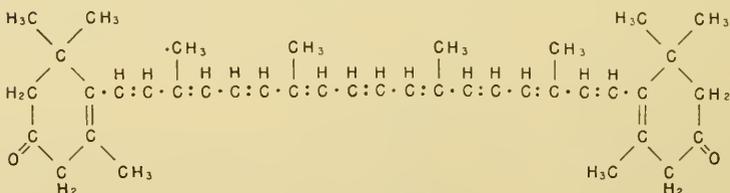
pure pigment from 30 kg. of the ripe fruit of the yew (*Taxus baccata*). It was present in the seed coverings.

a. Structure. Rhodoxanthin has been shown to have an empirical formula of $C_{40}H_{50}O_2$. The absorption of the longer wave lengths by rhodoxanthin solutions differs from that of the other carotenoids; this fact must be ascribed to variations in structure. It is impossible to form an ester of rhodoxanthin; on the other hand, it does form a well-crystallized dioxime with hydroxylamine, with the empirical formula $C_{40}H_{52}O_2N_2$. Since such polyene aldehydes as lycopene do not react with hydroxylamine, it must be assumed that rhodoxanthin has a ketone group. Both carbonyl groups must be in conjugation with the double bond system, since a considerable spectroscopic difference obtains between the pigment and its dioxime.

On catalytic hydrogenation, rhodoxanthin adds 12 molecules of hydrogen, indicating that it has a paired number of conjugated double bonds. In fact, if rhodoxanthin is further hydrogenated, it takes up two additional molecules of hydrogen, which are involved in the reduction of the ketones to secondary alcohols. It is therefore evident that rhodoxanthin is the most highly unsaturated of the carotenoids, since it will take up a total of 14 molecules of hydrogen. The structure postulated by Kuhn and Brockmann³⁶³ is supported by the fact that, on reduction to dihydrorhodoxanthin, the resulting compound is optically similar to β -carotene and zeaxanthin. This argues in favor of the identity of their conjugated double bond systems.



Rhodoxanthin



Dihydrorhodoxanthin

b. Occurrence. As noted earlier, one of the chief sources of rhodoxanthin is in the seed hulls of the yew (*Taxus baccata*). It has also been found in the reddish brown leaves of *Potamogeton natans* (floating-leaf pondweed),²⁵ as well as in leaf extracts of the moss-like herb *Selaginella*, and the

conifers *Taxus* (yew), and *Gnetum* (joint fir), from which Montéverdé and Lýubimenko^{358, 359} were able to prepare crystals. Tswett³⁵⁷ separated this pigment chromatographically from a series of plants, including *Taxus baccata* (English yew), the cypresses, *Cupressus nainocki* and *Chamaecypara* (*Retinispora*) *pisifera plumosa* (plume false cypress), and the eastern cedar (*Juniperus virginiana*). It was named *thujorhodin* because of its presence in the *Thuja orientalis* L. (Oriental arborvitae), whose leaves turn red after exposure to frost.

12. Capsanthin

As early as 1817, Braconnot³⁶⁴ carried out studies on the pigment of the paprika. Thudichum³³ was the first to recognize that this coloring matter is related to the carotenoids, and this theory was later substantiated by others.^{365, 366} Although a number of workers found that the spectroscopic absorption corresponded to that of lycopene,^{305, 359, 367} the proof that it was a new specific substance was established when Zechmeister and Cholnoky³⁶⁸ succeeded in crystallizing the pigment from the paprika (*Capsicum frutescens*). They chose the name *capsanthin* for this new pigment. Capsanthin appears to be formed when the green pods develop a strong red coloration. The biosynthesis of capsanthin results from the interaction of some precursor with oxygen.

A number of other polyene pigments appear to be associated with capsanthin. These include capsorubin and the carotenols, lutein, cryptoxanthin, and zeaxanthin. Capsanthin itself is an alcohol. In paprika, neither capsanthin nor the other carotenols occur as free alcohols, but they are present as the esters, since they cannot be extracted without saponification. Myristic, palmitic, stearic, oleic, and carnaubic acids are the chief acids with which the carotenols are esterified in the fruit. It is therefore evident that the polyene esters of the capsicum fruit do not form a single chemical compound but a mixture of a number of different esters of each of a series of carotenols. These have been referred to by Zechmeister²³ as colored fats; they are discussed at length in Chapter IV.

a. Structure. Zechmeister and Cholnoky³⁶⁸ were the first to demonstrate that the red pigment of capsicum fruit (*Capsicum frutescens* L.) is not a hydrocarbon. Although their first empirical formula was given as C₃₄H₄₈O₃, it was later revised to C₃₅H₅₀O₃,³⁶⁹ and finally again changed to

³⁶⁴ H. Braconnot, *Ann. chim.* [2], 6, 122-141 (1817).

³⁶⁵ T. Pabst, *Arch. Pharm.*, 230, 108-134 (1892).

³⁶⁶ F. G. Kohl, *Untersuchungen über das Carotin und seine physiologische Bedeutung in Pflanzen*, Borntraeger, Leipzig, 1902. Cited by P. Karrer and E. Jucker, *Carotinoide*. Birkhäuser, Basle, 1948, p. 245.

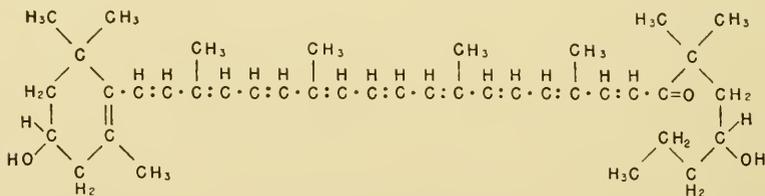
³⁶⁷ A. Tschirch, *Ber. deut. botan. Ges.*, 22, 414-439 (1904).

³⁶⁸ L. Zechmeister and L. v. Cholnoky, *Ann.*, 454, 54-71 (1927).

³⁶⁹ L. Zechmeister and L. v. Cholnoky, *Ann.*, 487, 197-213 (1931).

$C_{40}H_{58}O_3$,²⁵⁸ which is believed to represent the correct composition. It has been demonstrated by catalytic hydrogenation that there are ten double bonds; five acetic acid molecules also originate on strenuous oxidation²³ according to the procedure of Kuhn and L'Orsa.⁷⁰ Of the oxygen atoms, two occur in hydroxyl groups, as determined by the esterification reaction and the Zerewitinoff test. Although this carotenol will not react with hydroxylamine, as would be expected if the third oxygen were present in a carbonyl group, there is considerable evidence that a ketone group does exist. Kuhn and Brockmann⁸⁴ found that the diketone semi- β -carotenone only partially reacts with hydroxylamine, as it gives a monoxime instead of the expected dioxime. This latter compound exhibits a spectroscopic picture similar to that of capsanthin, which might suggest that a carbonyl group is present in the latter. The presence of the ketone group was proved by the demonstration that esterification of the reduced capsanthin resulted not in two but in three acetyl groups entering into combination. This must indicate that the unreduced pigment is a dihydroxy-ketone.

Since capsanthin possesses no biological activity, it would seem highly improbable that it contains an unsubstituted β -ionone ring. Presumably one hydroxyl occurs there, and it probably occupies the same position on the ionone ring as in zeaxanthin, with which capsanthin is frequently associated. On decomposition of capsanthin with permanganate, both α , α -dimethylsuccinic acid and dimethylmalonic acid are formed, but no acetone results.⁵⁰ Zechmeister and Cholnoky^{370,371} have proposed the following formula for capsanthin:



Capsanthin

b. Occurrence. Capsanthin occurs in a number of plants belonging to the *Solanaceae*. The principal source is the paprika grown in Hungary and Spain (*Capsicum frutescens*) from which its name is derived. The paprika is the same as the South American Perfection pimento.³⁷² Capsanthin also occurs in another pepper, the Japanese chili (*Capsicum frutescens japonicum*).³²⁶ The presence of capsanthin in the anthers of the tiger lily (*Lilium tigrinum*), in conjunction with antheraxanthin, was demonstrated by Karrer and Oswald.³³⁵ In all these cases, the capsanthin

³⁷⁰ L. Zechmeister and L. v. Cholnoky, *Ann.*, 516, 30-45 (1935).

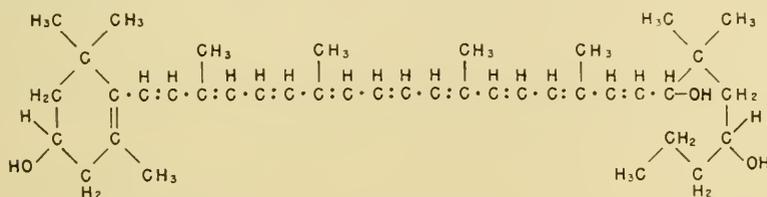
³⁷¹ L. Zechmeister and L. v. Cholnoky, *Ann.*, 523, 101-118 (1936).

³⁷² W. L. Brown, *J. Biol. Chem.*, 110, 91-94 (1935).

occurs in the esterified form. The presence of this carotenoid is not limited to the plant kingdom, as is indicated by the fact that it has been isolated from human fat.¹³⁴

c. Related Compounds. (a) *Perhydrocapsanthin*, C₄₀H₇₈O₃. When capsanthin is reduced with platinum, the pigment absorbs ten molecules of hydrogen, and changes to a thick, colorless oil. This product is more soluble in organic solvents than is the parent carotenoid. The carbonyl group is retained in perhydrocapsanthin; however, if this compound is further treated with sodium and alcohol, the carbonyl group is reduced, and the completely hydrogenated triol, C₄₀H₈₀O₃, results.³⁷⁰

(b) *Capsanthol*, C₄₀H₆₀O₃. When capsanthin is reduced with aluminum isopropoxide in isopropanol, capsanthol is formed.³⁷³ It can be purified through adsorption on calcium hydroxide from a benzene solution. When crystallized from ethanol, it forms brown-red leaflets which appear yellow under the microscope. It melts at 175–176°C. It is difficultly soluble in boiling alcohol.



(c) *Capsanthin Diesters*. Capsanthin diacetate, C₄₄H₆₂O₅, is prepared by treating capsanthin in pyridine solution with acetyl chloride.³⁶⁹ It can be purified chromatographically on calcium hydroxide; it crystallizes from methanol in platelets which melt at 146.5°C. It is very soluble in chloroform, diethyl ether, carbon disulfide, and benzene, and somewhat less so in methanol. When it is partitioned between methanol and petroleum ether, the ester passes quantitatively into the upper layer (petroleum ether). The following diesters are synthesized by an analogous procedure:

Capsanthin dipropionate, C₄₆H₆₆O₅ (m.p., 140°C.).

Capsanthin dicaprate, C₆₀H₉₄O₅ (violet-stippled red plates melting at 109°C.).^{258, 369}

Capsanthin dimyristate, C₆₈H₁₁₀O₅ (red needles melting at 88°C.).

Capsanthin dipalmitate, C₇₂H₁₁₈O₅ (Bordeaux red crystals melting at 95°C.).^{258, 374}

Capsanthin distearate, C₇₆H₁₂₆O₅ (m.p., 84°C.).

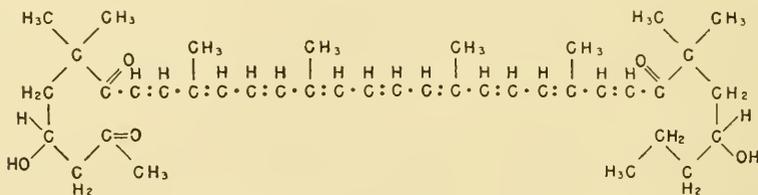
Capsanthin dibenzoate, C₅₄H₆₆O₅ (m.p., 121–122°C.).

(d) *Capsanthinone*, C₄₀H₅₈O₅. When capsanthin diacetate is oxidized with chromic acid, the triketone capsanthinone results.³⁷¹ Capsanthinone diacetate crystallizes from a benzene-hexane mixture in needles having a

³⁷³ P. Karrer and H. Hübner, *Helv. Chim. Acta*, 19, 474–479 (1936).

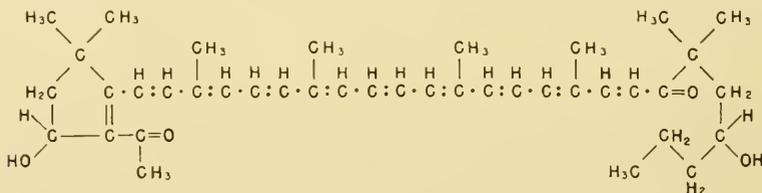
³⁷⁴ L. Zehmeister and L. v. Cholnoky, *Ann.*, 543, 248–257 (1940).

metallic luster, which melt at 123–124°C. The diacetate is hypophasic. It is quite soluble in ethanol, diethyl ether, benzene, and carbon disulfide, less so in acetone, and it is practically insoluble in hexane. When concentrated hydrochloric acid is added to an ethereal solution, it assumes a deep blue color.



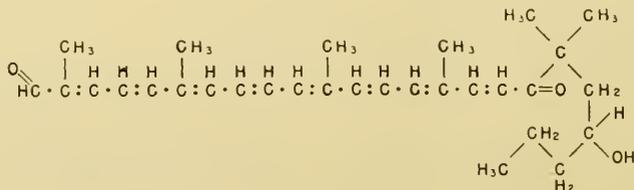
Capsanthinone

(e) *Anhydrocapsanthinone*, C₄₀H₅₆O₄. Anhydrocapsanthinone can be synthesized from capsanthinone diacetate by the removal of one molecule of water,³⁷¹ using a reaction analogous to that employed for the preparation of anhydro-β-semicarotenone from semi-β-carotenone.⁸² It can be separated from methanol in small red crystals which do not have a sharp melting point.²⁵ When partitioned between methanol and petroleum ether, the pigment passes into the lower layer (methanol).

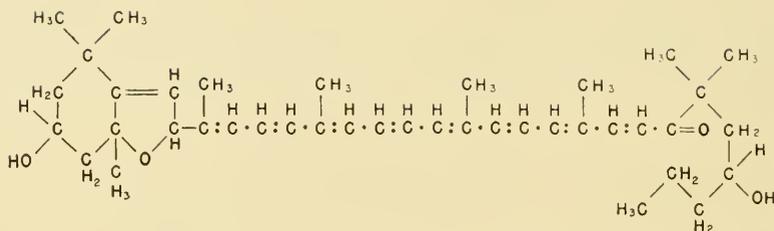


Anhydrocapsanthinone

(f) *Capsanthylal*, C₃₀H₄₂O₃. This aldehyde originates when capsanthin diacetate is oxidized with chromic acid, using an excess of the oxidizing agent.³⁷¹ Capsanthylal crystallizes from 80% methanol in star-shaped needles which melt at 127°C. It is quite soluble in benzene, ethanol, and carbon disulfide, but poorly so in petroleum ether. The aldehyde can be identified by converting it to the monoxime, C₃₀H₄₅O₃N, which melts at 184°C.



Capsanthylal

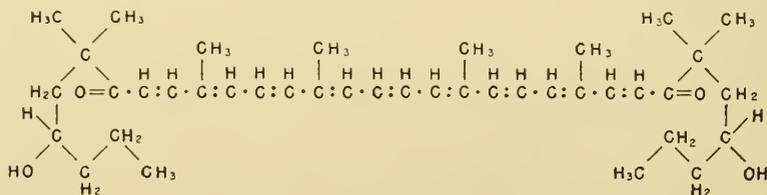


Capsochrome

The epoxide, capsochrome, and capsanthin are readily distinguished from each other by their absorption spectra. The following are the absorption peaks in $m\mu$, using carbon disulfide as the solvent: capsanthin, 542, 503; capsanthin epoxide, 534, 499; capsochrome, 513, 482.

(13) Capsorubin

In 1930, Zechmeister and Cholnoky,²⁵⁸ by the use of a chromatographic separation, isolated a second pigment from *Capsicum frutescens* (paprika), in addition to capsanthin. This violet-red chromogen was named "capsorubin." To date it has been found only in the paprika. Capsorubin has nine double bonds instead of the ten found in capsanthin; there are two alcohol groups and four methyl side chains and no isopropylidene groups. Apparently it is a diketone, since it has four oxygens; it has an absorption spectrum which compares quite closely with that of bixin dialdehyde. The marked difference between the positions of the absorption maxima in petroleum ether and in alcohol also argues in favor of the diketone structure. The empirical formula of capsorubin is $C_{40}H_{60}O_4$. On the basis of these data, capsorubin has been assigned the structure shown here.^{355, 370}



Capsorubin

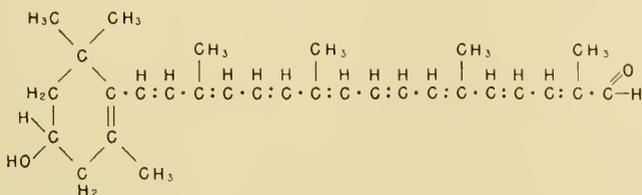
(14) β -Citrawin

Zechmeister and Tuzson^{255, 375} first noted the presence of an unknown pigment in Seville orange peels (*Citrus aurantium*) in addition to carotene, cryptoxanthin, zeaxanthin, lutein, and violaxanthin. They called it

³⁷⁵ L. Zechmeister and P. Tuzson, *Ber.*, 70, 1966-1969 (1937).

"citraurin," but Karrer *et al.*^{165,376} have suggested that it be referred to as " β -citraurin" to differentiate it from a somewhat similar product which is called α -citraurin. β -Citraurin has been found only in the orange peel.

β -Citraurin has an empirical formula of C₃₀H₄₀O₂. It is an aldehyde. Karrer and Solmssen¹⁶⁵ suggested that β -citraurin is 3-oxy- β -apo-2-carotenal, and this hypothesis was later proved to be correct.^{376,377} This compound is formed when zeaxanthin is hydrolyzed with strong alkali or when lutein is oxidized with permanganate.³⁷⁶ In the latter case, α -citraurin also results. König³⁷⁸ likewise obtained β -citraurin from capsanthin by permanganate oxidation.



β -Citraurin

β -Citraurin separates from a benzene-ligroin mixture as thin orange-to-yellow plates which appear almost colorless when viewed under the microscope. It melts at 147°C. It is readily soluble in acetone, ethanol, benzene, and carbon disulfide. β -Citraurin forms an oxime, C₃₀H₄₁O₂N, when treated with hydroxylamine. This melts at 188°C. It can also be characterized as the semicarbazone (C₃₁H₄₃O₂N₃). β -Citraurin semicarbazone crystallizes from benzene as reddish brown platelets which melt at approximately 190°C.

(15) *Myxoxanthin*

Heilbron, Lythgoe, and Phipers³⁷⁹ separated an unknown pigment from the fresh-water, blue-green alga (*Rivularia nitida*), which was found to be an epiphasic carotenoid. It was given the name "myxoxanthin." The same pigment was later identified in the purple fresh-water plankton alga (*Oscillatoria rubescens*)³⁸⁰; it is likewise probably the pigment found in the marine blue alga (*Calothrix scopulorum*).³⁸¹ Although the structure of myxoxanthin is not certain, the following formula is strongly suggested by the work of Heilbron and Lythgoe³⁸⁰ and of Karrer and Rutschmann.³⁸²

³⁷⁶ P. Karrer, A. Rügger, and U. Solmssen, *Helv. Chim. Acta*, 21, 448-451 (1938).

³⁷⁷ L. Zechmeister and L. v. Cholnoky, *Ann.*, 530, 291-300 (1937).

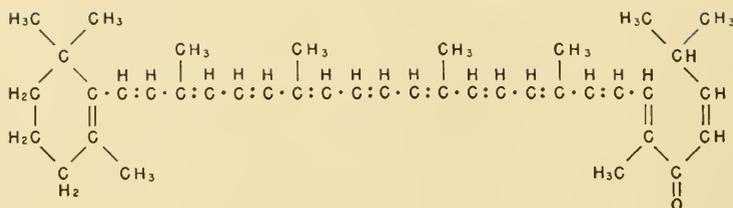
³⁷⁸ H. König, *Dissertation*, University of Zürich, 1940. Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 221.

³⁷⁹ I. M. Heilbron, B. Lythgoe, and R. F. Phipers, *Nature*, 136, 989 (1935).

³⁸⁰ I. M. Heilbron and B. Lythgoe, *J. Chem. Soc.*, 1936, 1376-1380.

³⁸¹ J. Tischer, *Z. physiol. Chem.*, 251, 109-128 (1938).

³⁸² P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, 27, 1691-1695 (1944).



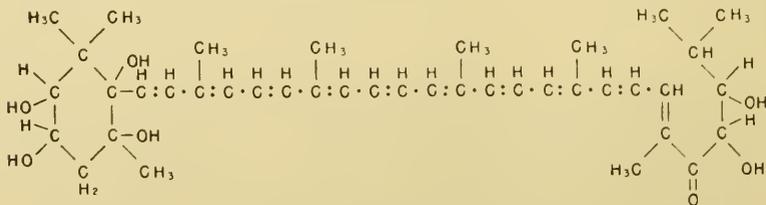
Myxoxanthin (?)

The empirical formula is $C_{40}H_{54}O$. The free pigment absorbs 12 molecules of hydrogen, whereas the myxoxanthin oxime will take up 13 moles, indicating that the pigment has 12 double bonds and a carbonyl group. When reduced with aluminum isopropoxide in isopropanol, it is changed to a secondary alcohol, myxoxanthol. Myxoxanthin must have an unsubstituted β -ionone ring, since it possesses activity as a provitamin A.

Myxoxanthin crystallizes in deep violet colored prisms, from a pyridine-methanol mixture, which melt at 168–169°C. Myxoxanthin dissolves well in a mixture of chloroform-diethyl ether or chloroform-petroleum ether, but only poorly in chloroform alone. It is epiphasic. Myxoxanthin oxime, $C_{40}H_{55}ON$, forms cinnabar-red platelets which melt at 195–196°C.

(16) Myxoxanthophyll

In addition to myxoxanthin, lutein, and β -carotene, Heilbron and Lythgoe³⁸⁰ found a new hypophasic pigment in the purple plankton alga (*Oscillatoria rubescens*) which they named myxoxanthophyll. It was found^{380,382} to have an empirical formula of $C_{40}H_{56}O_7$. The carotenoid absorbs 10 molecules of hydrogen rapidly and one mole slowly, which would indicate the presence of 10 double bonds and of a carbonyl group. The remaining 6 oxygens occur in hydroxyl groups. Myxoxanthophyll forms a tetraacetate, indicating that 4 of the hydroxyls are secondary alcohol groups; proof that the remaining 2 oxygens are components of tertiary alcohol groups is obtained by the Zerewitinoff reaction, which indicates that 2 free hydroxyl groups still remain on the tetraacetate. The tentative formula shown here is believed to indicate the probable structure.³⁸²

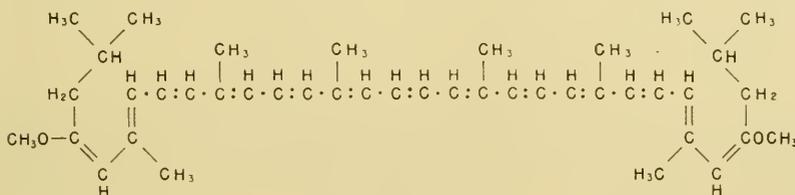


Myxoxanthophyll (?)

Myxoxanthophyll can be crystallized from acetone in violet needles which melt at 182°C. It is markedly levo-rotatory ($[\alpha]_{\text{Cd}} = -255^{\circ}\text{C.}$)_{ethanol}. The pigment dissolves readily in pyridine and ethanol, but it is somewhat less soluble in chloroform and acetone. It is insoluble in petroleum ether, diethyl ether, and benzene. Myxoxanthophyll tetraacetate, C₄₈H₆₄O₁₁, forms glistening violet leaflets which melt at 131–132°C.

(17) *Rhodoviolascin and Related Pigments*

As early as 1873, Lankester first investigated the nature of the pigment in purple bacteria.^{382a} However, it was not until the studies of Karrer and his associates³⁸³ that the composition of these pigments was clarified. The most interesting chromogen identified was rhodoviolascin, which was prepared both from *Rhodovibrio* species and from *Thiocystis* (sulfur bacteria).^{231,383–386} Rhodoviolascin has an empirical formula of C₄₂H₆₀O₂. On catalytic hydrogenation it takes up 13 moles of hydrogen. According to the absorption spectrum, it is apparent that all double bonds are in conjugation with each other. Neither of the original ionone rings is intact. Moreover, it is the only known carotenoid containing methoxy groups. There are two such groups in rhodoviolascin. There are no ketone groups, and consequently the pigment does not react with hydroxylamine.



Rhodoviolascin

When rhodoviolascin was subjected to stepwise degradation with permanganate, Karrer and Koenig³⁸⁶ were able to identify at least six products. Bixin dialdehyde was one of the compounds isolated and identified.

Rhodoviolascin separates from benzene in dark red, glistening crystals which are spindle-shaped and which melt at 218°C. It is very difficultly soluble in petroleum ether, ligroin, or methanol, but it dissolves somewhat better in hot benzene. Its behavior is epiphasic.

a. Rhodopin, C₄₀H₅₈O. Karrer and Solmssen²³¹ were the first to isolate rhodopin from the "purple" bacterium, *Rhodovibrio*.^{231,383–385} It presumably contains 12 double bonds, all of which are conjugated, and a single

^{382a} E. R. Lankester, *Quart. J. Microscop. Sci., n.s.*, 13, 408–425 (1873); 16, 27–40 (1876).

³⁸³ P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 18, 1306–1315 (1935).

³⁸⁴ P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 19, 3–5 (1936).

³⁸⁵ P. Karrer, U. Solmssen, and H. Koenig, *Helv. Chim. Acta*, 21, 454–455 (1938).

³⁸⁶ P. Karrer and H. Koenig, *Helv. Chim. Acta*, 23, 460–463 (1940).

hydroxyl group. As yet no one has succeeded in preparing an acetyl derivative. Rhodopin forms dark red crystals from a carbon disulfide-petroleum ether mixture. These appear as small clusters of prisms and needles. They melt at 171°C.

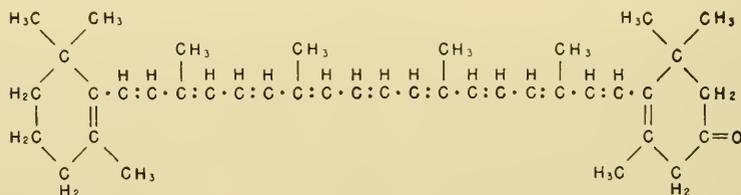
b. Rhodovibrin. Rhodovibrin can be separated from rhodopin chromatographically, since it is adsorbed more readily on calcium hydroxide than is rhodopin. Its structure is unknown, but presumably pure crystals have been shown to melt at 168°C.

c. Rhodopurpurin. Rhodopurpurin is a fourth distinct pigment present in the extracts from *Rhodovibrio*. It is probably a hydrocarbon with an empirical formula of $C_{40}H_{56}$ or $C_{40}H_{58}$.

(18) *Aphanin and Aphanacene*

Several carotenoid pigments are present in blue algae. Although a number of investigations have recently been made in this field, the results are still somewhat confusing. In 1927, Kylin³⁵⁶ was able to demonstrate three new pigments in the blue alga (*Calothrix scopulorum*), in addition to carotene, but these were not further characterized. Sometime later, Tischer^{381,387} separated four new pigments from another fresh-water blue-green alga (*Aphanizomenon flos-aquae*), as well as β -carotene. These new substances were called aphanin, aphanacene, flavacene, and aphanizophyll. The pigments have not been demonstrated elsewhere.

Aphanin is a monoketone having an empirical formula of $C_{40}H_{54}O$. Tischer³⁸⁷ proved that it has 12 double bonds and forms an oxime. Since it is active as a provitamin A,³⁸⁸ it is necessary that there be one intact unsubstituted β -ionone ring. The probable formula²⁵ is given here. Although



Aphanin

the principal pigment of the blue alga is β -carotene, 50 mg. of aphanin and 20 mg. of aphanacene were obtained per kilogram of dried alga.

Aphanacene is one of the pigments which Tischer³⁸¹ isolated from *Aphanizomenon flos-aquae*. It was considered to be a dicarotenoid made up of two molecules of aphanin joined by an oxygen bridge and having a composition of $C_{80}H_{106}O_3$. Aphanacene forms an oxime which demonstrates the presence of at least one carbonyl group. The provitamin A activity is

³⁸⁷ J. Tischer, *Z. physiol. Chem.*, **260**, 257-271 (1939).

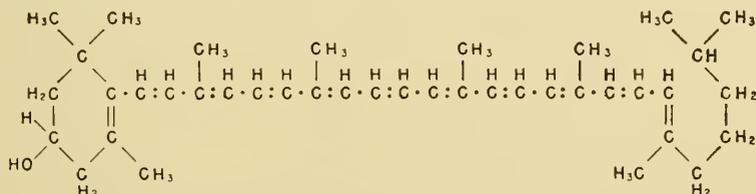
³⁸⁸ A. Scheunert and K. H. Wagner, *Z. physiol. Chem.*, **260**, 272-275 (1939).

considerably less than that of aphanin,³⁸⁸ suggesting that a considerable portion of the molecule is not concerned with its biological reaction.

Little is known about the composition or structure of flavacene or aphanizophyll. Flavacene has some properties in common with mutatochrome. Aphanizophyll has been crystallized from methanol in prism-like crystals which form rosettes. They melt at 172–173°C.

(19) *Gazaniaxanthin*

Gazaniaxanthin was discovered by Schön¹⁰² in the red-brown treasure-flower gazania (*Gazania rigens* R. Br.), which is an African composite. It has a structure indicated by the formula C₄₀H₅₆O, and appears from spectroscopic data to be similar to γ -carotene. However, it has only 11 double bonds in place of the 12 present in γ -carotene. The hydroxyl group is believed to be in the intact β -ionone ring, since it is biologically inactive. Zechmeister and Schroeder¹⁹⁷ believe that gazaniaxanthin may be a dehydrorubixanthin with the following structure (C₄₀H₅₅O).



Gazaniaxanthin (?)

Zechmeister and Schroeder¹⁹⁷ were able to isolate 1400 mg. of gazaniaxanthin per kilogram of gazania flowers raised in Southern California, while 100 mg. of γ -carotene and 60 mg. of β -carotene were obtained from the same amount of flower material. Cryptoxanthin and lutein were also found among the carotenoids in the flowers raised in this country. On the other hand, rubixanthin occurred in the yellow gazania (*Gazania rigens*) grown in Portugal, in place of the gazaniaxanthin. The other pigments found in the Southern California variety were also reported in the Portuguese flowers, with the exception of cryptoxanthin, which was replaced by an unknown pigment.

(20) *Petaloxanthin*

Zechmeister, Béres, and Ujhelyi³⁸⁹ separated a carotenoid from the flowers of the *Cucurbita pepo* (pumpkin) which was called petaloxanthin. Michaud and Tristan³⁹⁰ had likewise noted the presence of this pigment

³⁸⁹ L. Zechmeister, T. Béres, and E. Ujhelyi, *Ber.*, 69, 573–574 (1936).

³⁹⁰ G. Michaud and J. F. Tristan, *Arch. sci. phys. nat.* [4], 37, 47 (1914). Cited by P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 326.

some years earlier. It has been reported only in the pumpkin. Little is known about the structure of petaloxanthin. The empirical formula is $C_{40}H_{56}O_3$ or $C_{40}H_{58}O_3$. A number of its properties indicate that it is closely related to if not identical with antheraxanthin.

(21) Miscellaneous Plant Pigments

During the last decade, the number of different known plant carotenoids has been largely increased. This is due largely to improved methods of isolation, purification, and identification, which are mainly the result of the application of chromatographic adsorption methods,^{92,390} new absorption procedures,³⁹¹⁻³⁹⁴ and new microchemical methods.^{395,396}

a. Eschscholtziaxanthin, $C_{40}H_{54}O_2 \equiv H_2$. This has been separated from the petals of the California poppy (*Eschscholtzia californica*).³⁹⁷ It has 12 double bonds and contains 2 hydroxyl groups. It is very labile in the presence of oxygen. The pigment occurs in the poppy as an ester. Eschscholtziaxanthin melts at 185-186°C., $[\alpha]_{6678}^{18} = 225 \pm 12^\circ$ (in chloroform). The following esters have been prepared: eschscholtziaxanthin diacetate, m.p., 200-240°C., $[\alpha]_{6678}^{20} = +132^\circ$ (in chloroform); dipalmitate, m.p., 100-110°C.; dibenzoate, m.p., 133°C., $[\alpha]_{6678}^{20} = -142^\circ$; di-*p*-nitrobenzoate, m.p., 260°C., $[\alpha]_{6678}^{20} = -234^\circ$.

b. Spirilloxanthin, $C_{43}H_{66}O_3$. The pigment has been prepared from the purple bacteria from well-water (*Spirillum rubrum* Esmarch) by van Niel and Smith.³⁹⁸ It appears to be related to rhodoviolascin.

c. Euglenarhodon, $C_{40}H_{48}O_4$. Euglenarhodon is a tetraketone which was discovered and named by Härdtl.³⁹⁹ It has been prepared from the red alga "water-bloom" (*Euglena heliorubescens*).⁴⁰⁰ Tischer⁴⁰¹ reported the presence of euglenarhodon in the form of the dipalmitate ester in the resting spores of *Haematococcus pluvialis*. This would leave open to question whether the pigment concerned is a reduction product of euglenarhodon or whether two of the oxygens in the parent compound are actually present as hydroxyls rather than as ketones.

³⁹¹ J. H. C. Smith and H. W. Milner, *J. Biol. Chem.*, *104*, 437-447 (1934).

³⁹² E. S. Miller, G. MacKinney, and F. P. Zscheile, *Plant Physiol.*, *10*, 375-381 (1935).

³⁹³ J. H. C. Smith, *J. Am. Chem. Soc.*, *58*, 247-255 (1936).

³⁹⁴ K. W. Hausser, R. Kulin, A. Smakula, and K. H. Kreuchen, *Z. physik. Chem.*, *B29*, 363-370 (1935).

³⁹⁵ K. H. Slotta and E. Blanke, *J. prakt. Chem.*, *143*, 3-17 (1935).

³⁹⁶ H. Jackson and R. N. Jones, *J. Chem. Soc.*, 1936, 895-899.

³⁹⁷ H. H. Strain, *J. Biol. Chem.*, *123*, 425-437 (1938).

³⁹⁸ C. B. van Niel and J. H. C. Smith, *Arch. Mikrobiol.*, *6*, 219-229 (1935).

³⁹⁹ H. Härdtl, *Botan. Centr. Abt. A., Beihefte*, *53*, 606-619 (1935).

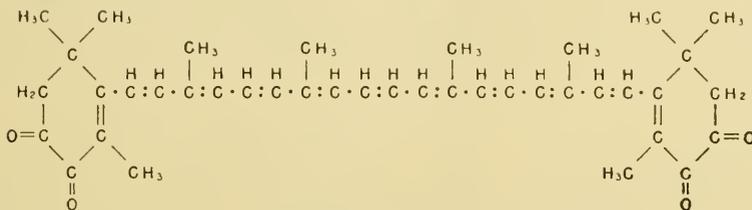
⁴⁰⁰ J. Tischer, *Z. physiol. Chem.*, *239*, 257-269 (1936).

⁴⁰¹ J. Tischer, *Z. physiol. Chem.*, *252*, 225-233 (1938).

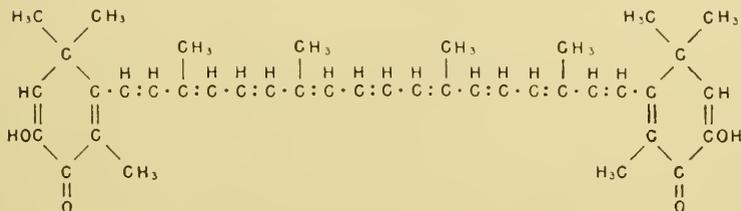
4. Structure and Occurrence of Carotenoids of the C₄₀ Series Primarily of Animal Origin

(1) Astacene

The coloring matter of *Crustacea* is of considerable interest to the chemist and the zoologist. One important pigment of this type was first isolated by Kuhn and Lederer in 1933⁴⁰² in the shell, hypodermis, and eggs of the Norwegian lobster (*Astacus gammarus*, also known as *Homarus vulgaris*). It was called astacenc. It was partly bound with protein and, in part, combined as a dipalmitate ester. The structure of astacene was largely clarified through the investigations of Karrer and his collaborators.⁴⁰³⁻⁴⁰⁶ Astacene was found to be a tetraketone with the composition C₄₀H₄₈O₄. It was shown to be 3,4,3',4'-tetraketo- β , β' -carotene.



Astacene (keto form)



Astacene (enol form)

Astacene has been demonstrated chiefly in the *Crustacea*. Karrer *et al.*⁴⁰⁵ found it in the esterified form (astacein) in the shells of lobsters and crabs. However, it has also been reported in the eggs of the spider crab (*Maia squinado*)⁴⁰⁷ and in the dark red brittle sea star (*Ophidiaster ophidianus*).⁴⁰³ Other crustacea in which astacene has been noted include some lobsters,⁴⁰⁵ such as *Palinurus vulgaris* (European rock lobster, or langouste), a European river crayfish (*Potamobius astacus* or *Astacus fluviatilis*), the small Norway lobster (*Nephrops norvegicus*),⁴⁰⁸ the red prawn (*Leander serratus*),

⁴⁰² R. Kuhn and E. Lederer, *Ber.*, 66, 488-495 (1933).

⁴⁰³ P. Karrer and F. Benz, *Helv. Chim. Acta*, 17, 412-416 (1934).

⁴⁰⁴ P. Karrer and L. Loewe, *Helv. Chim. Acta*, 17, 745-747 (1934).

⁴⁰⁵ P. Karrer, L. Loewe, and H. Hübner, *Helv. Chim. Acta*, 18, 96-100 (1935).

⁴⁰⁶ P. Karrer and H. Hübner, *Helv. Chim. Acta*, 19, 479-480 (1936).

⁴⁰⁷ R. Kuhn, E. Lederer, and A. Deutsch, *Z. physiol. Chem.*, 220, 229-235 (1933).

⁴⁰⁸ G. N. Burkhardt, I. M. Heilbron, H. Jackson, E. G. Parry, and J. A. Lovern, *Biochem. J.*, 28, 1698-1701 (1934).

and also the swimming crab (*Portunus puber*),^{409,410} and crayfish (*Nephrops* spp.).⁴¹⁰ Astacene has also been demonstrated in salmon oil and muscle,^{411,412} as well as in the eggs of the cod.⁴¹³ It occurs in the copepods (*Calanus finmarchicus*) of the plankton,⁴¹⁴ in the red shrimp (*Palaemonetes vulgaris*),⁴¹⁵ and in the red sponge (*Axinella crista-galli*).⁴¹⁶ Karrer and Solmssen⁴¹⁶ also suggested that astacene might be present in the clavuline sponge (*Suberites domuncula*), although this is at variance with the results of Lederer.¹⁵⁸ The presence of astacene in the liver oil and feces of the whale is attributed to the fact that this mammal consumes large amounts of the pigment in the "krill," which consists largely of small crustaceans.^{417,418} In addition, astacene has been recognized in several terrestrial animals. Its presence in the cones of the retina of the chicken, pigeon, and turtle, along with xanthophyll esters, represents a most unexpected source.⁴¹⁹ It is believed to function in some way to render color vision possible. An astacin-like compound has been separated from a red yeast (*Torula rubra*) by Lederer.⁴²⁰ It was accompanied by β -carotene and by "torulene."

(2) Astaxanthin

Although astaxanthin was first studied by Newbigin⁴²¹ in 1897, it was not until the investigations of Kuhn and Sørensen^{422,423} that its relationship to astacene became apparent. The latter workers proved that the pigment in the eggs of the lobster was not esterified astacene ("ovoester"), but rather a new pigment which was called astaxanthin. They indicated its constitution and demonstrated its close relationship to astacene.

The pigment apparently occurs as the prosthetic group of a conjugate protein,²⁸ in which the carrier protein is an albumin. Stern and Salomon^{424,425} have reported that the protein complex of astaxanthin, obtained

⁴⁰⁹ R. Fabre and E. Lederer, *Compt. rend. soc. biol.*, 113, 344-346 (1933).

⁴¹⁰ R. Fabre and E. Lederer, *Bull. soc. chim. biol.*, 16, 105-117 (1934).

⁴¹¹ N. A. Sørensen, *Z. physiol. Chem.*, 235, 8-11 (1935).

⁴¹² A. Emmerie, M. van Eekelen, B. Josephy, and L. K. Wolff, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, 4, 139-141 (1934).

⁴¹³ A. Emmerie, M. van Eekelen, and L. K. Wolff, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, 4, 5-6 (1934).

⁴¹⁴ H. v. Euler, H. Hellström, and E. Klusmann, *Z. physiol. Chem.*, 228, 77-89 (1934).

⁴¹⁵ F. A. Brown, *Biol. Bull.*, 67, 365-380 (1934).

⁴¹⁶ P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 18, 915-921 (1935).

⁴¹⁷ J. C. Drummond and R. J. MacWalter, *J. Exp. Biol.*, 12, 105-107 (1935).

⁴¹⁸ S. Schmidt-Nielsen, N. A. Sørensen, and B. Trumpy, *Kgl. Norske Videnskab. Selskab. Förh.*, 5, No. 30, 118-121 (1932).

⁴¹⁹ G. Wald and H. Zussman, *Nature*, 140, 197 (1937).

⁴²⁰ E. Lederer, *Compt. rend.*, 197, 1694-1695 (1933).

⁴²¹ M. I. Newbigin, *J. Physiol.*, 21, 237-257 (1897).

⁴²² R. Kuhn and N. A. Sørensen, *Ber.*, 71, 1879-1888 (1938).

⁴²³ R. Kuhn and N. A. Sørensen, *Z. angew. Chem.*, 51, 465-466 (1938); *Chem. Abst.*, 32, 7138 (1938).

⁴²⁴ K. G. Stern and K. Salomon, *J. Biol. Chem.*, 122, 461-475 (1938).

⁴²⁵ K. G. Stern and K. Salomon, *Science*, 86, 310-311 (1937).

from the eggs of the North American lobster (*Homarus americanus*), is stable from a pH of 4 to one of 8; it has an isoelectric point at a pH of 6.7, while its approximate molecular weight is 300,000. The name *ovoverdin* has been proposed for this chromoprotein.⁴²⁴

A somewhat simple protein-carotenoid complex has been described by Ball⁴²⁶ in the eggs of the goose barnacle (*Lepas fascicularis* L. *anatifera*). A dissociation of the blue-colored complex into the colorless protein and the red carotenoid apparently accounts for the change in color observed in these embryos. Ball⁴²⁶ believes that the prosthetic group of the chromoprotein is astaxanthin. A salt bridge combination of the pigment with protein, through the hydroxyl groups, similar to that suggested for ovoverdin, is assumed.

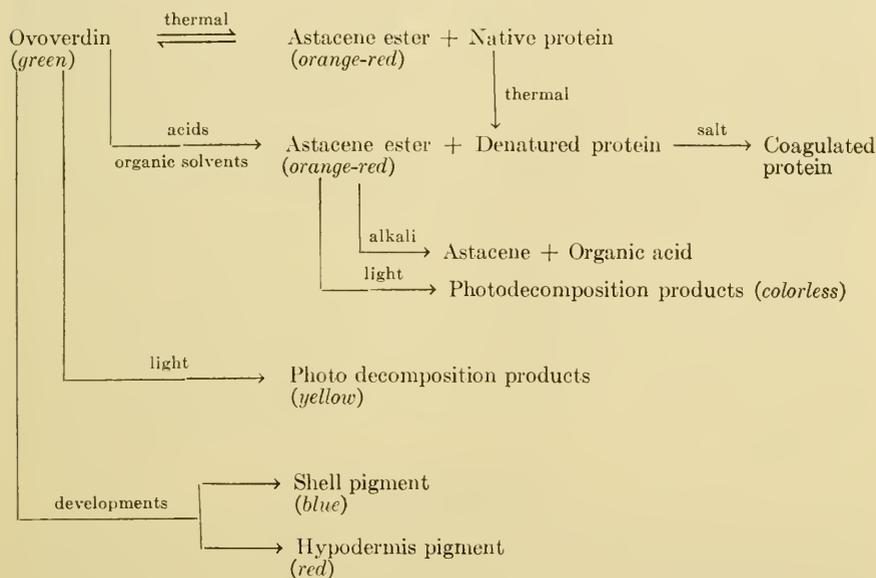


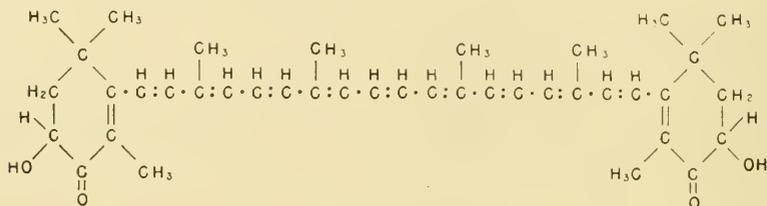
Fig. 1. The reactions of ovoverdin.⁴²⁴

Astaxanthin is closely related to astacene, which is formed when ovoverdin is broken down by acids or by heat. The simpler pigment does not occur as the protein complex. Presumably astacene has been reported in a number of cases whereas, as a matter of fact, the naturally occurring pigment actually was astaxanthin in the form of ovoverdin; in these cases astacene developed only as a result of chemical procedures occurring during its isolation. Figure 1 demonstrates the relationships between the pigments in lobster egg.⁴²⁴

a. Structure. Astaxanthin has an empirical formula of C₄₀H₅₂O₄. It

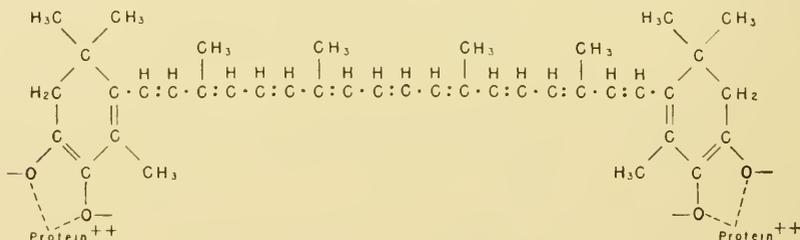
⁴²⁶ E. G. Ball, *J. Biol. Chem.*, 152, 627-634 (1941).

has been shown to be 3,3'-dihydroxy-4,4'-diketo- β -carotene,^{402,406} while its derivative, astacene, is a tetraketone.



Astaxanthin (free form)

Astaxanthin occurs in the protein complex as an ester with an as yet unknown fatty acid.^{402,406,407} It has been suggested by Kuhn and Sørensen⁴²² that, in ovoverdin, the hydroxyl groups of astaxanthin undergo an ionization which permits its linkage to protein through a salt-like bridge. Such a type of linkage accounts for the pronounced difference in color between the free and the combined forms of astaxanthin. The formula suggested by Kuhn and Sørensen⁴²² for the bound form of astaxanthin involves four hydroxyl groups and two additional double bonds, which are conjugated. Astaxanthin is probably identical⁴²⁷ with hematoxanthin,



Astaxanthin (bound form)
(ovoverdin)

which has been prepared by Tischer³¹⁷ from the red rain-water alga (*Haematococcus pluvialis*).

b. Occurrence. Astaxanthin is widely distributed in the *Crustacea*. It occurs as a chromoprotein in the brownish black complex in the shell, the red complexes of the hypoderm, and in the blue-green pigment of the eggs of the Norwegian lobster (*Astacus gammarus*).^{402,407} It is also present in the eggs of the spider crab (*Maja squinado*)⁴⁰⁷ and of the North American lobster (*Homarus americanus*),^{424,425} as well as of a number of other *Crustacea*,^{409,410} including a lake *Cladocera*, a copepod, the plankton crustacean (*Holopedium gibberum*), the fresh-water shrimp (*Gammarus pulex*), and the tentacled mussel (*Lima excavata*).⁴²³ In the latter three cases the pigment was in the form of an astacene ester.

⁴²⁷ R. Kuhn, J. Stene, and N. A. Sørensen, *Ber.*, 72, 1688-1701 (1939).

⁴²⁸ N. A. Sørensen, *Kgl. Norske Videnskab. Selskabs Skrifter*, 1936, No. 1, 1-14.

Recent investigations have demonstrated the wide distribution of astaxanthin among the fishes. It has been reported in the eggs of the rainbow trout (*Salmo irideus*),⁴²⁹ in the Atlantic salmon (*Salmo salar*),^{155,430} the Pacific sockeye salmon (*Onchorhynchus nerka*),⁴³⁰ the scythe fish or oarfish (*Regalecus glesne*),^{418,427} and the lump-sucker (*Cyclopterus lumpus*).⁴¹⁸ The latter species has astaxanthin in the liver oil, and appears to mobilize it in the skin and flesh during the spawning season. Lederer^{24,431} found this pigment in the red skin, gill, and mouth mucus, iris, and sclera of the gold mackerel or marine dorado (*Beryx decadactylus*). The common goldfish (*Carassius auratus*), some but not all varieties of fresh-water perch (*Perca fluviatilis*), and the red Norway haddock (*Sebastes marinus*) yield astaxanthin or astacene.²⁴ In the sea-devil fish or angler fish (*Lophius piscatorius*), astaxanthin (as an astacene ester) occurs with taraxanthin-like compounds,⁴³² while in the sunfish (*Orthogoriscus mola*) it is present in association with α -carotene and minute amounts of vitamin A, which may be accounted for by the food ingested.⁴³³ Astaxanthin has been found, also, in a number of terrestrial arthropods, including the mite (*Trombidium* spp.) and probably the Colorado potato beetle, *Doryphora* (*Leptinotarsa*) *decemlineata*.⁴³⁴ In addition, the pigment has been reported in birds. It has been found in the egg-yolk of the black-headed laughing gull, or peewit (*Larus ridibundus*) and of the stork (*Ciconia ciconia*), with only traces of carotene and lutein.³¹⁵ Moreover, an analogous pigment has been shown to be a component of the fat of the carmine flamingo (*Phoenicopterus roseus*).⁴³⁵ Kuhn *et al.*⁴²⁷ have also demonstrated that astaxanthin is to be found in the retina of the chicken and in the red sclera of the pheasant (*Phasianus colchicus*).⁴²⁷

(3) *Echinenone*

Lederer^{24,133} isolated a new pigment from the ovaries of the Atlantic sea urchin (*Strongylocentrotus lividus*) which proved to be a carotenoid. It was named echinenone from *Echinus esculentus*,⁴³⁶ which was at first erroneously believed to be the species from which it was obtained.

Echinenone is a monoketone having an empirical formula of C₄₀H₅₄O. Since the compound possesses provitamin A activity,^{436,437} it is as-

⁴²⁹ M. Hartmann, F. G. Medem, R. Kuhn, and H. J. Bielig, *Z. Naturforsch.*, **2b**, 330-349 (1947).

⁴³⁰ B. E. Bailey, *J. Biol. Board Can.*, **3**, 469-472 (1937).

⁴³¹ E. Lederer, *Les Caroténoïdes des Animaux*, Hermann, Paris, 1935.

⁴³² N. A. Sørensen, *Kgl. Norske Videnskab. Selskabs Skrifter*, 1934, No. 1, 1-14.

⁴³³ N. A. Sørensen, *Kgl. Norske Videnskab. Selskabs Førh.*, **6**, No. 40, 154-157 (1933).

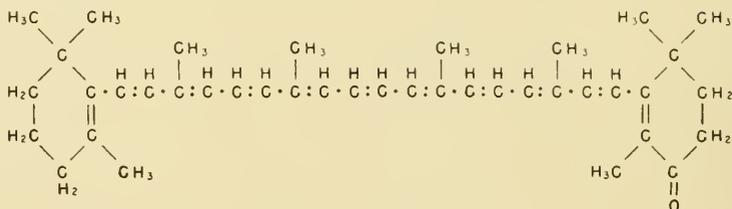
⁴³⁴ C. Manunta, *Nature*, **162**, 298 (1948).

⁴³⁵ C. Manunta, *Helv. Chim. Acta*, **22**, 1151-1153 (1939).

⁴³⁶ E. Lederer and T. Moore, *Nature*, **137**, 996 (1936).

⁴³⁷ S. M. Greenberg and B. T. Scheer, *Unpublished results* (1947-1948).

sumed that one β -ionone ring is unattached. The following structure has been postulated.³²



Echinenone

Echinenone can be crystallized from a mixture of petroleum ether and benzene, in violet needles with a metallic luster which melt at 178–179°C. Absorption maxima in carbon disulfide are 520, 488, and 450 m μ . Drumm and O'Connor²⁰⁸ and Drumm *et al.*¹⁵⁹ have prepared crystalline echinenone from the red rock sponge (*Hymeniacidon sanguineum* Grant). The demonstration that echinenone occurs in both the red sponge and the sea urchin provides a hitherto unrecognized chemical link between the *Porifera* and the *Echinodermata*.

(4) Other Animal Carotenoids

There are a number of animal pigments of the C₄₀ series about which information is still quite incomplete.

a. Sulcatoxanthin, C₄₀H₅₂O₈. This has been isolated from the sea anemone (*Anemonia sulcata*) by Heilbron and co-workers.⁴³⁸ The solubility of this carotenoid in 65% methanol is in harmony with its high oxygen content as indicated in the empirical formula. It is insoluble in petroleum ether, slightly soluble in carbon disulfide, and it dissolves readily in benzene and ethanol. It crystallizes from a mixture of ether and petroleum ether in deep scarlet-red needles which have no sharp melting point, but which shrivel at 110°C., soften at 125°C., and melt at 130°C.

b. Pentaxanthin, C₄₀H₅₆O₅ (\pm H₂). Lederer²⁴ isolated echinenone from the Atlantic sea urchin (*Strongylocentrotus lividus*). This same author¹³³ found a second, hitherto unknown carotenoid, pentaxanthin, in the edible sea urchin (*Echinus esculentus*). It possesses 3 hydroxyl groups and is believed to have 11 conjugated double bonds. It can be crystallized from benzene in red needles melting at 209–210°C. The pigment is readily soluble in chloroform and carbon disulfide, somewhat less so in benzene, and quite poorly in diethyl ether and petroleum ether.

c. Pectenoxanthin, C₄₀H₅₄O₂ (\pm H₂). Lederer^{202,439} has reported the presence of a carotenoid pigment which appears during the development

⁴³⁸ I. M. Heilbron, H. Jackson, and R. N. Jones, *Biochem. J.*, 29, 1384–1388 (1935).

⁴³⁹ A. Lederer, *Compt. rend. soc. biol.*, 116, 150–153 (1934).

of the sex organs of the mollusc, St. Jacques mussel or scallop (*Pecten maximus*). The same pigment apparently occurs in the sea-weed-inhabiting ascidian, *Botryllus schlosseri*.²⁰² The pigment has been named pectenoxanthin. It has 11 conjugated double bonds and 2 active hydrogens, probably in hydroxyl groups. This carotenoid is inactive as a precursor for vitamin A, which indicates that there are no unsubstituted β -ionone rings. It can be separated in long yellow-brown prisms from dilute pyridine; these melt at 182°C. The pigment dissolves better in 90% methanol than in petroleum ether. Benzene, carbon disulfide, and pyridine are excellent solvents. Karrer and Solmssen⁴¹⁶ found a pigment with similar absorption peaks in the red scallop (*Pecten jacobaeus*) which they considered might possibly be identical with pectenoxanthin.

d. Cynthiaxanthin. In connection with studies of the pigments of various types of *Ascidiae*, Lederer²⁰² reported that, in addition to astacene, another unknown carotenoid pigment is present in the red solitary fixed ascidian, *Cynthia (Halocynthia) papillosa*. He named the pigment cynthiaxanthin. Karrer and Solmssen⁴¹⁶ were unable to confirm the presence of the pigment in material of the same kind; it may have been masked by the bands for astacene.

5. Structure and Occurrence of Carotenoids Having Less Than Forty Carbon Atoms

It has long been known that several polyene acids having a smaller number of carbon atoms than the C₄₀ series exist in nature. Kuhn and Winterstein⁷⁹ have advanced the hypothesis that certain compounds in this category, such as bixin, crocetin, and azafrin, originate through the oxidative splitting of the carotenoids. There is some support for such a theory, since bixin dialdehyde and two molecules of methylheptenone originate on oxidation of lycopene with chromic acid. Bixin dialdehyde can be readily changed to bixin.²²¹ Moreover, it is believed that a glucoside of crocetin originates by the breakdown of a bicyclic C₄₀ carotenoid which has been paired with sugar. In this reaction, two molecules of picrocrocin are formed. The relationship of the C₄₀ polyenes to the simpler members of this class is schematically represented in Figure 2.

(1) Bixin and Related Compounds

Bixin has long been known as a dye present in the seed hulls of the tropical anatto tree (*Bixa orellana* L.). Up to the present time this constitutes the main commercial source of the dye anatto (also called orleán or orellin). This coloring matter was characterized as early as 1825 by Bossingault,⁴⁴⁰ while Etti⁴⁴¹ succeeded in crystallizing it in 1878. Later (1917) Heiduschka

⁴⁴⁰ J. B. Bossingault, *Ann. chim.* [2], 28, 440-443 (1825).

⁴⁴¹ C. Etti, *Ber.*, 11, 864-870 (1878).

TABLE 10. NOMENCLATURE, STRUCTURE, AND MELTING POINTS OF BIXIN AND SOME RELATED COMPOUNDS^a

Formula	Current name		Older names		Melting point, °C.	Geometric form
	Labile norbixin	Labile bixin	Karrer	Herzig and Faltis ^b		
C ₂₂ H ₃₆ (COOH) ₂	Labile norbixin		Norbixin	Norbixin	254-255	<i>cis</i>
C ₂₂ H ₃₆ { COOCH ₃ } { COOH }	Labile bixin		Bixin	Bixin	196	<i>cis</i>
C ₂₂ H ₃₆ (COOCH ₃) ₂	Labile bixin methyl ester		Bixin methyl ester	Bixin methyl ester	163-164	<i>cis</i>
C ₂₂ H ₃₆ (COOH) ₂	Stable norbixin		Isonorbixin	β-Norbixin	> 300	<i>trans</i>
C ₂₂ H ₃₆ { COOCH ₃ } { COOH }	Stable bixin		Isobixin	β-Bixin	220	<i>trans</i>
C ₂₂ H ₃₆ (COOCH ₃) ₂	Stable bixin methyl ester		Isobixin methyl ester	β-Bixin methyl ester	200-201	<i>trans</i>

^a Adapted from P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 265.

^b J. Herzig and F. Faltis, *Ann.*, 431, 40-70 (1923).

TABLE 11. NOMENCLATURE, STRUCTURE, AND MELTING POINTS OF CROCETIN AND RELATED COMPOUNDS^a

Formula	Current name		Old name	Melting point, °C.	Geometric form
	Stable crocetin	Labile crocetin monomethyl ester			
C ₁₃ H ₂₂ (COOH) ₂	Stable crocetin		α-Crocetin	285	<i>trans</i>
C ₁₃ H ₂₂ { COOCH ₃ } { COOH }	Stable crocetin monomethyl ester		β-Crocetin	218	<i>trans</i>
C ₁₃ H ₂₂ (COOCH ₃) ₂	Stable crocetin dimethyl ester		γ-Crocetin	222	<i>trans</i>
C ₁₃ H ₂₂ (COOCH ₃) ₂	Labile crocetin dimethyl ester		Pigment of Kuhn ^b	141	<i>cis</i>

^a Data adapted from P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, p. 281

^b R. Kuhn and A. Winterstein, *Ber.*, 66, 209-214 (1933); 67, 344-357 (1934).

The correctness of the Kuhn-Winterstein formula was proved by Karrer and associates,^{444,445} who demonstrated the identity of the synthetically prepared perhydrobixin ester with the compound formed on hydrogenation of the natural bixin.

The so-called "stable bixin" is a compound having a structural formula identical with that of the natural (labile) bixin. The occurrence of the isomer was first demonstrated by Herzig and Faltis⁴⁴⁶ during the preparation of bixin from orleán. In a single, unreproducible experiment, these workers failed to obtain the usual (labile) bixin, but obtained instead a substance which had a higher melting point, a greater stability, and a longer wave-length spectrum. They called this isomer " β -bixin"; the term "stable bixin" is now applied to it.

Labile and stable bixins are now known to be *cis-trans* isomers. Karrer, Helfenstein, Widmer, and van Itallie⁴⁴³ confirmed the occurrence of the stable form and were the first to suggest that the two types of bixin are geometrical isomers. The *cis-trans* relationship was indicated by the fact that the transformation of ordinary bixin to stable bixin (β -bixin) could be effected by iodine^{79,443} even when this was used in catalytic amounts, or by perbenzoic acid in chloroform.⁴⁴⁷ The structural identity of the two bixins was proved by the demonstration that when two hydrogens were added either to labile bixin or to stable bixin by treatment with zinc and glacial acetic acid or titanium chloride and ammonia, the same dihydrobixin resulted in both cases.^{448,449} If the dihydrobixin so formed is oxidized in the air in the presence of pyridine, stable bixin results. It is therefore possible to change the labile into the stable isomer by this procedure.

Zechmeister^{450,451} has presented proof that labile bixin (also called natural bixin, α -bixin, bixin II, and lower melting bixin) has the *cis* configuration, while the stable bixin (β -bixin, bixin I, and higher melting bixin) has an all-*trans* structure. The formulas for the two isomers are represented diagrammatically in Figure 3.

b. Norbixins. The norbixins have the same structural formulas as the bixins except that both carboxyl groups are free. The empirical formulas are $C_{24}H_{28}O_4$. The structural formula is represented here. Two forms of

⁴⁴⁴ P. Karrer, F. Benz, R. Morf, H. Raudnitz, M. Stoll, and T. Takahashi, *Helv. Chim. Acta*, **15**, 1218-1219 (1932).

⁴⁴⁵ P. Karrer, F. Benz, R. Morf, H. Raudnitz, M. Stoll, and T. Takahashi, *Helv. Chim. Acta*, **15**, 1399-1419 (1932).

⁴⁴⁶ J. Herzig and F. Faltis, *Ann.*, **431**, 40-70 (1923).

⁴⁴⁷ T. Takahashi, *J. Pharm. Soc. Japan*, **56**, No. 1, 352-355 (1936); in German, pp. 48-50; *Chem. Abstr.*, **30**, 6348 (1936). Cited by L. Zechmeister, *Chem. Revs.*, **34**, 324 (1944).

⁴⁴⁸ R. Kuhn and P. J. Drumm, *Ber.*, **65**, 1458-1460 (1932).

⁴⁴⁹ R. Kuhn, P. J. Drumm, M. Hoffer, and E. F. Möller, *Ber.*, **65**, 1785-1788 (1932).

⁴⁵⁰ L. Zechmeister and R. B. Escue, *J. Am. Chem. Soc.*, **66**, 322-330 (1944).

⁴⁵¹ L. Zechmeister, *Chem. Revs.*, **34**, 267-344 (1944).

One proof of the above structure is the observation that by α, α' -bromination of perhydrocrocetin followed by replacement of the halogen with hydroxyl, a diglycol is formed which, on treatment with lead tetraacetate, yields a diketone; the aldehyde group is completely absent. This proves that the methyl groups are present in the α -position to the carboxyls.²³

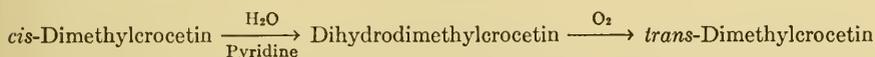
Better proof of the structure of crocetin has been presented by Karrer, Benz, and Stoll,⁴⁶³ who showed that synthetic perhydrocrocetin was identical with the perhydro compound prepared from the natural saffron dye-stuff.

b. Dimethylcrocetins. Kuhn and Winterstein⁴⁶⁴ first demonstrated that two types of dimethylcrocetin can be prepared by extraction and exchange esterification of the natural crocetin gentiobioside (crocine) with methyl alcohol.

1. Stable dimethylcrocetin (γ -crocetin, crocetin I). This is present in the larger amount (17–60 g. per kilogram of dry saffron). The dimethyl ester melts at 222°C. It appears to be the all-*trans* isomer.⁴⁵¹

2. Labile dimethyl crocetin (crocetin II, pigment of Kuhn). Labile crocetin is known only in the form of derivatives. The labile dimethyl ester can be prepared in comparatively small amounts from saffron (1 g. per kilogram of dry saffron); the dimethyl ester melts at 141°C. Zechmeister⁴⁵¹ believes that it contains one *cis* linkage.

cis-Dimethylcrocetin can be readily changed to the *trans* or stable form of the ester by heating the crystals of the *cis* form above the melting point and cooling,⁴⁶⁴ by catalysis of petroleum ether solution with iodine,⁴⁵¹ by irradiation of petroleum ether solution,⁴⁵¹ or by reduction of the labile isomer followed by oxidation of the dihydromethylcrocetin formed as indicated below:



c. Occurrence. Crocetin is present in the *Crocus sativus* (saffron), largely in the form of a glucoside (crocine) along with considerable amounts of carotene, lycopene, and zeaxanthin.^{62, 193, 454, 458} Both *cis* and *trans* forms are present.^{193, 464} It has also been prepared from two other members of the *Crocus* family: *Crocus candida* var. *luteus* (yellow crocus) where it appears in the petals,⁶¹ and *Crocus neapolitanus* (violet crocus) in which it is found in the stigma.^{76, 93} Flowers from other species such as mullein (*flores verbasci*)⁴⁶⁵ (also referred to as *Verbascum phlomoides*, clasping mullein or king's candle), the *Nyctanthes arbor-tristis* (night jasmine),^{466, 467} and the *Toona ciliata* or *Cedrela toona* Roxb. (Burma toon or Indian mahogany

⁴⁶⁴ R. Kuhn and A. Winterstein, *Ber.*, 66, 209–214 (1933).

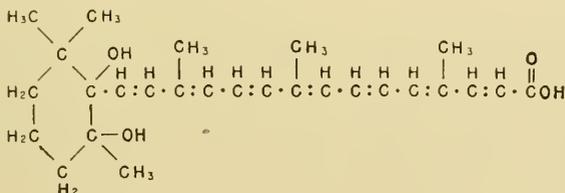
⁴⁶⁵ L. Schmid and E. Kotter, *Monatsh.*, 59, 341–356 (1932).

⁴⁶⁶ R. Kuhn and A. Winterstein, *Helv. Chim. Acta*, 12, 493–498 (1929).

⁴⁶⁷ E. G. Hill and A. P. Sikkar, *J. Chem. Soc.*, 91, 1501–1505 (1907).

to elucidate the structure of this pigment. More recent studies of Kuhn and his collaborators⁴⁷⁵ have succeeded in clarifying the structure of this dyestuff.

a. Structure. Azafrin, $C_{27}H_{38}O_4$, absorbs seven molecules of hydrogen on catalytic hydrogenation, forming perhydro-azafrin, $C_{27}H_{52}O_4$. The latter is a colorless, odorless, viscous oil which can be distilled unchanged *in vacuo*. When it is treated with bromine in chloroform, only four double bonds of the azafranillo dyestuff are saturated. On treatment with iodine in benzene, a greenish black, crystalline iodide is formed. Of the four oxygen atoms in azafrin, two are present in the carboxyl group. This is evident from the fact that, when titrated with thymol blue in alcohol, azafrin titrates as a monocarboxylic acid. Perhydro-azafrin behaves in a similar manner. Since the Zerewitinoff test shows the presence of three active hydrogens, it is evident that the other two oxygens are present as hydroxyl groups. Kuhn and Deutsch⁴⁷⁶ proved that the hydroxyl groups are on adjacent carbons and represent, in fact, a ditertiary glycol. On oxidation with chromic acid, azafrinone is formed, which is a diketone. Since azafrinone is optically inactive, it is evident that the optical activity of the azafrin resides in the alcoholic hydroxyl groups. Kuhn and Deutsch⁴⁷⁶ have assigned to azafrin the formula shown here.



Azafrin

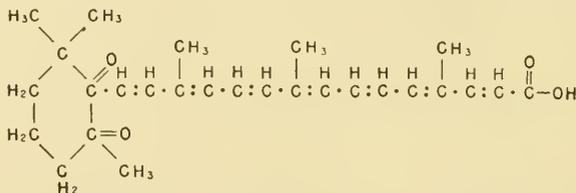
b. Occurrence. Azafrin is present in the root and stems of the figworts (*Scrophulariaceae*), *Escobedia scabrifolia* and *E. linearis* (ysipyu).⁴⁷²⁻⁴⁷⁵ These plants grow in tropical America and are used in Paraguay as "azafran" or "azafranillo" for the coloring of fats. The pigment occurs as bright yellow-orange spots in the wood of the roots and stems.

c. Related Compounds. (a) *Azafrin Methyl Ester.* Azafrin methyl ester, $(HO)_2C_{26}H_{35}COOCH_3$, has the same relation to azafrin as methylcrocetin to crocetin or as methylbixin to bixin. It can be prepared by dissolving azafrin in 0.1 *N* alkali and treating it with dimethyl sulfate, keeping the reaction slightly alkaline. The methylester forms glistening platelets (from methyl alcohol) which melt at 193°C. The ester is insoluble in alkali, but soluble in most solvents, with the exception of petroleum ether; it dissolves exceedingly well in chloroform. The ethyl ester of azafrin $((HO)_2C_{26}H_{35} \cdot COOC_2H_5)$, m.p. 182°C., is also known.

⁴⁷⁵ R. Kuhn, A. Winterstein, and H. Roth, *Ber.*, 64, 333-341 (1931).

⁴⁷⁶ R. Kuhn and A. Deutsch, *Ber.*, 66, 883-892 (1933).

(b) *Azafrinone*. This compound is the diketone of azafrin and has a formula of $C_{27}H_{36}O_4$. It forms orange-red platelets from a concentrated solution, but separates from more dilute solutions in star-shaped clusters of needle-like crystals. It melts at $191^{\circ}C$. and is optically inactive. The



Azafrinone

absorption spectrum corresponds to that of its methyl ester ($C_{28}H_{38}O_4$). It reacts with only one hydroxylamine molecule, forming a monoxime which crystallizes in platelets and melts at $194^{\circ}C$. Azafrinone aldehyde originates on chromic acid degradation of β -oxycarotene.⁴⁷⁷

6. Properties of the Carotenoids

(1) *Melting Points and Optical Rotation*

The carotenoids represent a group of substances which are solids at ordinary temperatures, having characteristic melting points which are usually between 150° and $200^{\circ}C$. However, in certain cases values as low as $80^{\circ}C$. and as high as $300^{\circ}C$. have been recorded. Data on the melting points, specific rotation, vitamin A activity, and double bonds for some of the more common carotenoids are summarized in Tables 12 and 13.

(2) *Spectral Absorption*

One of the most important physical properties of the carotenoids which helps in their identification is their spectral absorption. The intensity with which light of various wave lengths is absorbed is characteristic of each derivative and, in fact, even varies with the different stereochemical isomers. With the recent development of simple instruments for the quantitative measurement of the absorption spectra (such as the Beckman spectrophotometer and the Coleman photoelectric spectrophotometer), wide application of the extinction values at wave lengths of maximum absorption have been made for the quantitative determination of the carotenoids. Such procedures are exceedingly accurate and relatively simple.

The extinction coefficient (or simply the extinction value) is usually

⁴⁷⁷ R. Kuhn and H. Brockmann, *Unpublished work*, cited by L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, pp. 144, 271.

TABLE 12. SOME PROPERTIES OF C₄₀ POLYENE HYDROCARBONS AND THEIR DERIVATIVES

Compound	Empirical formula	Unsubstituted β-ionone rings	Vitamin A activity ^a	Double bonds	Melting point, °C.	[α] _D , degrees	Refractive index
α-Carotene	C ₄₀ H ₅₆	1	++	11	174-175 ^b 187-188 ^d	380 ^{b,e} ; 359 ^f	1.451 ^h
α-Oxycarotene	C ₄₀ H ₅₄ O ₂	0	0 ⁱ	10	183 ^d	—	—
β-Carotene	C ₄₀ H ₅₆	2	+++	11	182 ^{b,e} ; 183 ^d 184 ^g	Inactive	1.453 ^h
β-Carotene oxide	C ₄₀ H ₅₆ O	1	+ ^d	10	160-161 ^d	—	—
β-Oxycarotene	C ₄₀ H ₅₄ O ₂	1	+ ⁱ	10	184 ^d	—	—
Semi-β-carotene	C ₄₀ H ₅₆ O ₂	1	+ ⁱ	10	118-119 ^d	—	—
β-Carotenone	C ₄₀ H ₅₆ O ₄	0	0	9	174-175 ^d	—	—
β-Perhydrocarotene	C ₄₀ H ₇₀	0	0 ^j	0	—	Inactive ^k	—
γ-Carotene	C ₄₀ H ₅₆	1	+	12	178 ^{d,g}	—	—
Lycopene	C ₄₀ H ₅₆	0	0	13	173-175 ^{g,i}	—	—
Lycopenal	C ₃₃ H ₄₂ O	0	0	11	147 ^d	—	—

^a + + + + indicates 100% activity, + + 50%, and + 25%.

^b R. Kuhn and E. Lederer, *Ber.*, 64, 1349-1357 (1931).

^c P. Karrer, A. Helfenstein, H. Wehrli, B. Pieper, and R. Morf, *Helv. Chim. Acta*, 14, 614-632 (1931).

^d L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934.

^e R. Kuhn and E. Lederer, *Naturwissenschaften*, 19, 306 (1931); benzene, Cd light.

^f L. Zechmeister, *Chem. Revs.*, 31, 267-344 (1944).

^g R. A. Morton, *The Application of Absorption Spectra to the Study of the Vitamins, Hormones, and Coenzymes*, 2nd ed., Jarell-Ash Co., Boston, 1942.

^h H. v. Euler and B. Jansson, *Arkiv Kemi Mineral. Geol.*, B10, No. 17 (1931).

ⁱ I. M. Heilbron, W. E. Jones, and A. L. Bacharach, *Vitamins and Hormones*, 2, 155-213 (1944).

^j H. v. Euler, V. Demole, P. Karrer, and O. Walker, *Helv. Chim. Acta*, 13, 1078-1083 (1930).

^k L. Zechmeister and L. v. Cholnoky, *Ber.*, 61, 1534-1539 (1928).

^l P. Karrer and R. Widmer, *Helv. Chim. Acta*, 11, 751-752 (1928).

TABLE 13. SOME PROPERTIES OF C₄₀ POLYENE ALCOHOLS AND THEIR DERIVATIVES^a

Compound	Empirical formula	Groups				Furanoid	Vitamin A activity	Double bonds	Melting point, °C.	[α] _D , degrees
		—OH	>CO	Unsubstituted β-ionone rings						
Antheraxanthin	C ₄₀ H ₆₆ O ₃	1	—	0	—	0	10	211 ^b	0	
Aphanacene	C ₃₀ H ₁₀₆ O ₃ (?)	0	—	1	—	—	12	190–195	0	
Aphanin	C ₄₀ H ₆₄ O	0	1	1	—	—	11	176–180	0	
Astacene	C ₄₀ H ₄₈ O ₄	0	4	0	—	—	11	240–243 ^c 228 ^d	0	
Astaxanthin	C ₄₀ H ₅₂ O ₄	2	2	0	—	0	11	216	0	
Auroxanthin	C ₄₀ H ₅₆ O ₄	2	—	0	2	0	9	203	0	
Capsanthin	C ₄₀ H ₅₈ O ₂	2	1	0	—	0	10	175–176	+36 (CHCl ₃)	
Capsorubin	C ₄₀ H ₆₀ O ₄	2	2	0	—	0	9	201	0	
Celaxanthin	C ₄₀ H ₆₆ O	1	—	0	—	0	13	209–210	?	
Chrysanthemaxanthin	C ₄₀ H ₅₆ O ₃	2	—	0	1	0	10	184–185	+180 to +190 (benzene)	
Cryptoxanthin	C ₄₀ H ₅₆ O	1	—	1	—	—	11	169	0	
Dihydrodihydroxanthin	C ₄₀ H ₅₂ O ₂	0	2	0	—	—	11	219	0	
Echinenone	C ₄₀ H ₅₈ O (≠H ₂)	0	1	1	—	—	?	178–179	0	
Eschscholtziaxanthin	C ₄₀ H ₅₄ O ₂ (≠H ₂)	2	—	0	—	—	12	185–186	+225 ± 12 (CHCl ₃)	
Euglenarhodon	C ₄₀ H ₄₈ O ₄	0	—	0	—	—	?	227–228 ^e	0	
Flavoxanthin	C ₄₀ H ₅₆ O ₃	2	—	0	1	0	10	184	+190 (benzene)	
Fucoxanthin	C ₄₀ H ₅₆ O ₆	4	2	0	0	0	9	159.5– 160.5 ^f	+72.5 ± 9 ^g (CHCl ₃)	
Gazaniaxanthin	C ₄₀ H ₅₆ O	1	—	0	0	0	13	209–210	?	
Helenien	C ₇₂ H ₁₁₆ O ₄	2 ^h	—	0	—	—	11	92 ⁱ	0	
Lutein (xanthophyll)	C ₄₀ H ₅₆ O ₂	2	—	0	0	0	11	193	+160 (CHCl ₃); 145 (ethyl acetate)	
Lycophyll	C ₄₀ H ₅₆ O ₂	2	—	0	0	0	13	179	0	
Lycoxanthin	C ₄₀ H ₅₆ O	1	—	0	—	—	13	168	?	

Compound	Empirical formula	Groups					Vitamin A Activity	Double bonds	Melting point, °C.	[α] _D , degrees
		—OH	>CO	Unsubstituted β-ionone rings	Furanoid	A				
Mutatoxanthin	C ₄₀ H ₅₆ O ₃	2	—	0	1	0	10	177	?	
Myxoxanthin	C ₄₀ H ₅₄ O	0	1	1	0	?	12	168-169	0	
Myxoxanthophyll	C ₄₀ H ₅₆ O ₇	6	1	0	0	0	10	182	-255 (ethanol)	
Pectoxanthin	C ₄₀ H ₅₄ O ₃ (=H ₂)	3	—	0	0	0	11	182	?	
Pentaxanthin	C ₄₀ H ₅₆ O ₃ (=H ₂)	2?	2?	0	0	0	11	209-210	?	
Petaloxanthin	C ₄₀ H ₅₆ O ₃ (=H ₂)	2	?	0	0	0	?	211-212	?	
Physalene	C ₇₂ H ₁₁₆ O ₄	2 ^b	—	0	0	0	11	98.5-99.5	0	
Rhodopin	C ₄₀ H ₅₅ O (=H ₂)	0	—	0	0	0	12	171	?	
Rhodoviolasin	C ₄₂ H ₆₀ O ₂	2 ^l	—	0	0	0	13	218	0	
Rhodoxanthin	C ₄₀ H ₅₀ O ₂	0	2	0	0	0	12	219	0	
Rubixanthin	C ₄₀ H ₅₆ O	1	—	0	0	0	12	160	0	
Spirilloxanthin	C ₄₃ H ₆₆ O ₄ ^k	0	—	—	0	0	15	218-219 ^l	0	
Taraxanthin	C ₄₀ H ₅₆ O ₄	3-4?	—	—	0	0	11	184-185	+200 (ethyl acetate)	
Violaxanthin	C ₄₀ H ₅₆ O ₄ ^m	2	—	—	0	0	9	200	+35 (CHCl ₃)	
Zeaxanthin	C ₄₀ H ₅₆ O ₂	2	—	—	0	0	11	215.5	-40 to -45.2 (CHCl ₃) ⁿ	

^a Data are from P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, unless otherwise noted.

^b P. Karrer and A. Oswald, *Helv. Chim. Acta*, 18, 1303-1305 (1935).

^c R. Kuhn and E. Lederer, *Ber.*, 66, 488-495 (1933).

^d R. Kuhn, J. Stene, and N. A. Sørensen, *Ber.*, 72, 1688-1701 (1939).

^e J. Tischer, *Z. physiol. Chem.*, 239, 257-269 (1936).

^f R. Willstätter and H. J. Page, *Ann.*, 404, 237-271 (1914).

^g P. Karrer, A. Helfenstein, H. Wehrli, B. Pieper, and R. Morf, *Helv. Chim. Acta*, 14, 614-632 (1931).

^h As dipalmitate ester.

ⁱ Crystallized from alcohol.

^k As dimethyl ether.

^l L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934.

^m C. B. van Niel and J. H. C. Smith, *Arch. Mikrobiol.*, 6, 219-229 (1935).

ⁿ As a di-epoxide.

^o L. Zechmeister, L. v. Cholnoky, and A. Polgár, *Ber.*, B72, 1678-1685 (1939).

designated as E (1%, 1 cm.). It can be determined for each carotenoid for each specific wave length by calculation from the following general formula:

$$E (1\%, 1 \text{ cm.}) = (1/cd) \log (1/T)$$

where c = concentration of substance in grams per 100 milliliters, d = length of light path through solution in centimeters, and $\log (1/T)$ = optical density. $1/T$ is the reciprocal of the fraction of "incident" light which is transmitted through the solution. It is frequently replaced by the expression, $\log (I_0/I_x)$, where I_0 is the incident light and I_x is the transmitted light. If 90% of the incident light is absorbed in the solution, 10% is transmitted, and the value for $\log (1/T)$ becomes 1.00. Thus $\log 100\%$ (incident light) - $\log 10\%$ (transmitted light) = $2 - 1 = 1.00$. When the instrument is set at 100% transmittance for the pure solvent, the expression $\log (1/T)$ is the optical (or photometric) density. This figure is read directly from the dial setting on such instruments as the Beckman spectrophotometer. In most cases, one attempts to have solutions of such concentration that the optical densities recorded will be between 0.3 and 0.8. However, in many cases there is still a direct correspondence with Beer's law when solutions of such concentration as to give an optical density over 1.0 are employed. The maximum optical density possible when 100% absorption occurs is 2.0.

Because of the small concentrations of carotenoids usually employed, it has been the custom to express the extinction values in this case as molecular extinction values (E_{mol} , or ϵ). These may be calculated by the modified formulas given below:

$$\epsilon = (1/cd) \log (1/T)$$

where c = concentration in gram molecules per liter (moles), d = length of light path through the solution in centimeters, $\log (1/T)$ = optical density, and:

$$\epsilon = (1/cd) \ln (1/T)$$

The $\ln \epsilon$ formula is used in place of the $\log \epsilon$ when it is desirable to compress data in comparing substances having a widely different value for ϵ on the same graph.

The absorption spectrum of carotene and of xanthophyll is readily demonstrated from the spectrogram given in Plate 1. Thus, one sees that both carotene and xanthophyll, in alcoholic solution, exhibit three absorption bands of different widths, which also vary in position depending upon the particular carotenoid employed. When carbon disulfide is employed as the solvent, only two bands are visible, and these are placed at a somewhat higher wave length.

The absorption maxima and, in fact, the shape of the absorption curve, are to a great extent related to the molecular structure. The greater the number of conjugated double bonds which occur in an uninterrupted series, the longer are the wave lengths at which absorption will obtain, and the higher on the scale the color which will be exhibited by solutions of such carotenoids. Where the pattern of conjugated double bonds is identical for several compounds as, for example, is the case with β -carotene, cryptoxanthin, and zeaxanthin, the absorption spectra would be expected to be

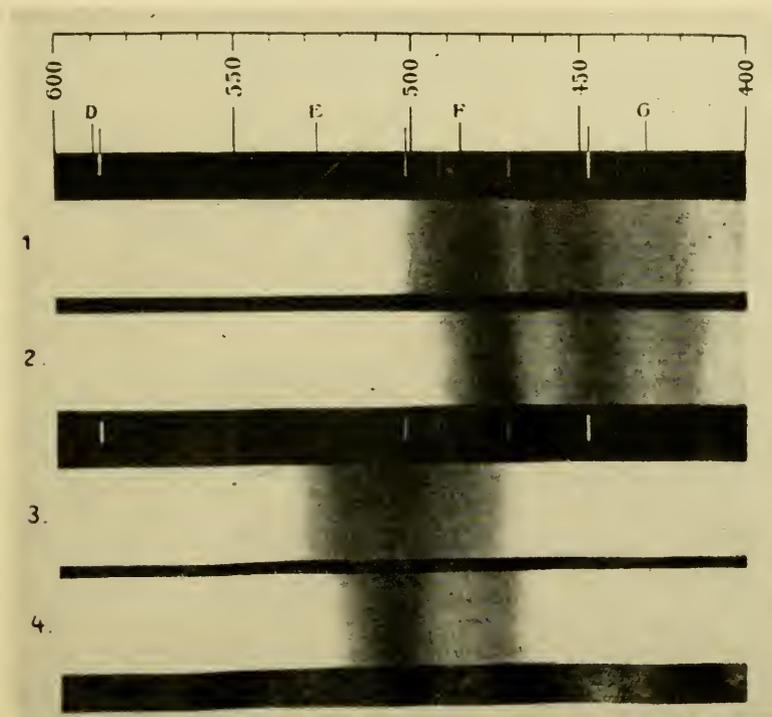


Plate 1. Spectrograms of carotene and xanthophyll according to Willstätter and Stoll²⁸⁷: (1) carotene in alcohol; (2) xanthophyll in alcohol; (3) carotene in carbon disulfide; (4) xanthophyll in carbon disulfide.²³

similar. This hypothesis finds support in the well-known fact that it is practically impossible to differentiate among the above three carotenoids solely on the basis of their spectrometric behavior.

When an alteration in the chromophore system occurs, a marked change in the absorption spectrum always results. On the other hand, a very considerable change in the structure of the molecule can be brought about without any change in the absorption properties, provided that the chromo-

phore system has not been altered. This is beautifully illustrated in the results of Kuhn and Broeckmann,^{56,363} Kuhn and Grundmann,²²⁰ and Kuhn and Deutsch,⁴⁷⁶ summarized in Table 14. The absorption spectra of rhodoxanthin dioxime are considerably shifted toward the ultraviolet portion of the spectrum as compared with those for free rhodoxanthin. The same has been shown to occur when lycopenal is treated with hydroxylamine with the resultant formation of lycopenal oxime. In both of these instances, it is certain that the ketone group which combines with the hydroxylamine must have been a part of the system of conjugated double bonds. On the other hand, the dioximes of dihydrorhodoxanthin and of β -carotenone, as well as the monoximes of semi- β -carotenone and of azafrinone, have absorption spectra identical with those of the corresponding free ketones. Since the β -carotenone is a tetraketone, while it forms only a dioxime, it must be assumed that the two combined ketone groups are not concerned with the conjugated system. A similar relationship exists in the case of the monoxime of the diketone, semi- β -carotenone.

TABLE 14
COMPARISON OF ABSORPTION SPECTRA OF OXIMES OF CAROTENOIDS WITH THOSE OF THE FREE KETONES^a

Compound	Solvent	Absorption maxima, m μ
Ketone groups as part of system of conjugated double bonds		
Rhodoxanthin	Hexane	524, 489, 458
Rhodoxanthin dioxime	Hexane	513, 479, 451
Lycopenal	Petr. ether	525.5, 490.5, 455.5
Lycopenal oxime	Petr. ether	503.5, 471, 442
Ketone groups combined as oxime independent of system of conjugated double bonds		
Dihydrorhodoxanthin	Hexane	480, 449, 422
Dihydrorhodoxanthin dioxime	Hexane	480, 449, 422
β -Carotenone	Petr. ether	502, 468, 440
β -Carotenone dioxime	Petr. ether	502, 468, 440
Semi- β -carotenone	Petr. ether	501, 470, 446
Semi- β -carotenone oxime	Petr. ether	501, 470, 446
Azafrinone	Petr. ether	454, 429
Azafrinone oxime	Petr. ether	454, 429

^a L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, p. 67.

Another factor which may profoundly affect the position of the absorption maxima is the solvent employed. Most dilute solutions of the carotenoids are an intense yellow, while concentrated solutions are deeply orange colored. In carbon disulfide, carotene is reddish brown, in chloroform a brownish yellow, and in ether it appears as a greenish yellow solution. When a cold-saturated solution of carotene in carbon disulfide is poured through filter paper, it produces orange-red spots. Under similar conditions, xanthophyll gives a yellow color, while the lycopene specks are from a flesh-red to a chocolate-brown.^{23,478}

⁴⁷⁸ H. H. Escher, *Zur Kenntnis des Carotins und des Lycopins*. Dissertation, Zürich, 1909. Cited by L. Zechmeister, *Die Carotinoide*, 1934, p. 155.

The effect of solvent on the color of the carotenoid solution is especially marked where the polyene carbonyl or carboxyl group exists in conjugation with the carbon-to-carbon double bond system. For example, rhodoxanthin exhibits a pure red color in alcohol solution, while it appears orange yellow when dissolved in petroleum ether. The absorption maxima of the longest wave lengths are 538 and 521 $m\mu$, respectively, in the two solvents. Kuhn and Brockmann³⁶³ explain these variations by suggesting a reciprocal effect between the polar carbonyl group of the carotenoid and the polar alcohol molecule. Such an effect cannot occur in a non-polar solvent such as petroleum ether. On the other hand, when two hydrogens are added to rhodoxanthin, the resulting dihydrorhodoxanthin no longer shows this anomaly, as the carbonyl groups are no longer conjugated. The latter conditions also explain the failure of zeaxanthin to exhibit such divergent absorption spectra in different solvents.

Marked changes result in the form of the absorption curves when *trans* \rightarrow *cis* changes occur. The absorption maxima occur at the longest wave lengths in the natural all-*trans* carotenoids. With the increase in the number of *cis* linkages, the color of the carotenoid is decreased, since the absorption maxima are shifted toward the ultraviolet end of the spectrum. One of the most characteristic changes which occurs with an increase in *cis* linkages is the development of the so-called "*cis* peak" in the ultraviolet region. Practically no absorption takes place in this area in the case of the all-*trans* compounds, but it may become an area in which the extinction coefficient is of considerable importance after isomerization. The location and intensity of this *cis* peak is of great value in establishing the position and number of the *cis* bonds. A correction for this effect is important when one is determining vitamin A in the presence of carotenoids, as the maximum absorption for the *cis* peak is frequently in close proximity to that employed for the estimation of vitamin A. A further discussion of this effect is included in the section on stereoisomerism of the carotenoids (see pages 621 ff.).

The absorption maxima of the more common C_{40} polyene hydrocarbons and of some of their derivatives in several solvents are included in Table 15, while Table 16 summarizes the same information for the simpler carotenoids.

Not only are the points of maximum absorption of considerable importance in establishing the identity of a substance, but likewise the relative intensities of the various maxima are of great help. In addition, it is frequently of as much value to know the points of minimum absorption. All these data are available in the molecular extinction curves.⁴⁷⁹⁻⁴⁸¹ Data

⁴⁷⁹ L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, **65**, 1522-1528 (1943).

⁴⁸⁰ H. J. Deuel, Jr., C. Johnston, E. R. Meserve, A. Polgár, and L. Zechmeister, *Arch. Biochem.*, **7**, 247-255 (1945).

^{480a} P. Karrer and E. Würgler, *Helv. Chim. Acta*, **26**, 116-121 (1943).

⁴⁸¹ A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, **66**, 186-190 (1944).

TABLE 15. ABSORPTION MAXIMA OF C₄₀ POLYENE HYDROCARBONS AND THEIR DERIVATIVES^a

Compound	Absorption maxima (Å)					λ max. (SbCl ₅ in CHCl ₃)
	Carbon disulfide	Petroleum ether	Chloroform	Hexane	Benzene	
Antheraxanthin	510, 478	—	490.5, 460.5	—	—	—
Aphanacene	533, 494	494, 462	504, 474	—	505, 474	—
Aphanin	533.5, 494	494, 460, (432)	504, 474	—	505, 472	—
Astacene	510	—	—	—	—	—
Auroxanthin	454, 423	—	—	—	—	—
Capsanthin	542, 503	505, 475	—	—	520, 486	—
Capsorubin	541.5, 503, 468	506, 474, 444	—	—	520, 486, 455	—
α-Carotene	509, 477	478, 447.5, 420 ^b , 395 ^b	485, 454	—	—	542 ^c
α-Carotenone	(535), 502, 471	270 ^b	484, 454	—	—	—
β-Carotene	520, 485, 450	483.5, 452, 426	497, 466	482, 451	—	590 ^c
β-Carotenone	538, 499, 466	502, 468, 440	527, 489, 454	500, 466, 436	522, 486, 453	—
β-Carotene oxide	486, 456, 427 ^d	—	465, 437, 410 ^e	—	—	—
γ-Carotene	533.5, 496, 463	495, 462, 431	508.5, 475, 446	494, 462, 431	510, 477, 447	590 ^b
Celaxanthin	562, 521, 487, 455	520, 486.5, 456, (429)	—	—	—	—
Chrysothemenaxanthin	479, 449	450, 421	459, 430	—	—	—
Cryptoxanthin	519, 483, 452	485.5, 452, 424	497, 463, 433	484, 451, 423	—	590 ^e
Dihydrophodoxanthin	514, 479, 448	483, 452, 425	492, 460, 431	—	—	—
Echinone	(520), 488, (450)	—	—	—	—	—
Escholtziaxanthin	536, 502, 475	—	513, 484, 456	—	516, 485, 458	—
Flavoxanthin	479, 449	450, 421	459, 430	—	—	—
Fucoxanthin	510, 477, 445	—	492, 457	—	—	—
Gazaniaxanthin	531, 494.5, 461	494.5, 462.5, 434.5	—	—	509, 476, 447.5	—

Compound	Absorption maxima (m)					λ max. (SpCh ₂ in CHCl ₃)
	Carbon disulfide	Petroleum ether	Chloroform	Hexane	Benzene	
Lutein	508, 475, 445	477.5, 447.5, 420	487, 456, 428	—	—	620, 585 ^b
Lycopene	548, 507.5, 477	506, 475.5, 447	517, 480, 453	—	522, 487, 455	585 ^b
Lycophyll	546, 506, 472	504, 473, 444	—	—	521, 487, 456	—
Lyoexanthin	546, 506, 472	504, 473, 444	—	—	521, 487, 456	—
Mutatoxanthin	488, 459	456, 426	468, 437	—	468, 439	—
Myxoxanthin	488	465	473	—	—	—
Myxoxanthophyll	518, 484, 454	—	—	—	—	—
Pectenoxanthin	518, 488, 454	488, 458	—	—	496, 464, 434	—
Pentaxanthin	506, 474, 444	—	—	—	487, 456, 424	—
Petaloxanthin	514.5, 481	—	492, 460.5	—	494, 460.5	—
Rhodopin	547, 508, 478	501, 470, 440	521, 486, 453	—	—	—
Rhodopurpurin	550, 511, 479	502, 472	527, 487, (458)	—	527, 490	—
Rhodoviolaicin	573.5, 534, 496	—	544, 507, 476	—	548, 511, 482	—
Rhodoxanthin	564, 525, 491	521, 487, 456	546, 510, 482	—	542, 503.5, 474	—
Rubixanthin	533, 494, 461	495.5, 463, 432	509, 474, 439	494, 462, 432	—	595, 535 ^c
Taraxanthin	501, 469, 441	472, 443	—	—	—	—
Violaxanthin	501, 470, 440	472, 443, 417.5	482, 451.5, 424	—	—	615, 585 ^b
Zeaxanthin	517, 482, 450	483.5, 451.5, 423	495, 462, 429	—	—	621, 587 ^b

^a Data from P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basle, 1948, unless otherwise noted.

^b R. A. Morton, *The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes*, 2nd ed., Jarrell-Ash Co., Boston, 1942, pp. 60-61, Chart V.

^c P. Karrer and O. Walker, *Helv. Chim. Acta*, 16, 641-643 (1933).

^d I. M. Heilbron, W. E. Jones, and A. L. Bacharach, *Vitamins and Hormones*, 2, 155-213 (1944).

^e J. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934.

TABLE 16
 ABSORPTION MAXIMA OF POLYENES CONTAINING LESS THAN 40 CARBONS IN SEVERAL SOLVENTS^a

Compound	Absorption maxima (λ)		
	Carbon disulfide	Petroleum ether	Chloroform
Bixin, labile	523.5, 489, 457	503, 470 ^b	503, 469.5, 439
stable	526.5, 491, 457	508.5, 475 ^b	509.5, 475, 443
Bixin dialdehyde	539.5, 502, 467.5	502, 468, 437.5	528, 490
Norbixin, labile	527, 491, 458	—	503, 469.5, 440
stable	527.5, 492, 457.5	—	509, 474.5, 442
Methyl bixin, labile	519.5, 485.5, 454	490, 458, 429.5	503, 470, 441
stable	525.5, 490, 456.5	—	509.5, 476, 444
	526.5, 490, 457	—	—
	429 ^c	—	—
Crocein, labile	—	—	—
stable	482, 453, 426 ^d	—	463, 435.5 ^e
	—	—	463, 434.5 ^d
Crocein dimethyl ester, labile	—	445, 422 ^e	458, 432.5 ^e
stable	478.5, 448, 421 ^c	450.5, 424.5 ^e	463, 434.5 ^e
Azafrin	—	—	464, 436, 411 ^c
Azafrin methyl ester	476, 445.5, 419 ^e	447, 422.5 ^e	458, 428 ^e

^a Data are from R. Kuhn and A. Winterstein, *Ber.*, 65, 646-651 (1932), unless otherwise noted.

^b L. Zeelmeister, *Chem. Revs.*, 34, 267-344 (1944).

^c R. Kuhn, A. Winterstein, and H. Roth, *Ber.*, 64, 333-341 (1931).

^d P. Karrer and E. Jucker, *Carotinoide*, Birkhauser, Basle, 1948.

^e R. Kuhn and A. Winterstein, *Ber.*, 66, 209-214 (1933).

are included in Figure 4 for ordinary (*all-trans*)- β -carotene in hexane, while the curves for some of the commoner carotenoids are in Figures 5 to 13 on pages 614–618.

The values reported for the molecular extinction of various carotenoids in several solvents are found in Table 17 and in Table 18 on page 619.

TABLE 17
EXTINCTION COEFFICIENTS OF THE CAROTENES AT POINTS OF MAXIMUM AND MINIMUM ABSORPTION^a

Carotene	Solvent ^b	Absorption A.	Extinction coefficients	
			<i>ε</i> _{mol.}	<i>E</i> (1%, 1 cm.)
α-	20% ether, 80% eth.	Max. 2470	23,900	420
		Min. 3100	5,680	100
		Max. 4450	146,500	2,580
		Min. 4630	106,800	1,880
		Max. 4750	129,500	2,280
β-	20% ether, 80% eth.	Min. 5050	0	0
		Max. 2500	21,400	400
		Min. 3200	6,440	120
		Max. 4530	113,400	2,500
		Min. 4720	112,600	2,100
	Chloroform	Max. 4800	118,000	2,200
		Min. 5200	0	0
		Max. 2800	20,100	385
		Min. 3200	6,970	130
		Max. 3500	15,510	289
		Min. 3625	12,000	224
		Max. 4350	94,400	1,764
		Min. 4450	93,200	1,730
γ-	Hexane	Max. 4620	101,400	1,892
		Min. 4800	91,200	1,700
		Max. 4920	94,300	1,760
		Max. 4075	11,500	229
		Min. 4125	11,000	205
		Max. 4350	23,900	429
		Min. 4400	22,500	420
		Max. 4600	34,000	634
		Min. 4800	23,000	429
		Max. 4900	28,500	516
		Min. 5250	0	0

^a Adapted from J. R. Loofbourov, *Vitamins and Hormones*, 1, 109–155 (1943).

^b Eth. = ethanol.

(3) Solubility

The carotenoids are characterized as lipids because of their ready solubility in the fat solvents. However, marked differences in solubility exist between the carotenoids belonging to the group which are strictly hydrocarbons and the group of carotenols. These differences are sufficiently pronounced to offer a satisfactory criterion for the quantitative differentiation of one group from the other when they are present in the form of a mixture.

a. Separation of the Carotenes from the Xanthophylls. When the



Fig. 4. Molecular extinction curve for β -carotene in hexane.⁴⁷⁹

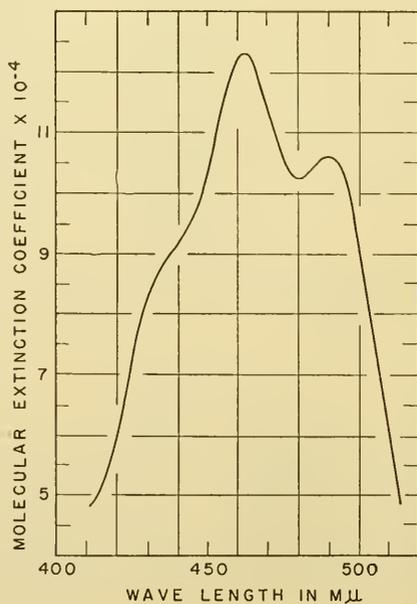


Fig. 5. Molecular extinction curve for β -carotene in Wesson oil.⁴⁸⁰

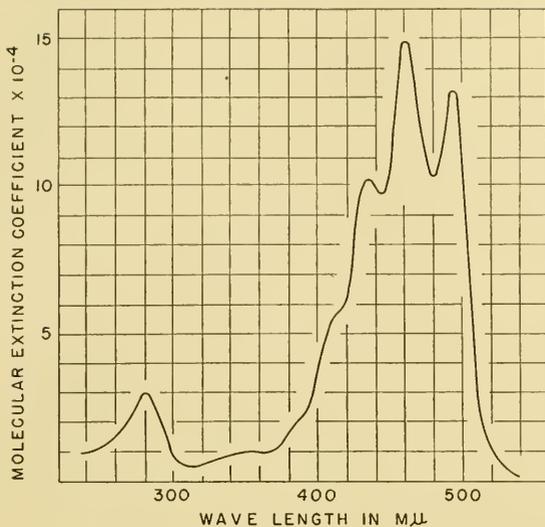


Fig. 6. Molecular extinction curve for α -carotene in hexane.⁴⁷⁹

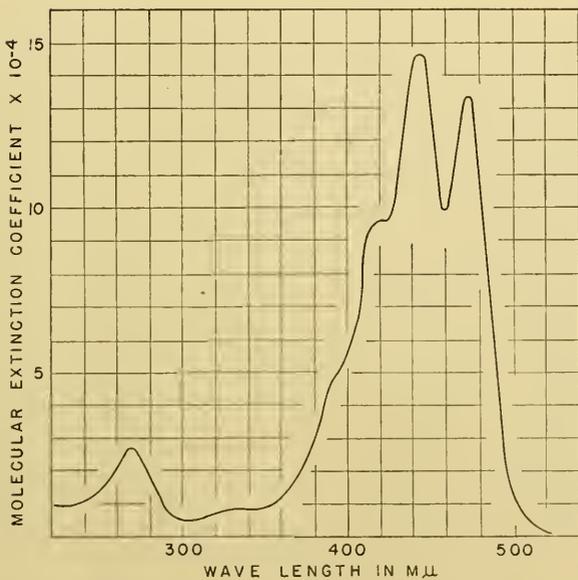


Fig. 7. Molecular extinction curve for γ -carotene in hexane.²⁴³

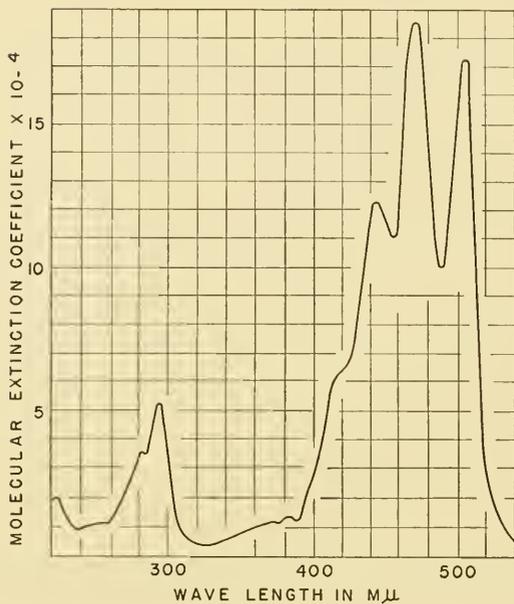


Fig. 8. Molecular extinction curve for lycopene in hexane.²⁴²

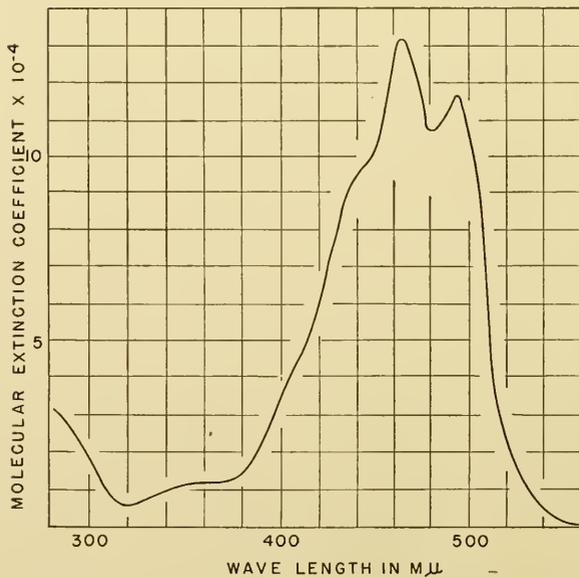


Fig. 9. Molecular extinction curve for cryptoxanthin in benzene.²⁷¹

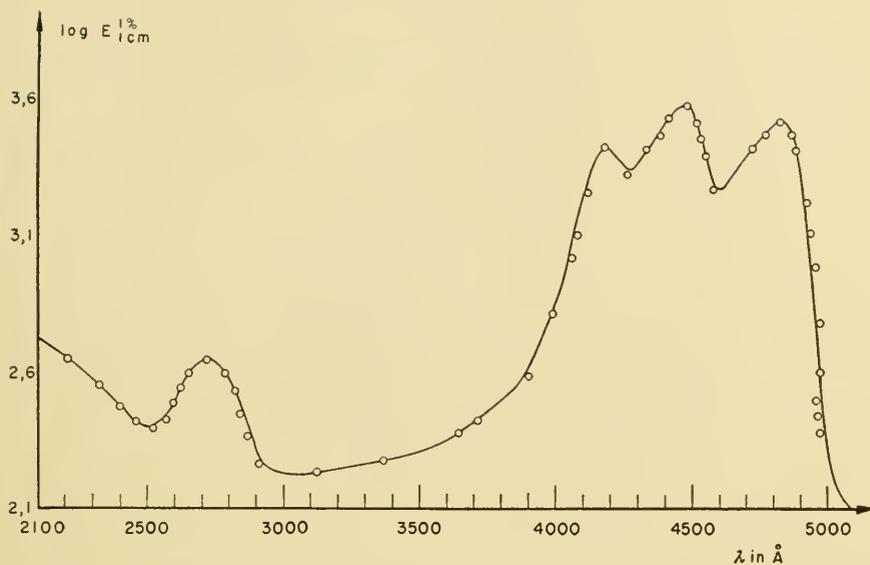


Fig. 10. Molecular extinction curve for lutein in hexane.⁴⁸⁰⁴

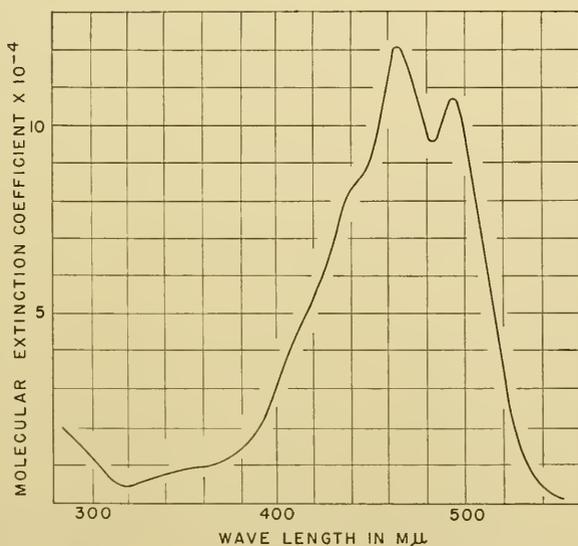


Fig. 11. Molecular extinction curve for zeaxanthin in benzene.²⁷¹

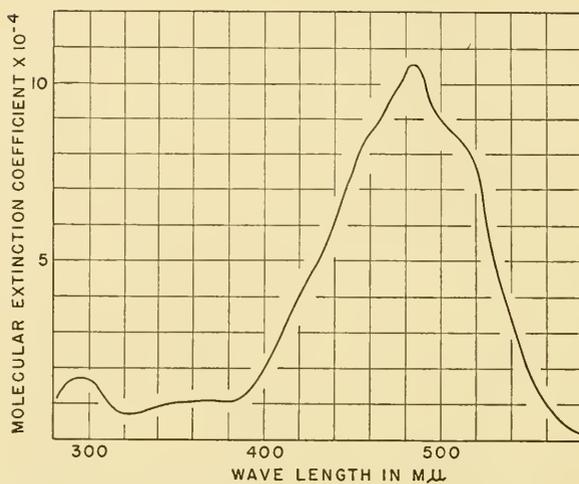


Fig. 12. Molecular extinction curve for capsanthin in benzene.⁴⁸¹

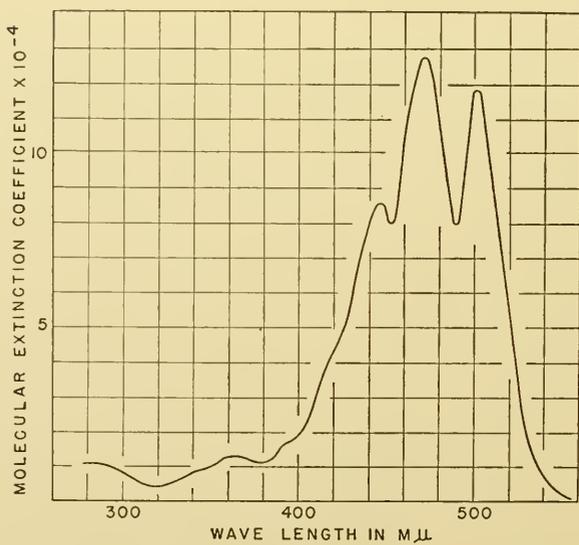


Fig. 13. Molecular extinction curve for natural methylbixin in benzene.⁴⁵⁰

TABLE 18
MOLECULAR EXTINCTION COEFFICIENTS OF SOME CAROTENOIDS AS MEASURED IN
BECKMAN SPECTROPHOTOMETER

Carotenoid	Molecular extinction $\times 10^{-4}$ at λ , max.		
	Hexane	Benzene	Ethanol
α -Carotene	14.6 ^a (445-446)	—	—
Neo- α -carotene U	11.8 ^b (441)	—	—
β -Carotene	14.2 ^a (452)	—	—
Neo- β -carotene U	13.5 ^b (446-447)	—	—
γ -Carotene	14.6 (461-462)	—	—
Pro- γ -carotene	11.2 ^b (457)	—	—
Lycopene	18.6 ^a (472-473)	—	—
Polycopene	10.3 ^b (438)	—	—
Cryptoxanthin	—	13.4 ^c (452.5)	—
Neo-cryptoxanthin U	—	11.3 ^d (458)	—
Neo-cryptoxanthin A	—	10.8 ^d (456)	—
Neo-cryptoxanthin B	—	12.4 ^d (456)	—
Zeaxanthin	—	12.0 ^c (462.5)	—
Capsanthin	—	10.5 ^c (484)	10.3 ^c (476)

^a L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1944).

^b Personal communication from Professor L. Zechmeister.

^c L. Zechmeister and R. M. Lemmon, *J. Am. Chem. Soc.*, 66, 317-322 (1944).

^d A. Chatterjee and L. Zechmeister, *J. Am. Chem. Soc.*, 72, 254-256 (1950).

^e A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, 66, 186-190 (1944).

carotenoids are distributed between a mixture of diethyl ether and petroleum ether on the one hand, and 85% methyl alcohol on the other hand, one finds that α -, β -, and γ -carotene, lycopene, and esters of the carotenols (such as the colored fats) are present almost exclusively in the upper ether layer. They are referred to as being *epiphasic*. The carotenols, such as lutein (xanthophyll), zeaxanthin, violaxanthin, taraxanthin, fucoxanthin, and unesterified capsanthin, are to be found practically quantitatively in the aqueous methanol layer on the bottom (*hypophasic*). Although the monohydroxy carotenoids may be epiphasic with respect to 85% methanol, they are hypophasic, or largely so, with respect to 95% methyl alcohol. One can effect a further separation by saponification of the carotene fraction, after which the carotenols set free by hydrolysis of the colored fats can be extracted from the carotene with 85% methanol.

b. Solubilities of Individual Carotenoids. A consideration of the method of separation of the hydrocarbon carotenoids from the carotenols demonstrates the fundamental differences in the solubility properties of the two groups. The hydrocarbons dissolve readily in diethyl and petroleum ethers, while they are only sparingly soluble in ethanol, and only slightly so in methanol. The carotenols, on the other hand, are quite soluble in alcohol but are relatively insoluble in petroleum ether. The carotenoid alcohols are, however, quite soluble in diethyl ether.

The solubilities of carotene (chiefly β -carotene), lycopene, xanthophyll, and zeaxanthin in some of the commoner solvents are summarized in Table 19.

TABLE 19
SOLUBILITIES OF SOME CAROTENOIDS IN SEVERAL LIPID SOLVENTS IN THE COLD AND WHEN HEATED

Carotenoid	Solubility, ^a mg. per liter solvent							
	Petr. ether		Diethyl ether		CS ₂	Ethanol	Methanol	
	Hot	Cold	Hot	Cold	Cold	Cold	Hot	Cold
Carotene	667 ^b	—	1111 ^b	—	RS	15.5 ^c	—	—
Lycopene	101 ^d	—	333 ^d	—	RS	SD	—	—
Lutein (xanthophyll)	—	9.5 ^e	3333 ^d	952 ^e	SD	201.5 ^e	1430 ^f	134.9 ^e
Zeaxanthin	—	—	—	—	—	—	645 ^f	—

^a RS, readily soluble. SD, soluble with difficulty.

^b R. Willstätter and W. Mieg, *Ann.*, *355*, 1-28 (1907).

^c F. M. Schertz, *J. Agr. Research*, *30*, 469-474 (1925).

^d R. Willstätter and H. H. Escher, *Z. physiol. Chem.*, *64*, 47-61 (1910).

^e F. M. Schertz, *J. Agr. Research*, *30*, 575-585 (1925).

^f R. Kuhn, A. Winterstein, and E. Lederer, *Z. physiol. Chem.*, *197*, 141-160 (1931).

Chloroform is an excellent solvent for most carotenoids. Glacial acetic acid will dissolve xanthophyll readily when heated, but only slowly in the cold. Fats are also good solvents, although it must be recalled that, in the case of carotenols, the pigments in natural fats do not usually occur in simple solution but esterified as the so-called colored fats. Glycerol is devoid of any solvent action for the carotenoids in the cold or when heated. Benzene is a good solvent, especially when heated.

c. Colloidal Suspensions of Carotene. Water is entirely ineffective as a solvent for carotene or for any of the other carotenoids. However, carotene can be prepared in colloidal form by the method of Fodor and Schoenfeld,⁴⁸² as well as by the procedure of Karrer *et al.*⁴⁸³ Sexton and co-workers⁴⁸⁴ have also made fairly permanent aqueous carotene suspensions by stabilizing with lecithin or propylene glycol.

⁴⁸² A. Fodor and R. Schoenfeld, *Biochem. Z.*, *233*, 243-244 (1931).

⁴⁸³ P. Karrer, H. v. Euler, H. Hellström, and M. Rydbom, *Svensk Kem. Tid.*, *43*, 105-109 (1931).

⁴⁸⁴ E. L. Sexton, J. W. Mehl, and H. J. Deuel, Jr., *J. Nutrition*, *31*, 299-319 (1946).

(4) *Stereochemical Behavior of the Carotenoids*

a. Introduction. The phenomenon of stereochemical or geometrical isomerism has been recognized for a number of years as a property of organic compounds possessing a double bond. Thus, the interconversion of oleic and elaidic acids has long been explained as a *trans-cis* rearrangement. However, the application of this type of isomerism to the carotenoids has been recognized only recently. The first interpretation of such a relationship among the carotenoids was furnished by Karrer and his co-workers⁴⁴³ in 1929. They suggested that the second form of bixin, discovered by Herzig and Faltis⁴⁴⁶ six years earlier, and the original bixin had a *cis-trans* relationship.

In 1935, Gillam and El Ridi⁴⁸⁵ were the first workers to point out the stereochemical isomerism in the C₄₀ carotenoids. When homogeneous β -carotene was washed from an alumina column with petroleum ether and then reabsorbed on such a column, it was found to separate into two bands which showed the characteristics of β - and of α -carotene (pseudo- α -carotene), respectively. On subsequent elution of either band followed by rechromatography, two similar zones were again formed. Such isomerization was therefore shown to be reversible and never complete. These workers named the new isomer "pseudo- α -carotene"; they recognized it as being distinct from α -carotene. On similar treatment, α -carotene exhibited a like phenomenon, but the new pigment possessed maxima at shorter instead of at longer wave lengths. In further studies on the *cis-trans* rearrangements of β -carotene, Gillam and co-workers^{486,487} proved the reversibility of the change, and discussed the possibility of geometrical isomerism.

Although the British workers attributed this isomerism to the action of the adsorbent in the chromatographic column, subsequent work of Zechmeister and his collaborators has demonstrated that the changes are spontaneous, and are independent of the process of adsorption. Thus, it was shown that capsanthin solutions undergo a spontaneous and reversible change.³⁷⁷ Furthermore, it was almost simultaneously demonstrated that similar changes could be produced, in β -carotene, lycopene, and cryptoxanthin solutions, which were not related to the adsorption process.^{488,489} Finally, where such a *cis-trans* rearrangement was accompanied by a simultaneous alteration in optical rotation, Zechmeister and Tuzson⁴⁹⁰ were able to demonstrate the change without the use of the Tswett column

⁴⁸⁵ A. E. Gillam and M. S. El Ridi, *Nature*, **136**, 914-915 (1935).

⁴⁸⁶ A. E. Gillam and M. S. El Ridi, *Biochem. J.*, **30**, 1735-1742 (1936).

⁴⁸⁷ A. E. Gillam, M. S. El Ridi, and S. K. Kon, *Biochem. J.*, **31**, 1605-1610 (1937).

⁴⁸⁸ L. Zechmeister and P. Tuzson, *Nature*, **141**, 249-250 (1938).

⁴⁸⁹ L. Zechmeister and P. Tuzson, *Biochem. J.*, **32**, 1305-1311 (1938).

⁴⁹⁰ L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340-1346 (1939).

for the separation of the components. The marked effect of heat in producing *cis-trans* isomerism was demonstrated by Strain,³¹¹ while the use of iodine as a catalyst of *cis-trans* changes for the C₄₀ carotenoids was discovered by Zechmeister and Tuzson.⁴⁹⁰ The subject of *cis-trans* isomerization and stereochemistry of the carotenoids has recently been reviewed by Zechmeister.⁴⁵¹

b. Nomenclature. Although considerable confusion previously existed in the terminology of various stereoisomers of the carotenoids, it would seem that such difficulties can now be avoided by the application of the nomenclature employed by Zechmeister.⁴⁹¹ This system is a logical one, since it is based upon the structural relationship of the various stereoisomers.

According to the Zechmeister usage, when the pigment is referred to by the usual name, for example, β -carotene, this designation refers to the all-*trans* form in which every double bond possesses a *trans* configuration. The all-*cis* form is applied to compounds in which all the double bonds, which are spatially unhindered, have assumed the *cis* arrangement; however, these all-*cis* compounds will still possess a number of *trans* linkages which cannot be changed to the *cis* form because of spatial considerations. In addition to these two types of stereoisomers, there are a great number of isomers in which one or more of the double bonds have assumed the *cis* arrangement. Each double bond in the chromophore system is numbered serially, and these numbers are italicized to avoid confusion with the numbering of the carbon atoms. The lowest number is applied to the unsaturated linkage nearest the β -ionone ring. If such a cyclic system is absent, the lowest number is assigned to the linkage adjacent to an α -ionone group. The conjugated linkages in or near open groups or terminal aliphatic groups are given the highest numbers. Special rules apply in each individual case for numbering the double bonds in entirely aliphatic or unsymmetrical molecules.

In addition to the terminology based upon the known configuration of the double bonds, Zechmeister has employed a temporary nomenclature for the differentiation of the isomers, based upon relative ease of adsorbability on the chromatographic column. The isomers which have a weaker adsorbability than the all-*trans* form are referred to as neo-isomers A, B, C, etc. Thus, neo- β -carotene A will appear on the chromatographic column directly under all-*trans*- β -carotene; the isomer having weaker adsorption than A and appearing directly under it in the chromatographic column is called neo- β -carotene B. The isomers with progressively weaker adsorbability are called C, D, E, etc. In the cases in which the stereoisomers are more readily adsorbed than the all-*trans* form, those with increased affinity

⁴⁹¹ L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, 66, 137-144 (1944).

are designated as neo-T, U, V, etc. Some inconsistency does result, however, since the stereoisomers of lutein, zeaxanthin, and capsanthin which are adsorbed more strongly than the all-*trans* forms are assigned letters at the beginning of the alphabet.

In addition to the "neo"-*cis* isomers, which are admittedly synthetic products, certain partial *cis* derivatives have been found in nature. Such compounds are designated by the prefix "pro" in place of "neo." Two such compounds which have been reported in a number of natural products are pro- γ -carotene and prolycopene.

c. Configuration of Natural Carotenoids. By far the largest proportion of the carotenoids which occur in nature is of the all-*trans* variety. Proof that such is the case has been established by x-ray studies on crystalline carotenoids,^{492,493} by interpretation of their absorption spectra,⁴⁹⁴⁻⁴⁹⁷ and most recently by an examination of solutions of such pigments on a chromatographic column. A preponderance of the all-*trans* form would be expected, since these isomers have the greatest stability and the lowest energy content.

A number of exceptions to the general rule of all-*trans* isomers have been reported. One of the first observations was of the occurrence of both *trans*- and *cis*-crocetin in the gentiobiosides of the saffron stigma. Bixin, which has been isolated from the seed hulls of the tropical anatto tree (*Bixa orellana* L.), is the *cis* form. Herzig and Faltis⁴⁴⁶ also prepared the *trans* product from orleán.

In the case of C₄₀ carotenoids, Zechmeister *et al.*²⁴² were the first to obtain qualitative evidence of the presence of a *cis*-lycopene (prolycopene) and a *cis*- γ -carotene (pro- γ -carotene). The final proof that such carotenoids exist in the plant was afforded by the isolation of the crystalline product from several natural sources. While the absorption curves of the *cis-trans* pairs of bixin and crocetin have visible spectral maxima only a few millimicrons apart, the maxima of prolycopene and of pro- γ -carotene are 35 m μ and 31 m μ lower, respectively, than the corresponding all-*trans* isomers. Prolycopene has been shown to contain 6 *cis* bonds, while pro- γ -carotene has 5 such linkages.

The comparatively wide distribution of the *cis* carotenoids is indicated by the fact that, in the 6 plants from which they have been isolated, 5 families are represented. There is also some indication that the procarotenoids may occur during development and that possibly they exist as

⁴⁹² J. Hengstenberg and R. Kuhn, *Z. Kryst. Mineral. Petrog.*, **A75**, 301-310 (1930); **A76**, 174-176 (1931).

⁴⁹³ G. MacKinney, *J. Am. Chem. Soc.*, **56**, 488 (1934).

⁴⁹⁴ R. S. Mulliken, *Science*, **87**, 427-428 (1938).

⁴⁹⁵ R. S. Mulliken, *J. Chem. Phys.*, **7**, 364-374 (1939).

⁴⁹⁶ R. S. Mulliken, *Rev. Modern Phys.*, **14**, 265-274 (1942).

⁴⁹⁷ L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, **3**, 203-235 (1939).

TABLE 20
 OCCURRENCE OF POLYCOPENE AND PRO- γ -CAROTENE IN SOME PLANTS^a

Family	Plant	Common name	Organ	Crystals isolated (mg. per kg.)	
				Polycopene	Pro- γ - carotene
<i>Solanaceae</i>	<i>Lycopersicon esculentum</i> Mill.	Tangerine tomato, common tomato	Fruit pulp	20 ^b	—
<i>Palmae</i>	<i>Butia capitata</i> Becc.	Brazilian butia palm	Fruit pulp	Some ^c	0.3 ^d
	<i>Butia eritospatha</i> Becc.	Woolly butia palm	Fruit pulp	Some ^c	Some ^d
<i>Pomoideae (Rosaceae)</i>	<i>Pyracantha angustifolia</i> Schneid.	Narrow-leaf firethorn	Whole fruit	28.4 ^e	27.7 ^e
	<i>Evonymus fortunei</i> L.	Winter creeper	Seeds	11 ^f	0.5 ^f
<i>Celastraceae</i>	<i>Mimusulus longiflorus</i> Grant	Bush monkey-flower	Petals	Some ^g	Some ^g

^a Modified from Table of L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1944).

^b A. L. Le Rosen and L. Zechmeister, *J. Am. Chem. Soc.*, 64, 1075-1079 (1942).

^c L. Zechmeister and W. A. Schroeder, *Science*, 94, 609-610 (1941).

^d L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.*, 64, 1173-1177 (1942).

^e L. Zechmeister and W. A. Schroeder, *J. Biol. Chem.*, 144, 315-320 (1942).

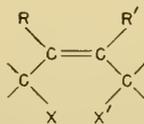
^f L. Zechmeister and R. B. Escue, *J. Biol. Chem.*, 144, 321-323 (1942).

^g L. Zechmeister and W. A. Schroeder, *Arch. Biochem.*, 1, 231-238 (1942).

intermediates. In line with this idea, Schroeder¹⁹⁶ showed that if the buds and stems of the monkey-flowers (*Mimulus longiflorus* Grant) are placed in water and allowed to open in diffuse light, substantial amounts of prolycopene and pro- γ -carotene are present, while only lycopene and γ -carotene result when the buds are allowed to develop on the intact plant in the open. Zechmeister⁴⁵¹ also calls attention to another similar example, *i.e.*, that some flowers show a little yellow color if there is a reduced amount of sunshine during their developmental period, while the usual deep orange color characteristic of the all-*trans* carotenoids develops only during prolonged clear weather. Probably such changes *in situ* are related to the external light available. However, it is known that the *cis* structures can be maintained for long periods in the vegetable tissues exposed to intense sunlight, although the same pigments would be extremely sensitive when insolated in solutions.

d. Possibilities for Steric Changes in the Polyenes. As recently as 1931, it was stated by Wittig and Wiemer⁴⁹⁸ that when the number of conjugated double bonds increases, the phenomenon of *cis-trans* isomerism becomes less important and finally disappears as a result of the increased mobility of the valence electrons. This hypothesis was questioned by Kuhn,⁴⁹⁹ who stated: "The fact that the higher diphenylpolyenes are known only in one spatial form is due to the inadequacy of the preparative methods." Zechmeister⁴⁵¹ concluded that the "length of the conjugated double bond system does not affect its ability to assume various *cis-trans* configurations; in some carotenoid sets a dozen stereoisomers have been observed."

Pauling⁴⁹⁷ demonstrated that only certain of the double bonds are available for spatial rearrangement. The unsaturated linkages are thus divided into two groups, the first of which is "stereochemically effective" and the second of which is "stereochemically ineffective." In the C₄₀ carotenoid which is composed of dehydrogenated isoprene residues, it can be shown that only one double bond in each C₅ unit in the aliphatic chain is able to assume a *cis* configuration. Only those double bonds having hydrogen atoms on the two carbon atoms adjacent to the carbons joined by the double bonds are capable of existing in *cis* form. In a chain with the structure, CX—CR=CR'—CX, the *cis* form would assume the following spatial arrangement:



⁴⁹⁸ G. Wittig and W. Wiemer, *Ann.*, 483, 144-156 (1930).

⁴⁹⁹ R. Kuhn, in K. Freudenberg, *Stereochemie*, Vol. II, Deuticke, Leipzig, and Vienna, 1932, pp. 915-920.

It has been calculated that if X and X' are both hydrogen atoms, they would be 1.7 Å. apart. Although this is somewhat less than the usual distance of the van der Waals contact, a molecule of this type can exist by removing the strain to a considerable extent by small rotations out of the coplanarity. If, on the other hand, X or X' is replaced by a methyl group, the distance is only 1.6 Å., which is about one-half the distance of the van der Waals contact. Such a compound would therefore be highly unstable and could not exist.⁴⁹⁷ The spatial relationship when two hydrogens or one hydrogen and one methyl group occupy the positions α and α' in relation to the double bond is illustrated graphically in Figure 14.

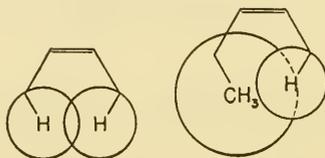


Fig. 14. A graphic illustration of the positions occupied by two hydrogen atoms or by one hydrogen atom and one methyl group in α, α' -positions to a double bond when a *cis* configuration exists.^{451, 497}

The number of theoretically possible stereoisomers is therefore much lower in the carotenoid molecule which contains branched chains than in the unbranched aliphatic compound containing the same number of conjugated double bonds. The number is also lower when the carotenoids are symmetrical, *i.e.*, the two halves of the molecule are identical, than when the compounds are unsymmetrical, *i.e.*, the two halves of the molecule are dissimilar. If n is the number of stereochemically effective double bonds, the possible isomers can be calculated by the following equations:

$$\text{Unsymmetrical: } N = 2^n$$

Symmetrical:

$$n = \text{odd} \quad N = 2^{(n-1)/2} \cdot (2^{(n-1)/2} + 1)$$

$$n = \text{even} \quad N = 2^{(n/2)-1} \cdot (2^{n/2} + 1)$$

The number of such possible isomers is calculated in Table 21.

Since the natural carotenoids contain only 4 to 7 stereochemically effective double bonds, the largest number of stereoisomers possible is 128, which is the case with lycopanthin and rhodoviolascin.²⁴⁰ The total number of isomers for the common carotenoids is summarized in Table 22.

TABLE 21
NUMBERS OF *cis-trans* ISOMERS FOR UNSYMMETRICAL AND SYMMETRICAL CHAINS
CONTAINING n STEREOCHEMICALLY EFFECTIVE DOUBLE BONDS^a

Unsymmetrical chains		Symmetrical chains	
n	Number of isomers	n	Number of isomers
1	2	1	2
2	4	2	3
3	8	3	6
4	16	4	10
5	32	5	20
6	64	6	36
7	128	7	72
8	256	8	136
9	512	9	272
10	1024	10	528
11	2048	11	1056
12	4096	12	2080

^a L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1944), p. 277.

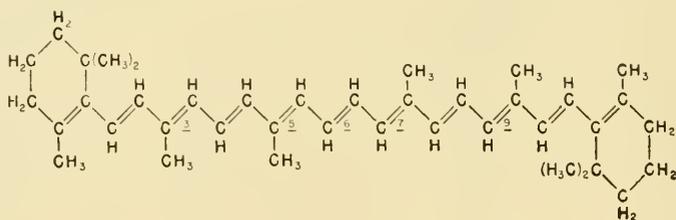
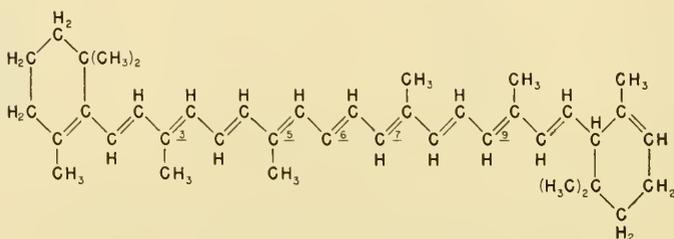
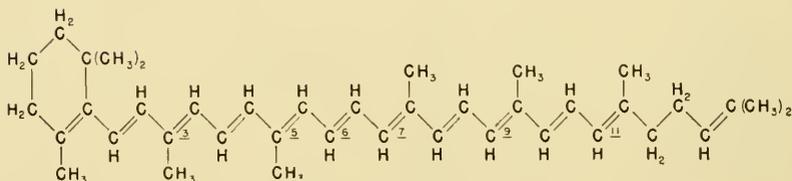
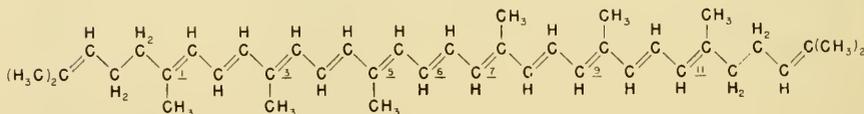
TABLE 22
CALCULATED NUMBER OF STEREOISOMERS FOR NATURALLY OCCURRING CAROTENOIDS
AND FOR SOME OF THEIR CONVERSION PRODUCTS^a

Unsymmetrical molecules ^b			Symmetrical molecules ^b		
Pigment	(1)	(2)	Pigment	(1)	(2)
α -Carotene	5	32	β -Carotene	5	20
γ -Carotene	6	64	Lycopene	7	72
Cryptoxanthin	5	32			
Rubixanthin	6	64			
Gaxaniaxanthin	6	64			
Lyeoxanthin	7	128			
Lutein	5	32	Zeaxanthin	5	20
Rhodoviolascin	7	128	Physalien	5	20
			Lycophyll	7	72
Capsanthin	5	32	Rhodoxanthin	5	20
			Capsorubin	5	20
			Astacene	5	20
			Astaxanthin	5	20
			β -Carotenone	5	20
Semi- β -carotenone	5	32			
α -Citaurin	5	32			
β -Citaurin	5	32			
Bixin	5	32	Methylbixin	5	20
			Norbixin	5	20
Methylcrocetin	5	32	Crocetin	5	20
			Dimethylcrocetin	5	20
Azafrin	4	10			
Vitamin A	2	4			

^a L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1944), p. 278.

^b Column (1): number of effective double bonds. Column (2): calculated number of isomers.

The structural formulas for all-*trans* α -, β -, and γ -carotenes as well as for all-*trans*-lycopene are given here, showing the stereochemically effective double bonds, which are numbered by underlined figures.

All-*trans*- β -caroteneAll-*trans*- α -caroteneAll-*trans*- γ -caroteneAll-*trans*-lycopene

Skeleton models of the 20 possible stereoisomers of β -carotene are included in Figure 15. In addition to all-*trans*- β -carotene (I), these include the three mono-*cis*- β -carotenes, the six di-*cis*- β -carotenes, the six *tri-cis*- β -carotenes, the three tetra-*cis*- β -carotenes, and the all-*cis*- β -carotene.

The structural formulas for all-*trans*-lycopene and for several stereochemical isomers are included in Figure 16. This method of presentation attempts to represent the relative space occupied by the hydrogen atoms and the methyl groups, and affords the most accurate picture of molecular shape which can be given on a two-dimensional drawing.

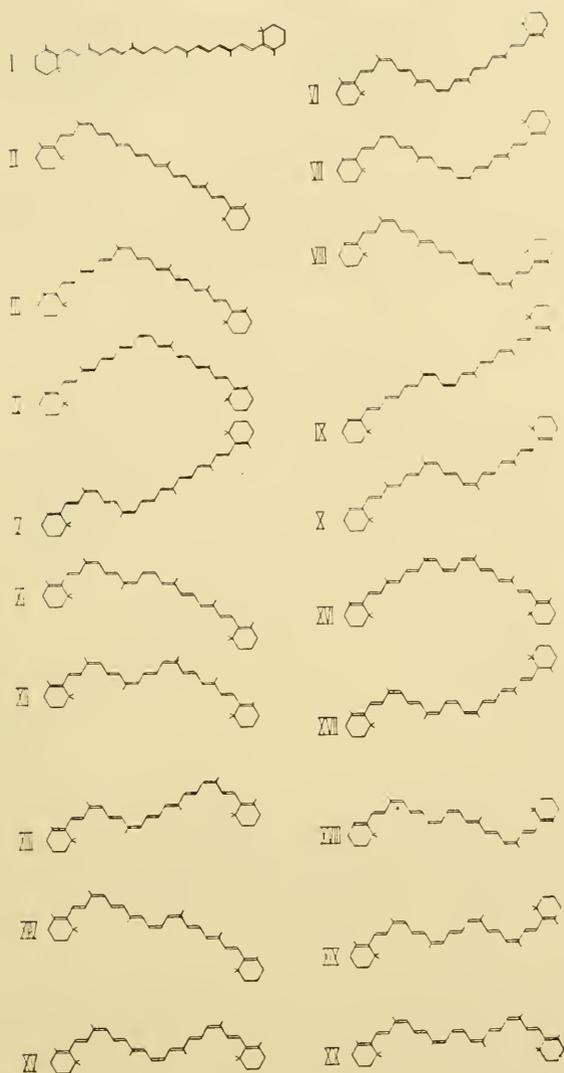


Fig. 15. Skeleton models of the 20 possible stereoisomers of β -Carotene, according to Zechmeister.⁴⁵¹

e. Methods of Producing Stereoisomeric Changes. There are a number of methods which can be employed to bring about stereochemical changes in the all-*trans* isomers. Under such treatment, an equilibrium mixture of a number of different isomers results, the all-*trans* form usually being present in the preponderant amount. If one uses as the starting material one of the *cis* isomers instead of the all-*trans* compound, a similar

equilibrium mixture obtains after treatment, since the isomerization is reversible. When any stereoisomer is subjected to the treatment by which it was produced, only members of the same stereochemical set can appear.

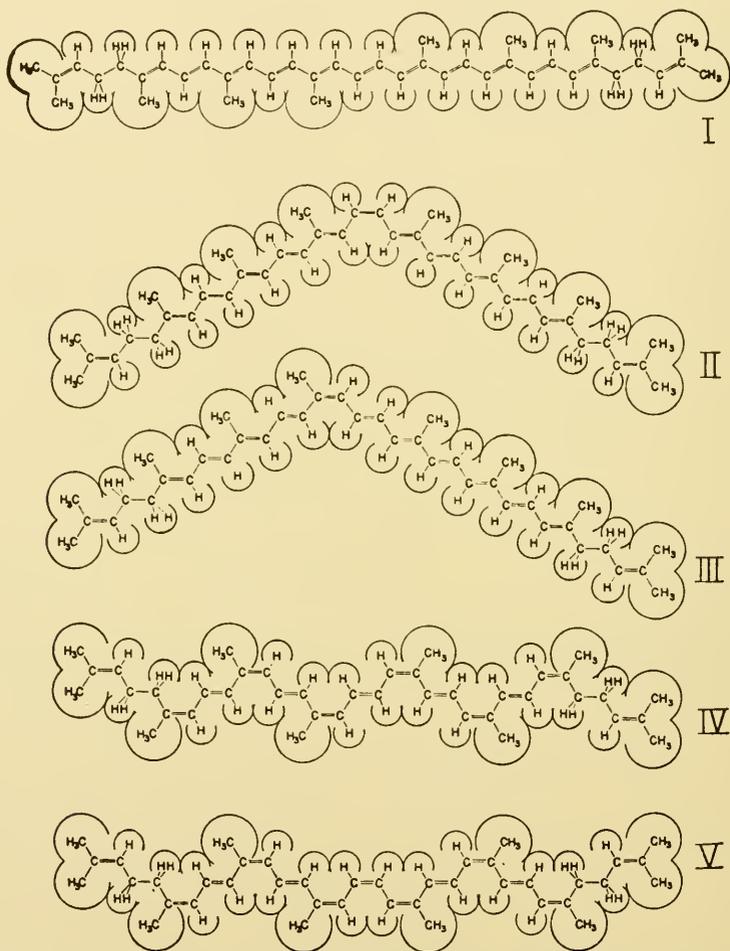


Fig. 16. Suggested stereochemical structures of some members of the lycopene set; these include all-*trans*-lycopene (I), neolycopene A (II), 5-*cis*-lycopene (III), prolycopene (IV), and all-*cis*-lycopene (V).²⁴²

The all-*trans* carotenoids are stable as long as they are in the crystalline state and are not subjected to heating or over-exposed to oxidation. However, as soon as they are dissolved, they become readily susceptible to such catalysts as iodine and acid, as well as to photo-isomerization, or to thermal isomerization in solution.

TABLE 23
STEREISOIMERS FORMED BY MELTING CRYSTALS OF CAROTENOIDS^a

Starting material	Temperature, °C.	Duration of fusion, min.	All-trans	Ratio of stereoisomers												
				Neo U	Neo V	Neo W	Neo X	Neo A	Neo B	Neo C	Neo D	Neo E	Labile	Mixed		
All-trans- α -carotene ^b	195-200	15	35	12	6	19	7	—	—	14	—	—	7	—	—	—
Neo- α -carotene U ^b	74	15	23.5	66	—	—	7	—	—	3.5	—	—	—	—	—	—
All-trans- β -carotene ^c	190	15	33	19	4	—	—	8	24	—	—	—	—	8	4	—
Neo- β -carotene U ^c	135	15	22	40	—	—	—	—	—	—	—	—	—	—	—	38 ^d
All-trans-cryptoxanthin ^e	170	2	Mostly unchanged	0	—	—	—	Some	—	—	—	—	—	—	—	—
	170	10	48	22	—	—	—	30	—	—	—	—	—	—	—	—
	175	15	49	7	—	—	—	38	6	—	—	—	—	—	—	—
	115	5	Mostly unchanged	0	—	—	—	Some	Less	—	—	—	—	—	—	—
With naphthalene ^e																
All-trans-zeaxanthin with naphthalene ^e	160	15	56	—	—	—	—	17	17	—	—	—	—	—	—	—
All-trans-capsanthin ^f	180	1	29	—	—	—	—	—	—	—	—	—	—	—	—	71 ^{g,o}

^a L. Zechmeister, *Chem. Revs.*, *34*, 267-344 (1944), p. 284.
^b L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, *66*, 137-144 (1944).
^c A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, *64*, 1856-1861 (1942).
^d 7 minor isomers.
^e L. Zechmeister and R. M. Lemmon, *J. Am. Chem. Soc.*, *66*, 317-322 (1944).
^f A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, *66*, 186-190 (1944).
^g Neo A + Neo B + Neo C.

(a) *cis-trans* Isomerization Produced by Melting Crystalline Carotenoids. When crystals of a carotenoid are kept at a temperature a few degrees above their melting point, both reversible and irreversible changes occur. The change is manifested by a loss in color, which may amount to more than 50% of the original value. Simultaneously fluorescent compounds appear which are decomposition products resulting from irreversible changes. The amount of such destruction is less in the cases in which the carotenoid melts at a lower temperature. It is also decreased when the time during which the carotenoid is subjected to the elevated temperature is reduced to a minimum. The inclusion of naphthalene to lower the temperature of the fusion mixture also has the effect of reducing the extent of destruction of the carotenoid molecule.

In practice, the carotenoid is placed in a sealed tube with carbon dioxide, heated to the desired temperature and maintained for five or ten minutes or longer, after which it is rapidly cooled. Following solution in petroleum ether, the stereoisomers may be separated from the original carotenoid and from the decomposition products, by chromatography. In contradistinction to the results obtained by other procedures for producing stereoisomeric changes, true equilibrium mixtures do not result from the fusion method. Results which have been obtained by the application of melting crystals are summarized in Table 23.

In some cases not only a *trans-cis* rearrangement results on heating of the crystalline carotenoids, but other more profound changes take place in the molecule. Thus, Zechmeister and Sease⁵⁰⁰ have reported that when lutein prepared from the Aztec marigold (*Tagetes erecta*) was melted with naphthalene, several new polyenes containing one less oxygen were formed. These are referred to as desoxyluteins; *trans-cis* isomerization was proved to have occurred in the case of desoxylutein I.

(b) *Thermal cis-trans* Isomerization in Solution. Spontaneous isomerization starts as soon as carotenoids are dissolved in a solvent; it proceeds

TABLE 24
PERCENTAGE OF β -CAROTENE STEREOISOMERS WITH DECREASED ADSORPTION AFFINITY FORMED AT SEVERAL TEMPERATURES IN BENZENE-PETROLEUM ETHER SOLUTION^a

Temp., °C.	Per cent isomerized after following periods				
	1 hr.	3 hrs.	24 hrs.	7 days	49 days
20	—	—	<1	5.5	11.1
40	4.0	5.4	11.2	—	—
60	7.5	9.7	—	—	—
80	8.5	31.9	34.1	—	—

^a Data from G. P. Carter and A. E. Gillam, *Biochem. J.*, 33, 1325-1331 (1939).

⁵⁰⁰ L. Zechmeister and J. W. Sease, *J. Am. Chem. Soc.*, 65, 1951-1955 (1943).

TABLE 25
cis-trans ISOMERISM OF ALL-*trans* CAROTENOIDS CAUSED BY REFLUXING SOLUTIONS^a

Pigment	Solvent	Duration of refluxing, min.	Ratio of stereoisomers, per cent							
			All- <i>trans</i>	N ^{neo} _U	N ^{neo} _A	N ^{neo} _B	N ^{neo} _E	Labile	All neo-forms	
α -Carotene ^b	Petroleum ether (b.p., 60-70°C.)	30	92	4	—	4	—	—	—	—
β -Carotene ^c	Petroleum ether (b.p., 60-70°C.)	60	86	4	—	8	—	1	—	—
Lycopene ^d	Petroleum ether (b.p., 70-80°C.)	30	45	—	—	—	—	—	—	55
Cryptoxanthin ^e	Ligroin (b.p., 120°C.)	30	62	—	—	32	—	—	—	—
Gazaniaxanthin ^f	Benzene	30	70	—	—	—	—	—	—	30
Lutein ^{g,h}	Benzene	30	89	—	—	—	—	—	—	11
Zeaxanthin ^h	Benzene	30	70	—	—	24	—	—	—	—
Physalene ^h	Petroleum ether (b.p., 70-80°C.)	60	58	—	—	—	—	—	—	42
Taraxanthin ^g	Benzene	30	85	—	—	—	—	—	—	15
Capsanthin ⁱ	Benzene	50	80	—	—	—	—	—	—	20
Capsanthin dipalmitate ⁱ	Petroleum ether (b.p., 70-80°C.)	30	64	—	—	—	—	—	—	36
Capsorubin ⁱ	Benzene	30	80	—	—	—	—	—	—	20

^a L. Zechmeister, *Chem. Revs.*, **34**, 267-344 (1944), p. 283.
^b L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, **66**, 137-144 (1944).
^c A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, **64**, 1856-1861 (1942).
^d L. Zechmeister and P. Tuzson, *Biochem. J.*, **32**, 1305-1311 (1938).
^e L. Zechmeister and R. M. Lemmon, *J. Am. Chem. Soc.*, **66**, 317-322 (1944).
^f L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.*, **65**, 1535-1540 (1943).
^g L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340-1346 (1939).
^h L. Zechmeister, L. v. Cholnoky, and A. Polgár, *Ber.*, **72**, 1678-1685 (1939).
ⁱ L. Zechmeister and L. v. Cholnoky, *Ann.*, **543**, 248-257 (1940).

TABLE 26. RELATIVE PROPORTIONS OF STEREOISOMERS OF SEVERAL CAROTENOIDS WHEN CATALYZED BY IODINE AND STARTING WITH VARIOUS COMPONENTS OF EACH SET^a

Starting material	Relative colorimetric values (per cent of pigment recovered)									
	Neo U	Neo V	Neo W	All- <i>trans</i>	Neo A	Neo B	Neo C	Neo E	Neo C, D, and E	Labile isomer
<i>α</i> -Carotene, petroleum ether, 30 minutes, daylight ^{b,c}										
Neo U	11	3.5	21	49.5	—	12	—	—	3	—
Neo V	11.5	4	19.5	50	—	13	—	—	2	—
Neo W	15.5	3.5	18.5	43.5	—	17	—	—	2	—
All- <i>trans</i>	14.5	3	15.5	51.5	—	13	—	—	2.5	—
Neo B	10.5	3	15	57	—	12.5	—	—	2	—
Neo forms C + D + E	10	2.5	21	51.5	—	9.5	—	—	5.5	—
<i>β</i> -Carotene, petroleum ether, 60 minutes, daylight ^{a,b}										
Neo U	24	—	—	47	—	24	—	3	—	2
All- <i>trans</i>	22	—	—	48	—	25	—	3	—	2
Neo B	21	—	—	51	—	23	—	3	—	2
Neo E	20	—	—	48	—	24	—	4	—	4
Labile isomer	18	—	—	45	—	16	—	13	—	8
Cryptoxanthin, petroleum ether, 60 minutes, daylight ^d										
Neo U	23	—	—	55	22	—	—	—	—	—
All- <i>trans</i>	18	—	—	59	18	5	—	—	—	—
Neo A	20	—	—	57	23	—	—	—	—	—
Neo B	21	—	—	55	17	7	—	—	—	—
Lutein, petroleum ether, 30 minutes, daylight ^e										
All- <i>trans</i>	—	—	—	60	17	23	—	—	—	—
Neo A	—	—	—	58	20	22	—	—	—	—
Neo B	—	—	—	56	20	24	—	—	—	—
Zeaxanthin, benzene, 30 minutes, daylight ^e										
All- <i>trans</i>	—	—	—	66	10	21	—	3	—	—
Neo A	—	—	—	52	30	15	—	3	—	—
Neo B	—	—	—	68	10	19	—	—	—	—
Neo C	—	—	—	67	12	18	—	3	—	—

^a Adapted from L. Zechmeister, *Chem. Revs.*, 34, 287 (1944).^b A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, 64, 1856-1861 (1942).^c L. Zechmeister and A. Polgár, *ibid.*, 66, 137-144 (1944).^d L. Zechmeister and R. M. Lemmon, *ibid.*, 66, 317-322 (1944).^e L. Zechmeister and P. Tuzson, *Ber.*, 72, 1340-1346 (1939).

without the aid of a catalyst and in the absence of light. This phenomenon was first observed by Zechmeister and Tuzson⁴⁸⁹ for lycopene, cryptoxanthin, and β -carotene, while the behavior of zeaxanthin and lutein, under similar conditions, was described by Strain³¹¹ as well as by Zechmeister *et al.*^{490,501,502} The speed at which isomerization takes place was found to depend upon the solvent employed but most of all upon the temperature at which the solution was maintained. While some spontaneous changes occur at room temperature, such reactions are extremely slow. Zechmeister⁴⁵¹ reports the following per cent of isomerization after 24 hours in a benzene or petroleum ether solution: α -carotene, β -carotene, cryptoxanthin, and capsanthin, 1–2; gazaniaxanthin and zeaxanthin, 4–5; capsorubin, 8; and lycopene, 10. If β -carotene solutions are kept at -2°C . in the dark and protected from air, it has been reported⁵⁰³ that less than 3–4% will undergo *trans-cis* rearrangement in the course of 3 months. The effect of temperature on the rate of isomerization is given in Table 24, while the comparative effects of refluxing solutions of different carotenoids are recorded in Table 25 (see pages 632 and 633).

When all-*trans* compounds are refluxed in a dilute benzene or petroleum ether (b.p., $60\text{--}80^{\circ}\text{C}$.) solution, equilibrium is reached in from 15 to 60 minutes. Compounds with some *cis* linkages have varying stability. For example, polycopene and pro- γ -carotene are so thermostable that their absorption curves show no alteration after refluxing in petroleum ether for 30 minutes.²⁴² The rate of isomerization of β -carotene has been shown by Hunter *et al.*⁵⁰⁴ to be greater in the non-polar solvent, toluene, than in the polar solvent nitromethane.

(c) *cis-trans* Isomerization Produced by Iodine Catalysis at Room Temperature. The effect of iodine on the stereoisomerism was first established by Zechmeister and his associates^{374,490,501} with a large number of C_{40} carotenoids. When iodine is added to a solution of the all-*trans* carotenoid in amounts approximating 1 to 2% of the amount of pigment, an equilibrium mixture of stereoisomers results. Not all the theoretically possible isomers are produced; in practice only from 2 to 12 of the more stable ones are formed in detectable quantities.

The equilibrium mixture is different from that produced when such procedures as fusion or refluxing of the solutions are used to produce *trans-cis* rearrangements. The differences are not only quantitative but possibly also qualitative, since some isomers may be formed—as a result of iodine catalysis—which are completely absent when the thermal methods are employed. The most important characteristic of the equilibrium mixture

⁴⁹¹ L. Zechmeister, L. v. Chohnoky, and A. Polgár, *Ber.*, **72**, 1678–1685 (1939).

⁴⁹² L. Zechmeister, L. v. Chohnoky, and A. Polgár, *Ber.*, **72**, 2039–2041 (1939).

⁴⁹³ G. P. Carter and A. E. Gillam, *Biochem. J.*, **33**, 1325–1331 (1939).

⁴⁹⁴ R. F. Hunter, A. D. Scott, and J. R. Edisbury, *Biochem. J.*, **36**, 697–702 (1942).

produced by iodine is the reversibility of the process. As shown in Table 26, the mixtures which result are in the same ratio, irrespective of whether one starts with the all-*trans* form or with any of a number of the *cis* isomers.

The speed at which equilibrium is reached depends upon several factors. When the weight of the pigment is about 1/5000 molar (0.1 milligram per milliliter of petroleum ether or benzene), and the weight of iodine is 1-3% of the pigment, equilibrium is reached in 15 to 60 minutes at 25°C. Zechmeister⁴⁵¹ reports that when iodine is present in the following percentages of polyycopene, the extent of the isomerization complete at one minute is as follows: 0.0013, 2%; 0.013, 37%; and 0.13, 93%. Another factor which influences the rate of the *trans-cis* reaction is temperature, the higher temperatures obviously favoring the reaction. Light, which by itself will promote the formation of stereoisomers, seems to promote the action of iodine to a marked extent. It has been stated⁴⁵¹ that, for most practical purposes, daylight or illumination with a Mazda lamp is necessary. However, over-exposure may cause destruction. Lycopene is especially sensitive to such a change when the effects of iodine and light are combined. Iodine itself may cause a certain amount of destruction in addition to the stereoisomeric changes; this is usually negligible, but in the case of polyycopene, losses as high as 30% have been reported.⁴⁵¹ The composition of the equilibrium mixtures when the all-*trans* or *cis* forms of α - or β -carotene, cryptoxanthin, lutein, and zeaxanthin are subjected to iodine catalysis is summarized in Table 26.

(d) *cis-trans* Isomerization Produced by Acid Catalysis. When carotenoids are subjected to the action of strong acids at ordinary temperatures, a stereoisomeric mixture is produced. Thus, when a β -carotene solution in benzene or petroleum ether was treated with commercial concentrated hydrochloric acid under carbon dioxide for 30 minutes, during which the mixture was constantly shaken, Polgár and Zechmeister⁵⁰⁵ found little destruction of the carotenoid; the resulting mixture of isomers was as follows: all-*trans*, 50, neo U, 23, neo B, 23, neo E, 3, labile isomer, 1.

However, certain acids such as hydriodic acid may cause considerable or complete destruction of the carotenoid without producing the stereoisomers. Commercial concentrated hydriodic acid in the cold causes a reduction of the carotenoids, while the use of boric acid melt results in the conversion of luteins to desoxyluteins.⁵⁰⁰ Xanthophylls and hydroxyketones are more sensitive to concentrated acids than are the hydrocarbon polyenes; the dark blue coloration after treatment with strong hydrochloric acid is a test for violaxanthin, fucoxanthin, capsanthin, and capsorubin. Lutein is very sensitive to organic acids when methanol solutions are refluxed.²⁷⁴ The optical rotation decreases as the action of the acid is prolonged. Strain⁵⁰⁶

⁵⁰⁵ A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, 64, 1856-1861 (1942).

⁵⁰⁶ H. H. Strain, *J. Am. Chem. Soc.*, 63, 3448-3452 (1941).

has found that the effect of acids in producing isomerization can be prevented by the addition of such bases as pyridine, quinoline, or dimethylaniline.

(c) *cis-trans Isomerization Produced by Photochemical Action.* All known carotenoids are more or less sensitive to the effect of photochemical action. However, marked variations in susceptibility to the effect of such light rays occur between the different carotenoids. In general, the *cis* compounds (particularly the poly-*cis* derivatives) would appear to be more sensitive to such treatment than are the all-*trans* isomers. Thus, it was shown⁴⁹¹ that when all-*trans*- β -carotene was insolated for 45 minutes in a petroleum ether solution, only 2% of stereoisomers resulted. When neo- β -carotene U was similarly treated, 55% was changed to the all-*trans* form, 6% to the neo- β isomer, and 2.5% to the mixed C + D neo compounds. When neo- β -carotene B was used as the starting material, only 27% remained after insolation for 45 minutes, while the following isomers were present: Neo V, 2.5%; all-*trans*, 60%; neo B, 5%; and mixed C + D neo isomers, 5.5%. The distribution of isomers after insolation of various members of the α -carotene set is given in Table 27. The marked susceptibility of such poly-*cis* compounds as polycopene and pro- γ -carotene to insolation was shown by the fact that the almost colorless pigments turned intensely yellow after exposure to sunlight for a few minutes.²⁴² This change was accompanied by a marked change in the absorption maxima, which were found at longer wave lengths.

TABLE 27
RELATIVE COLORIMETRIC VALUES (PER CENT OF PIGMENT RECOVERED) OF ALL-*trans*- α -CAROTENE AND OF SOME OF ITS STEREOISOMERS FORMED BY INSOLATION OF PETROLEUM ETHER SOLUTIONS FOR 45 MINUTES^a

Starting material	Neo U	Neo V	Neo W	Neo X	Neo Y	All- <i>trans</i>	Neo A	Neo B	Neo C + D, etc.
Neo U	64.5	1.5	3.5	2	3	24	—	1.5	—
Neo V	33	43	4	2	—	16	—	2	—
Neo W	7.5	5	32.5	1	—	41	4	7.5	1.5
All- <i>trans</i>	1.5	—	2	—	—	93.5	—	2.5	0.5
Neo B	1.5	—	34	—	—	56.5	—	8	—

^a L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1944).

The photochemical action is produced more efficiently by the visible than by the ultraviolet spectrum. The effect can be shown to be independent of the thermal process, since it proceeds equally well when precautions are taken to prevent heating. Considerable destruction may also accompany the photochemical process, particularly if some oxygen is present, or catalysts are accidentally available. In practice, the effect of

oxygen is avoided by insolation in closed quartz flasks where the air has been replaced by carbon dioxide.

f. Properties of Carotenoids Containing *cis* Bonds. (a) *General Properties.* The stereoisomeric configurations resulting from a spatial rearrangement of the double bonds of the all-*trans* carotenoids are characterized by a markedly lower color intensity and an increased solubility. Although such poly-*cis* compounds as prolycopene and pro- γ -carotene crystallize readily, in general the *cis* isomers are less readily crystallizable than the all-*trans* isomers. The melting points of the *cis* forms are considerably lower than are those of the corresponding all-*trans* forms. Adsorption on the chromatographic column shows a considerable variation from the all-*trans* form. This property is of extreme importance, as it not only affords a means of characterizing a stereochemical mixture, but also offers an excellent method for the separation of the various components and for their preparation in pure form. Some of the isomers, such as prolycopene and pro- γ -carotene, apparently have an increased thermal stability as compared with the all-*trans* form, when their solutions are refluxed. One excellent procedure for following stereochemical changes in the molecules that exhibit optical activity is by alteration of their specific rotation. The initial rotation may be changed in either direction if their molecules are bent. Examples of this change are given in Table 28.

TABLE 28
COMPARATIVE OPTICAL ROTATION OF ALL-*trans* FORMS AND *cis* ISOMERS OF SOME CAROTENOIDS^a

Stereoisomeric Set	Solvent	[α] _{Cd} ²⁵ or [α] _C ²⁰					
		All- <i>trans</i>	Neo U	Neo A	Neo B	Neo C	Neo group I
α -Carotene ^b	Chlor.	+359°	+224°	—	—	—	—
Gazaniaxanthin ^c	Petr. ether	\pm 0°	—	—	—	—	+220°
Zeaxanthin ^{a,d}	Chlor.	- 42.5°	—	+120°	\pm 0°	—	—
Capsanthin ^e	Benzene	\pm 0°	—	+ 89°	+21°	+27°	—
Capsanthin dipalmitate ^e	Petr. ether	- 30°	—	- 22°	-20°	—	—
Capsorubin ^e	Benzene	\pm 0°	—	-134°	-69°	—	—
Capsorubin dipalmitate ^e	Petr. ether	\pm 0°	—	- 75°	-15°	—	—

^a L. Zechmeister, *Chem. Revs.*, *34*, 267-344 (1944), p. 294.

^b L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, *66*, 137-144 (1944).

^c L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.*, *65*, 1535-1540 (1943).

^d L. Zechmeister, L. v. Cholnoky, and A. Polgár, *Ber.*, *73*, 1678-1685 (1939).

^e L. Zechmeister and L. v. Cholnoky, *Ann.*, *543*, 248-257 (1940).

(b) *Spectral Characteristics as Affected by trans-cis Isomerism.* In all cases, the extinction maxima for the all-*trans* compounds are at longer wave lengths than are those for the *cis* isomers. One of the most marked altera-

tions, therefore, which accompanies a *trans*→*cis* rotation is the shifting of the absorption curve toward the ultraviolet end of the spectrum. This is also accompanied by a decrease in the extinction values at the maxima, since the actual intensity of the absorption of the *cis* form is less than that of the corresponding all-*trans* isomer. The change in position of the maxima and of the height of the extinction curve is so pronounced as to be visible to the naked eye when the solution of the all-*trans* carotenoid is sufficiently concentrated to be visible. The addition of a drop of iodine to catalyze the stereoisomeric change is immediately followed by a noticeable decrease in intensity of the color of the solution.

On the other hand, when stereoisomeric changes are produced in carotenoids having *cis* bonds, a definite increase in color intensity is noted. This results from the reversible reaction whereby some of the *cis* isomer is changed back to the all-*trans* compound. However, because such solutions always consist of an equilibrium mixture which contains the various *cis* isomers as well as the all-*trans* carotenoid, the color intensity produced by catalytic action on a *cis* isomer never reaches the intensity of that given by a corresponding amount of the all-*trans* form. The accomplishment of a *cis*→*trans* rotation results not only in the shift of absorption maxima away from the ultraviolet region toward the longer wave lengths, but also in an increase in the intensity of such absorption maxima. It is, therefore, an exact reversal of the phenomena which accompany a *trans*→*cis* rotation. Although the single bonds in a conjugated double bond system like that present in a carotenoid attempt to maintain a coplanar arrangement, there is some overlapping of the hydrogen atoms at the *cis* linkages which tends to force the *cis* isomer out of coplanarity.

The first evidence of the relationship of structure to spectroscopic behavior was given by Gillam and El Ridi.⁴⁸⁶ Many data on the spectral characteristics of natural and isomerized carotenoids down to a wave length of 380 m μ have been reported by Strain,^{124,311,397} Beadle and Zscheile,⁵⁰⁷ White, Zscheile, and Brunson,²¹⁰ and White, Brunson, and Zscheile.⁵⁰⁸

The effects of isomerization of several all-*trans* carotenoids with a catalytic amount of iodine on the absorption curves are shown in Figures 17–24. Similar but less pronounced alterations are produced in each case by refluxing in darkness for 45 minutes. The curve for the isomeric mixture produced when all-*trans*- γ -carotene is so treated is included in Figure 19.

The comparative spectra maxima of some members of the methylbixin and of the dimethylcrocetin sets are included in Table 29 (see page 644).

In addition to the absorption in the visible portion of the spectrum, all-*trans* carotenoids exhibit extinction maxima in the ultraviolet region be-

⁵⁰⁷ B. W. Beadle and F. P. Zscheile, *J. Biol. Chem.*, **144**, 21–33 (1942).

⁵⁰⁸ J. W. White, A. M. Brunson, and F. P. Zscheile, *Ind. Eng. Chem., Anal. Ed.*, **14**, 798–801 (1942).

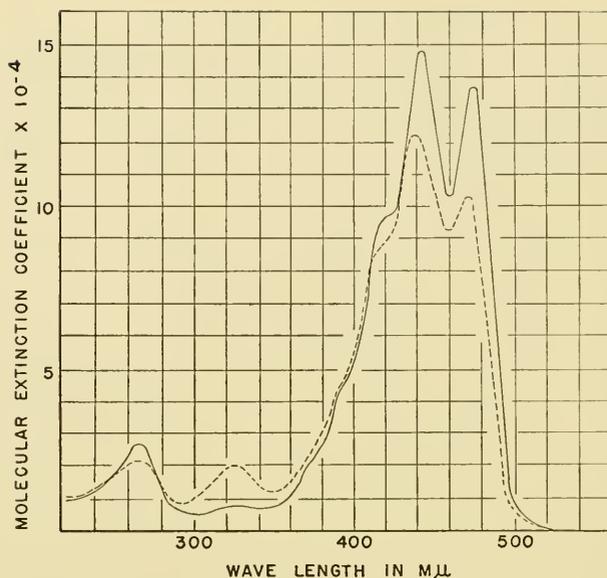


Fig. 17. Molecular extinction curves for α -carotene in hexane: (—) fresh solution of all-*trans* compound; (---) mixture of stereoisomers obtained from Neo U, after iodine catalysis at room temperature in light.⁴⁷⁹

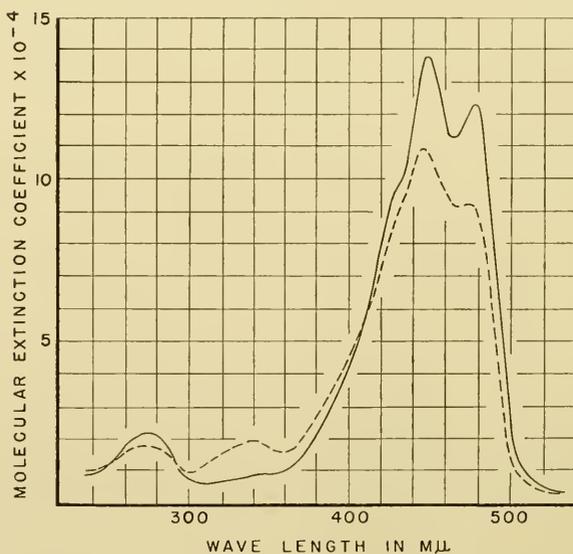


Fig. 18. Molecular extinction curves for β -carotene in hexane: (—) fresh solution of all-*trans* compound; (---) mixture of stereoisomers obtained from Neo U, after iodine catalysis at room temperature in light.⁴⁷⁹

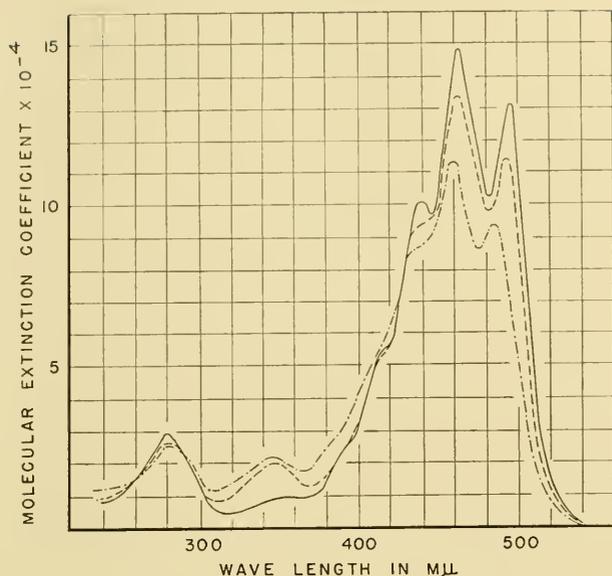


Fig. 19. Molecular extinction curves for γ -carotene in hexane: (—) fresh solution of all-*trans* compound; (---) mixture of stereoisomers after refluxing in dark for 45 minutes; (-·-) mixture of stereoisomers after iodine catalysis at room temperature in light.²⁴²

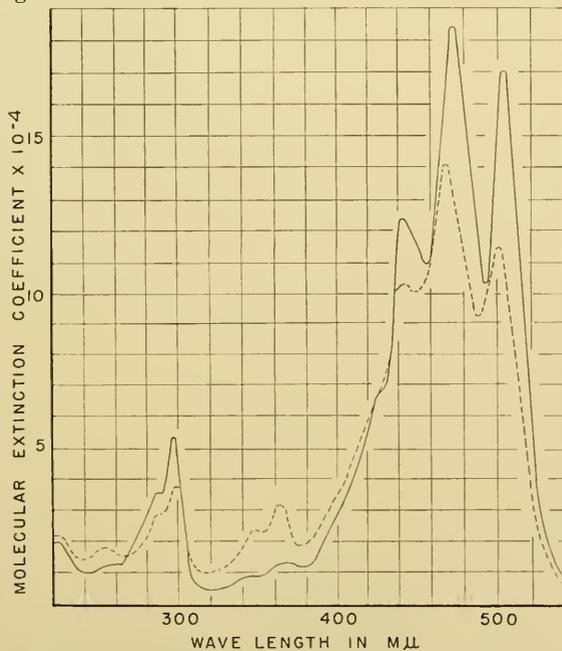


Fig. 20. Molecular extinction curves for lycopene in hexane: (—) fresh solution of all-*trans* compound; (---) mixture of stereoisomers after iodine catalysis at room temperature in light.²⁴²

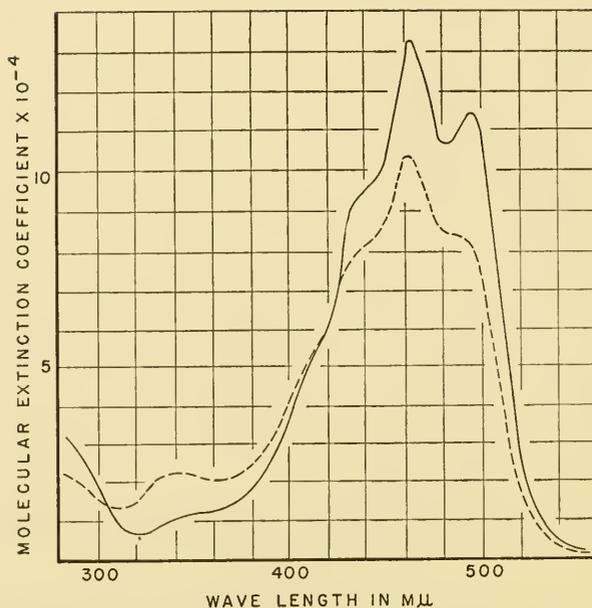


Fig. 21. Molecular extinction curves for cryptoxanthin in benzene: (—) fresh solution of all-*trans* compound; (- -) mixture of stereoisomers after iodine catalysis at room temperature in light.²⁷¹

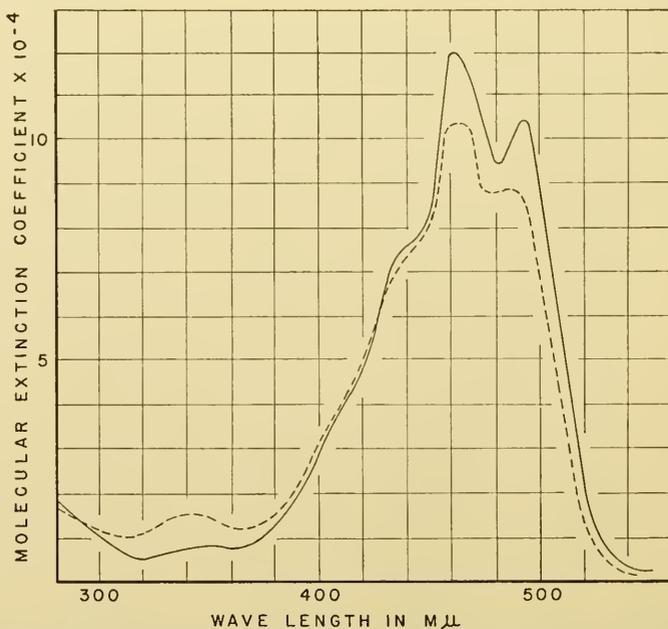


Fig. 22. Molecular extinction curves for zeaxanthin in benzene: (—) fresh solution of all-*trans* compound; (- -) mixture of stereoisomers after iodine catalysis at room temperature in light.²⁷¹

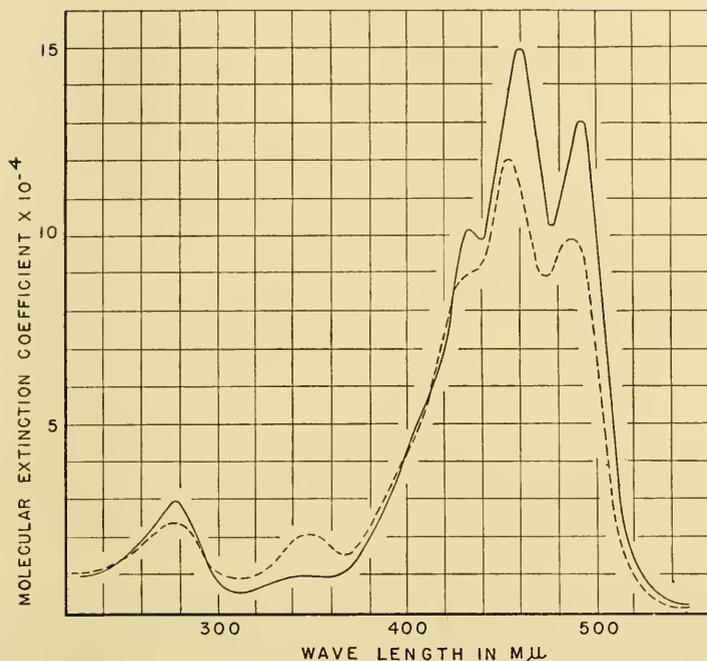


Fig. 23. Molecular extinction curves for gazaniaxanthin in hexane: (—) fresh solution of all-*trans* compound; (---) mixture of stereoisomers after iodine catalysis at room temperature in light.¹⁹⁷

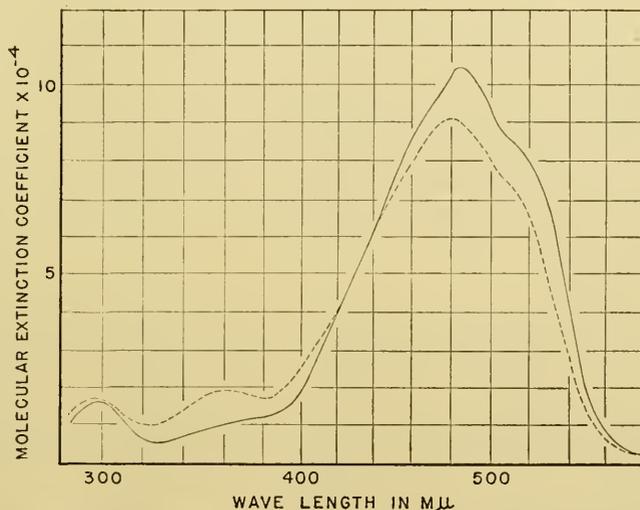


Fig. 24. Molecular extinction curves for capsanthin in benzene: (—) fresh solution of all-*trans* compound; (---) mixture of stereoisomers after iodine catalysis at room temperature in light.⁴⁸¹

TABLE 29

VISUALLY OBSERVED ABSORPTION MAXIMA OF SOME MEMBERS OF THE METHYLBIXIN SET, ARRANGED IN ORDER OF DECREASING AFFINITIES, AND OF THE DIMETHYLCROCETIN SET^a

Member of the set	Absorption maxima, m μ , in	
	Petr. ether, b.p., 60-70°	Benzene
Methylbixin set		
Natural methylbixin (labile)	485, 453.5	503, 470
All- <i>trans</i> -methylbixin (stable)	490, 457	508.5, 475
Neomethylbixin A	485, 454	502.5, 469
Neomethylbixin B	471, 444.5	491, 458
Neomethylbixin C	479.5, 449	496, 463
Dimethylcrocetin set		
Member of the set	Petr. ether, b.p., 70-80°	Chloroform
All- <i>trans</i> -dimethylcrocetin (stable)	450.5, 424.5	463, 434.5
Labile dimethylcrocetin	445, 422	458, 432.5

^a Data from L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1944), pp. 324, 334.

tween 250 and 300 m μ .⁴⁷⁹ When a *trans*→*cis* isomerization occurs, the intensity of the extinction values in the lower ultraviolet area is decreased to the same degree as in the visible portion of the spectrum. However, there is no corresponding shift of the extinction maxima of the ultraviolet area to shorter wave lengths such as occurs with the maxima in the visible portion of the spectrum.

(c) *The cis-Peak Effect.* The area between 320 and 380 m μ has practically no inflection in the all-*trans* carotenoids. However, Zechmeister and Polgár⁴⁷⁹ discovered that *trans*→*cis* changes were associated with the formation of maxima of considerable magnitude in this area, the intensity of which was proportional to the extent to which isomerization had occurred. This new maximum has been referred to as the "*cis* peak," and the range of wave lengths at which it appears is called the "*cis*-peak region." Figure 25 furnishes an example of the nature of the *cis* peak and the rapidity with which it develops.

It has also been demonstrated by Zechmeister and Polgár⁴⁷⁹ that the position of the maximum absorption of the *cis* peak bears a definite relationship to the extinction maxima in the visible spectrum. The difference between the wave length of the *cis* peak and that of the extinction maximum having the longest wave length of the all-*trans* isomer was found to be 142 ± 2 m μ , in hexane. The extraordinary uniformity of this value is illustrated by the data recorded in Table 30.

As illustrated in Figure 25, the *cis*-peak effect may develop almost instantaneously when the solution containing the all-*trans* compound is exposed to light. Thus, with lycopene in hexane, 92% of the maximum effect is

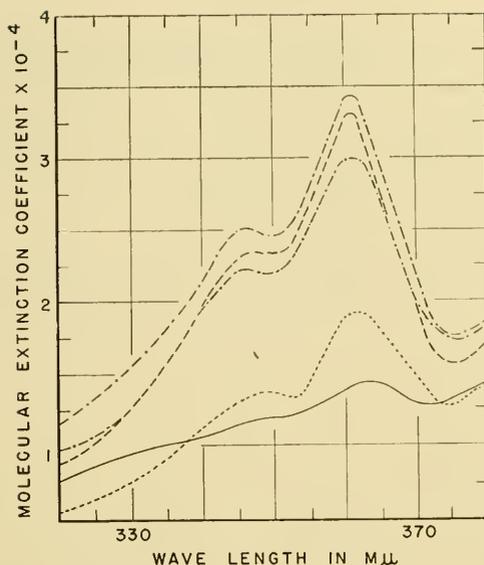


Fig. 25. Development of the *cis* peak in a hexane solution of all-*trans*-lycopen in an iodine-catalyzed solution exposed to light: (—) molecular extinction curve of all-*trans*-lycopen; (· · ·) mixture of isomers after 0 seconds; (--) after 5 seconds; (- · - · -) after 30 seconds; (- - - -) after 15 minutes illumination.⁴⁸¹

TABLE 30
POSITION OF *cis* PEAK OF STEREOISOMERIC EQUILIBRIUM MIXTURES OF SOME C₄₀
CAROTENOIDS OBTAINED AFTER IODINE CATALYSIS^a

Pigment	$m\mu$ in hexane solution		$m\mu$ in benzene solution	
	Position of <i>cis</i> peak	Distance between <i>cis</i> peak and max. at longest wave length of all- <i>trans</i> form	Position of <i>cis</i> peak	Distance between <i>cis</i> peak and max. at longest wave length of all- <i>trans</i> form
5,6-Dihydro- α -carotene ^b	328	141	—	—
5,6-Dihydro- β -carotene ^b	331	143	—	—
α -Carotene	331	143	—	—
Lutein	331	143	—	—
β -Carotene	339.5	141	—	—
Cryptoxanthin	339	141	348	146
Physalien	338	141	—	—
γ -Carotene	349	143	—	—
Gazaniaxanthin ^c	349	142	—	—
Lycopene	361	141	—	—
Capsanthin	354	144	363	145
Celaxanthin ^a	380	144	—	—
Zeaxanthin	—	—	348	145

^a Adapted from L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1944), p. 302.

^b Data from L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, 65, 1522-1528 (1943), p. 1523.

^c L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc.*, 65, 1535-1540 (1943).

apparent within 5 seconds. However, no inflection of the *cis* peak was observed over an interval of one hour when iodine catalysis was employed in the dark. Moreover, the solvent is also of importance in determining this effect. Whereas benzene, petroleum ether, and hexane all seemed to allow the *cis*-peak alteration to occur with equal effectiveness, lycopene, when dissolved in carbon disulfide,⁴⁵¹ gave practically no response when refluxed or catalyzed with iodine in the light.

The isomers which adsorb below the all-*trans* form on the chromatographic column are mainly responsible for the *cis*-peak effect.⁴⁷⁹ In fact, 55 to 95% of the total effect has been demonstrated to be the result of one

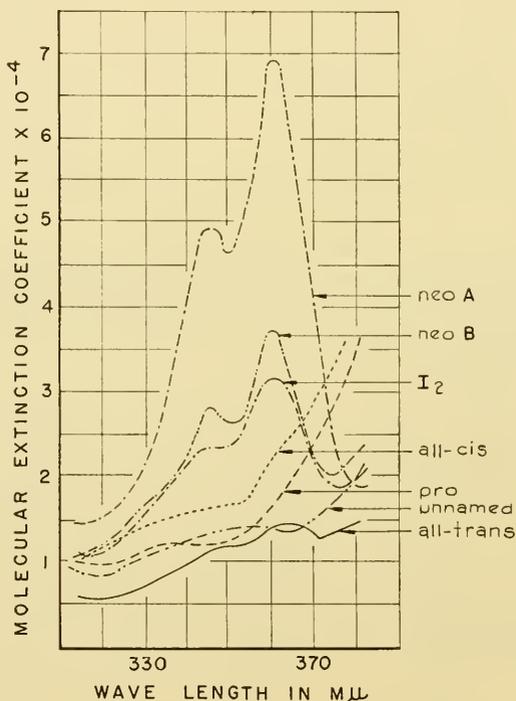


Fig. 26. Molecular extinction curves of some members of the stereoisomeric lycopene set in the *cis*-peak region in hexane. Curve marked I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light.⁴⁹¹

or two isomers (Table 32), namely neo A and neo B. In the poly-*cis* forms such as polycopene and pro- γ -carotene, the *cis* peak is negligible. The molecular extinction coefficients at the *cis*-peak wave lengths of the known isomers are included in Table 33. The comparison of adsorption curves in the *cis*-peak region of an equilibrium mixture of lycopene isomers and of the individual components is given in Figure 26.

TABLE 31
INFLUENCE OF LIGHT ON DEVELOPMENT OF *cis* PEAK IN IODINE-CATALYZED SOLUTIONS
OF ALL-*trans* CAROTENOIDS^a

Pigment	Duration of illumination						
	0 sec.	5 sec.	30 sec.	150 sec.	15 min.	30 min.	60 min.
Molecular extinction coefficient at longest wave length max., E (mol., 1 cm.) $\times 10^{-4}$							
α -Carotene ^b	14.6	—	13.7	13.4	13.2	12.7	11.3
β -Carotene ^b	14.2	—	13.2	13.0	12.3	10.9	—
Lycopene ^b	18.6	15.5	15.6	15.4	14.0	—	—
Cryptoxanthin ^c	13.4	13.1	12.7	12.0	11.0	10.0	—
Zeaxanthin ^c	12.0	11.8	11.4	11.1	10.8	10.5	10.1
Capsanthin ^c	9.9	9.8	9.5	9.3	9.0	9.0	8.9
Molecular extinction coefficient at the <i>cis</i> -peak wave length, E (mol., 1 cm.) $\times 10^{-4}$							
α -Carotene ^b	0.8	—	1.94	2.11	2.16	2.30	2.26
β -Carotene ^b	0.8	—	1.95	2.04	2.22	2.22	—
Lycopene ^b	1.47	3.2	3.4	3.2	3.0	—	—
Cryptoxanthin ^c	0.70	0.81	1.16	1.62	1.77	1.79	—
Zeaxanthin ^c	0.89	1.25	1.47	1.64	1.74	1.71	1.74
Capsanthin ^c	1.02	1.29	1.47	1.72	1.80	1.89	1.90
Difference of molecular extinction coefficient of <i>cis</i> -peak from 0 sec. value Expressed in per cent of maximum value							
α -Carotene ^b	0	—	75	87	90	100	97
β -Carotene ^b	0	—	80	87	100	100	—
Lycopene ^b	0	92	100	92	81	—	—
Cryptoxanthin ^c	0	10	42	84	98	100	—
Zeaxanthin ^c	0	42	68	88	100	97	100
Capsanthin ^c	0	31	51	80	89	99	100

^a L. Zechmeister, *Chem. Revs.*, *34*, 267-344 (1944), p. 305.

^b Hexane solution.

^c Benzene solution.

TABLE 32
STEREISOIMERS RESPONSIBLE FOR THE MAJOR PART OF *cis*-PEAK EFFECT OF TOTAL
EQUILIBRIUM MIXTURE OBTAINED BY IODINE CATALYSIS^a

Stereoisomeric set	Member of set	Approx. percentage of member in equil. mixt.	Approx. percentage of total <i>cis</i> -peak effect caused by member	Ref.
α -Carotene	Neo B	13	55	<i>a, b</i>
β -Carotene	Neo B	25	75	<i>c</i>
Lycopene	Neo A	30-40	95	<i>d</i>
Lutein	Neo A	17	70	<i>d</i>
Cryptoxanthin	Neo A	23	60	<i>e</i>
Zeaxanthin	Neo A	30	90	<i>f, a</i>
	+Neo B			
Capsanthin	Neo A	20	80	<i>g</i>

^a L. Zechmeister, *Chem. Revs.*, *34*, 267-344 (1944), p. 311.

^b L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, *66*, 137-144 (1944).

^c A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, *64*, 1856-1861 (1942).

^d L. Zechmeister and P. Tuzson, *Ber.*, *72*, 1340-1346 (1939).

^e L. Zechmeister and R. M. Lemmon, *J. Am. Chem. Soc.*, *66*, 317-322 (1944).

^f L. Zechmeister, L. v. Cholnoky, and A. Polgár, *Ber.*, *72*, 1678-1685 (1939).

^g L. Zechmeister and L. v. Cholnoky, *Ann.*, *543*, 248-257 (1940).

TABLE 33
TYPICAL *cis*-PEAK EFFECTS IN SOME STEREOCHEMICAL SETS OF CAROTENOIDS

Stereoisomeric set	Solvent	Molecular extinction coefficient at <i>cis</i> -peak wave length, ^a						
		Neo U	Neo V	All- <i>trans</i> s	Neo A	Neo B	Neo C	Miscellaneous
α -Carotene ^b	Hexane	1.2 (0.4)	1.1 (0.3)	0.8	3.8 (3.0)	3.8 (3.0)	4.5 (3.7)	Neo W: 1.6 (0.8); Neo X: 2.7 (1.9)
β -Carotene ^b	Hexane	1.3 (0.5)	0.8 (0)	0.8	—	3.4 (2.6)	—	Neo E: 3.4 (2.6)
γ -Carotene ^c	Hexane	—	—	0.95	—	—	—	Pro: 1.3 (0.35)
Lycopene ^b	Hexane	—	—	1.4	6.8 (5.4)	3.7 (2.3)	—	Unnamed crystal: 1.3 (-0.1); all- <i>cis</i> : 2.2 (0.8); prolycopenes: 1.6 (0.2)
Cryptoxanthin ^d	Hexane	1.5 (0.3)	—	1.2	4.2 (3.0)	4.5 (3.3)	—	—
Cryptoxanthin ^d	Benzene	1.7 (0.7)	—	1.0	3.4 (2.4)	4.6 (3.6)	—	—
Zeaxanthin ^d	Benzene	—	—	0.7	4.4 (3.7)	2.4 (1.7)	3.9 (3.2)	—
Lutein ^b	Hexane	—	—	0.8	4.9 (4.1)	2.1 (1.3)	—	—
Capsanthin ^e	Benzene	—	—	1.05	4.4 (3.4)	2.7 (1.6)	1.9 (0.8)	—

^a Values in parentheses are differences from that of all-*trans* form.

^b L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, **66**, 137-144 (1944).

^c L. Zechmeister, A. L. Le Rosen, W. A. Schroeder, A. Polgár, and L. Pauling, *J. Am. Chem. Soc.*, **65**, 1940-1951 (1943).

^d L. Zechmeister and R. M. Lemmon, *J. Am. Chem. Soc.*, **66**, 317-322 (1944).

^e A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.*, **66**, 186-190 (1944).

The greatest effect on absorption in the *cis*-peak region occurs with mono-*cis* isomers in which the new linkage is in the middle of the molecule, *i.e.*, in the center of the conjugated double bond system. The greater the distance from this center at which a mono-*cis* linkage occurs on either side, the less pronounced will the resulting absorption be in the *cis*-peak region. In the case of all-*cis* compounds, the *cis*-peak region is practically as flat as it is in the case of the all-*trans* compound.²⁴² This is in line with the idea that a bending of the all-*trans* molecule, especially in the middle of the conjugated system, is mainly responsible for the appearance of the high *cis* peak.

(d) *Effect of Isomerization of cis Compounds on Spectral Characteristics.* The reversibility of the *trans-cis* rearrangement is beautifully illustrated by the effect on the spectral properties when isomerization is produced, starting with a neo isomer. Because this will result in the production of a substantial amount of the all-*trans* form, the absorption maxima of such an equilibrium mixture of isomers is shifted to the right, *i.e.*, to higher wave lengths, the extinction coefficients of such maxima are also increased, and the *cis*-peak effect is markedly lessened. The effect of iodine catalysis on the molecular extinction curves of such natural poly-*cis* isomers as polycopene and pro- γ -carotene, as well as all-*cis*-lycopene, is illustrated in Figures 27-29.

g. Structural Configuration of Stereoisomers. Thermal and photochemical isomerization methods, as well as data on absorption spectra.

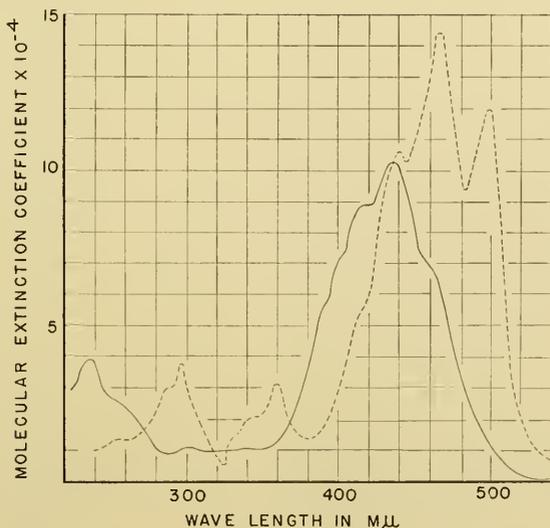


Fig. 27. Molecular extinction curves of polycopene in hexane: (—) fresh solution of polycopene; (- -) mixture of stereoisomers in polycopene solution after iodine catalysis at room temperature in light.²⁴²

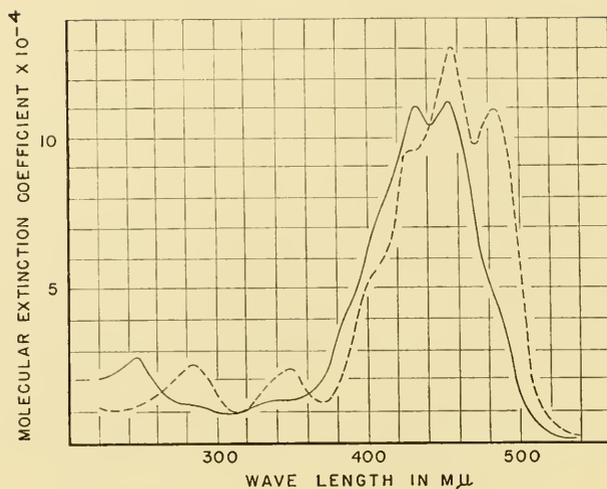


Fig. 28. Molecular extinction curves of pro- γ -carotene in hexane: (—) fresh solution of pro- γ -carotene; (---) mixture of stereoisomers after iodine catalysis at room temperature in light.²⁴²

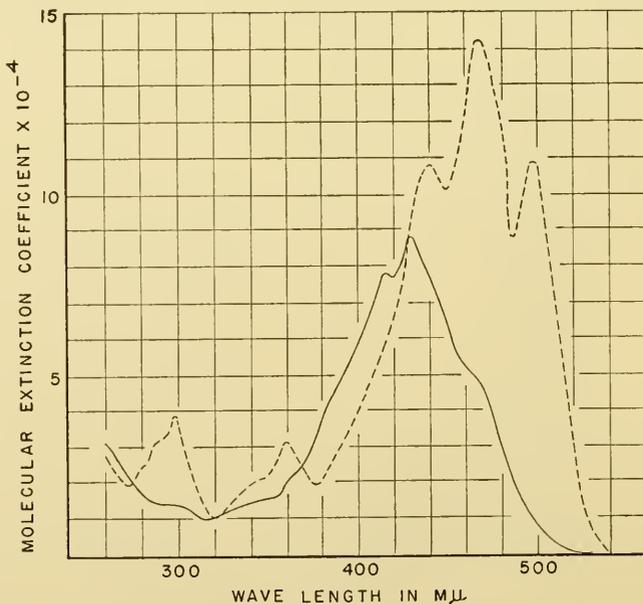


Fig. 29. Molecular extinction curves of all-*cis*-lycopene in hexane: (—) fresh solution of all-*cis*-lycopene; (---) mixture of stereoisomers after iodine catalysis at room temperature in light.²⁴²

have been marshalled to assist in the interpretation of the structure of the stereoisomers. The absorption data on shifts of the absorption maximum in the visual area have been of use in establishing the number of *cis* linkages. When the maximum has been shifted about $5\text{ m}\mu$ ($\approx 1\text{ m}\mu$), it is reasonably certain that the compound is a mono-*cis* derivative. Similarly, in the case of the di-*cis* compounds, the new absorption maximum is about $10\text{ m}\mu$ lower than that for the all-*trans* form. In the case of pro- γ -carotene with 5 *cis* linkages, the shift of the maximum in the visual area is 31; for prolycopenene with 6 *cis* bonds it is 34, and in all-*cis*-lycopenene having 7 *cis* linkages, the change in location of the absorption maximum is $38\text{ m}\mu$.

The information gained from the *cis*-peak change aids in establishing not only the number but also the location of the *cis* bonds. The effect of a given *cis* linkage varies with its position in the molecule. As noted earlier, the greatest effects are upon a centrally located mono-*cis* derivative.

Chiefly on the basis of data on absorption curves, Zechmeister⁴⁵¹ has suggested the structure of a number of isomers. The probable structures of a number of the well-known isomers are summarized in Table 34.

TABLE 34
PROBABLE STRUCTURAL RELATIONSHIPS OF SOME STEREOISOMERS OF A NUMBER OF CAROTENOID SETS^a

Stereoisomeric set	Member	Probable structure
α -Carotene	Neo U	9-Mono- <i>cis</i> - α -carotene
	Neo V	3,9-Di- <i>cis</i> - α -carotene
	Neo X	7,9-Di- <i>cis</i> - α -carotene
	Neo A and B	5,9-, 6,9-, 3,5-, or 3,6-Di- <i>cis</i> - α -carotene
	Neo C	5- or 6-Mono- <i>cis</i> - α -carotene
β -Carotene	Neo U	3-Mono- <i>cis</i> - β -carotene
	Neo V	3,9- (or 5,7-) Di- <i>cis</i> - β -carotene
	Neo B	3,6-Di- <i>cis</i> - β -carotene
γ -Carotene	Pro-	3,5,7,9,11-Penta- <i>cis</i> - γ -carotene
Lycopenene	Neo A	6-Mono- <i>cis</i> -lycopenene
	Neo B	1,6- (or 3,6-) Di- <i>cis</i> -lycopenene
	Prolycopenene	1,3,5,7,9,11-Hexa- <i>cis</i> -lycopenene
	All- <i>cis</i>	1,3,5,6,7,9,11-Hepta- <i>cis</i> -lycopenene
Cryptoxanthin	Neo U	3- (or 9-) Mono- <i>cis</i> -cryptoxanthin
	Neo A	Probably di- <i>cis</i> with one central and one peripheral <i>cis</i> bond
Zeaxanthin	Neo B	6-Mono- <i>cis</i> -cryptoxanthin
	Neo A	6-Mono- <i>cis</i> -zeaxanthin
	Neo B	5-Mono- <i>cis</i> -zeaxanthin
	Neo C	3,6-Di- <i>cis</i> -zeaxanthin
Lutein	Neo A	5- (or 6-) Mono- <i>cis</i> -lutein
	Neo B	5- (or 9-) Mono- <i>cis</i> -lutein
Capsanthin	Neo A	6-Mono- <i>cis</i> -capsanthin
	Neo B	5- (or 7-) Mono- <i>cis</i> -capsanthin
	Neo C	Di- <i>cis</i> -capsanthin
Bixin	Neo A	5-Mono- <i>cis</i> -methylbixin
	Natural methylbixin	2-Mono- <i>cis</i> -methylbixin
	Neo C	2,5-Di- <i>cis</i> -methylbixin
Crocetin	"Labile"-dimethylcrocetin	?-Mono- <i>cis</i> -dimethylcrocetin

^a Adapted from L. Zechmeister, *Chem. Revs.*, 34, 267-344 (1946).

(5) *Adsorption Phenomena (Chromatography)*

The carotenoids show marked variations in their ability to be adsorbed on a chromatographic column. This variation is exhibited not only by carotenoids having gross differences in composition, but even by the stereoisomers in which the change in structure is confined to the replacement of a single *trans* bond by a *cis* bond. This procedure thus presents an excellent and simple means, not only for the differentiation of the components of a stereoisomeric mixture, but also for the separation and preparation of pure carotenoids. No other chemical or physical procedures offer as much opportunity for the purification of the carotenoids as does chromatography. It is safe to say that little would be known about stereoisomerism of these polyenes were it not for this especially useful method.

The use of the chromatographic column in the separation of plant pigments is generally attributed to the botanist Tswett,⁵⁰⁹⁻⁵¹¹ whose first report was published in 1906. However, Weil and Williams^{511a} have recently called attention to the work of Day^{511b} who, in 1897, employed a column of powdered limestone for the fractionation of crude petroleum. Furthermore, Engler and Albrecht^{511c} reported the use of a liquid chromatogram as early as 1901. However, it must be admitted that the practical application of the method can be credited to Tswett. When petroleum ether, benzene, or carbon disulfide extracts were poured through columns of calcium carbonate, inulin, or sucrose, the most strongly adsorbed pigments were held fast at the top of the column, while the weakly adsorbed components were carried for varying distances down the column. When the column was washed with pure solvent, definite zones appeared where colored pigments were concentrated; these were separated from each other by interspersed white zones. This record of a mixture is spoken of as its chromatogram. Tswett prepared the components chromatographed individually in this manner by cutting out each zone from the column which had been pressed out of the glass tube, and by elution of the adsorbed pigment with an appropriate solvent.

Little use was made of the Tswett column in the study of carotenoids for the next 25 years, until its application by the Karrer and Kuhn schools. In 1931 Kuhn and Lederer,⁴⁶ as well as Kuhn, Winterstein, and Lederer,²⁷⁴ successfully introduced chromatography into the preparation of carotenoids. By the application of this method, these workers were able to demonstrate that a carotene preparation which was believed for 100 years to be a

⁵⁰⁹ M. Tswett, *Ber. deut. botan. Ges.*, 24, 316-323 (1906).

⁵¹⁰ M. Tswett, *Ber. deut. botan. Ges.*, 24, 384-393 (1906).

⁵¹¹ M. Tswett, *Die Chromophylle in der Pflanzen- und Tierwelt* (in Russian), Warsaw 1910. Cited by L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, p. 94 ff.

^{511a} H. Weil and T. I. Williams, *Nature*, 166, 1000-1001 (1950).

^{511b} D. T. Day, *Proc. Amer. Phil. Soc.*, 36, 112-115 (1897).

^{511c} C. Engler and E. Albrecht, *Z. angew. Chem.*, 1901, 889-893.

single substance was actually composed of two fractions: α - and β -carotene. Karrer and Walker¹⁷¹ were the first to employ calcium oxide in place of calcium carbonate as an adsorbent for the separation of carotene. The development of an understanding of the stereoisomers of carotenoids by Zechmeister and his co-workers was to a considerable extent based upon this application of chromatography.

Reviews on chromatography include the early work of Winterstein,⁵¹² and the more recent ones of Zechmeister,⁵¹³ Cook,⁵¹⁴ and Koschara.⁵¹⁵ The most complete treatment of the subject is given in the admirable treatises of Zechmeister and v. Cholnoky.^{516,517} Another excellent monograph is *Chromatographic Adsorption Analysis*, by Strain.³²³ In 1947 Williams⁵¹⁸ published a shorter treatise which includes some of the more recent developments in the field. The latest contribution to the subject is the book by Cassidy.^{519a}

a. Applications of the Chromatographic Technic. There are a number of problems which can be solved by the use of chromatographic methods and for which no other procedures are available.

One important use is for testing the homogeneity of a product. If a substance cannot be broken up into two or more components when solutions of it are percolated through an adsorption column, the material is chromatographically homogeneous. Although this is not an absolute proof of genuine chemical purity, it is at least presumptive evidence. The amount of the adsorption affinity depends to a much greater extent upon molecular structure than do such properties as melting point, boiling point, solubility, or even spectral absorption. It is conceivable, however, that two substances might have an identical adsorption coefficient and so not be resolvable into their separate components on the Tswett column. Such a condition can be excluded, however, if one finds a single zone when the material is chromatographed in several solvents. Tswett⁵¹¹ points out that it is extremely unlikely that the adsorption isotherms of two sub-

⁵¹² A. Winterstein, *Fraktionierung und Reindarstellung von Pflanzenstoffen nach dem Prinzip der chromatographischen Adsorptionsanalyse*, in G. Klein's *Handbuch der Pflanzenanalyse*, Vol. IV, Part 2, 1403-1437, Springer, Vienna, 1933.

⁵¹³ L. Zechmeister and L. v. Cholnoky, *Monatsh.*, **68**, 68-80 (1936).

⁵¹⁴ A. H. Cook, *Chemistry & Industry*, **14**, 724-726 (1936).

⁵¹⁵ W. Koschara, *Z. physiol. Chem.*, **239**, 89-96 (1936).

⁵¹⁶ L. Zechmeister and L. v. Cholnoky, *Die chromatographische Adsorptionsmethode, Grundlage, Methodik, Anwendungen*, Springer, Vienna, 1937. *Principles and Practice of Chromatography*, translated from the second and enlarged German edition by A. L. Bacharach and F. A. Robinson, Wiley, New York, 1941.

⁵¹⁷ L. Zechmeister, *Progress in Chromatography, 1938-1947*, Chapman & Hall, London, 1950.

⁵¹⁸ T. I. Williams, *An Introduction to Chromatography*, Chemical Pub. Co., Brooklyn, 1947.

^{519a} H. G. Cassidy, *Adsorption and Chromatography*, Interscience, New York, 1951, 360 pp.

stances change in exactly the same way when one varies the adsorbing phase and the solvent.

A second application of the method is for establishing the identity or non-identity of two substances. When a solution of the unknown is mixed with a known substance, the formation of a single zone on subsequent chromatography indicates that the two products are the same. Such a mixed chromatogram is of very great value in supplementing the mixed melting point technic for establishing identity. In cases in which a solution contains two components, the identity of one of the zones with a known substance can be verified by the addition of a large amount of the known compound to the solution, followed by rechromatography. By determining which of the zones is widened, one can establish which layer corresponds to the known substance.

Another important application of the chromatographic method is for the concentration of a product occurring in a high degree of dilution. Several hundred liters of such a solution can be put through a relatively small column, and the filtrate can then be rejected. By a single procedure, the desired substance is quantitatively removed without resorting to evaporation, which is time-consuming, and which may alter the structure of labile substances.

The chief importance of chromatography is for the separation of a mixture into its components. Each fraction may then be identified, and in most cases quantitatively estimated. At the scale of operations usually carried out in the laboratory, sufficient material of a homogeneous nature can be prepared from each zone of separation for microanalyses. The quantity of the product obtained can be increased by the use of larger batches of adsorbent. The procedures can be carried out practically without loss in the separation or concentration of materials. This renders such a method even more satisfactory.

Still another application of this technic is in the purification of products. If the substance desired is adsorbable on the chromatographic column, it can be eluted from that portion of the column; if a clean-cut separation cannot be made in a single operation, the eluate can be rechromatographed, and this procedure repeated several times. On the other hand, when the desired product is not adsorbable, it can frequently be freed from some or all of the contaminants, if the latter are adsorbable on the column.

b. Theoretical Basis for Chromatography. Tswett recognized the theoretical principles involved in chromatography. The positions occupied by a series of adsorbable components were explained as being related to the adsorption affinities of the several substances in a common solvent. The pigments tend to form layers in the descending order of their affinities, *i.e.*, those with the highest affinity are at the top of the column. Colorless materials follow the same laws of adsorption as do the colored products.

It is generally agreed that the capacity of a given amount of adsorbent is limited. If all the surfaces of the particles in a thin layer are surrounded by superficial active molecules, they will immediately adsorb them and become exhausted, so that no additional material can be taken up on that portion of the column. However, if the adsorbable molecules are in excess, only that portion of the pigment with the strongest affinity will be retained and the pigments with a lower adsorption coefficient will pass further down the column. When these latter molecules are no longer subject to competition on the part of the molecules of higher adsorption affinity, they become firmly adsorbed, forming a second zone of concentration. This phenomenon continues until all the adsorbable material is removed. Those molecules with the lowest adsorbability will appear furthest down on the column.

It is not believed that, under the actual conditions of the experiment, all the particles of the adsorbent immediately become exhausted by the molecules of highest affinity. Some molecules of lower adsorbability will also be retained. However, when new quantities of the solution are allowed to percolate through the column, the molecules of highest activity will displace those of lower activity from the uppermost adsorption band. The latter molecules will then pass further down the column. This same separation also occurs when the column is washed with large amounts of the solvent; this brings about the development of the chromatogram, with the clean-cut separation of the various pigments into separate layers.

The experimental evidence for this hypothesis has been beautifully demonstrated by Zechmeister and v. Cholnoky.⁵¹⁶ When zeaxanthin in a 1:4 benzene-petroleum ether solution is passed through a calcium chloride column, it forms a broad yellow band at the top of the column. If, after the development of this chromatogram, a capsanthin solution in a similar solvent is introduced into the tube, the red ring of the latter carotenoid is immediately adsorbed in the portion of the column occupied by the zeaxanthin; the latter pigment, which has a lower adsorption affinity than capsanthin, is displaced and forms a new yellow ring further down the column. When the reverse test is carried out, that is, by pouring a zeaxanthin solution into a column in which capsanthin has been chromatographed, no displacement occurs—the zeaxanthin passes through the upper red layer occupied by the capsanthin and forms a yellow band some distance below on the chromatographic column.

c. Practical Considerations in Chromatography. The general procedure for chromatography involves the passage of a dilute solution of the substance to be chromatographed in a suitable solvent through a vertical column of the adsorbent of sufficient length (at least 10 cm.) to allow differences in adsorbability to be manifest. Carbon disulfide (b.p., 46°C.), petroleum ether, and benzene (b.p., 80°C.) are the solvents most frequently

employed, although chloroform (b.p., 61.2°C.), carbon tetrachloride (b.p., 76°C.), 1,2-dichloroethane (b.p., 84°C.), ethanol, anisole, acetone, and diethyl ether may be used. It is usually advisable to moisten the column with the solvent before the mixture to be chromatographed is poured onto it. The column should not be allowed to become dry during the procedure, as this will cause the upper portion to crack. Hence, any new solution will not have an opportunity to be adsorbed at the same portion of the column, since it may pass into the column lower down at the base of the cracks. For this reason, pressure is frequently employed, rather than suction, to force the solution through the column.

After the mixture has all passed through the tube, one usually attempts to separate the zones as much as possible. This can sometimes be done by washing the column with an excess of the pure solvent originally employed. Frequently, if the zones separate too slowly when the first solvent is used, an increase in eluent activity can be brought about by the use of a new solvent. One continues to wash the column until the development is optimal in all zones.

In order to obtain the desired pigment, the column can then be washed with an eluent, which will dissolve the pigment and allow it to be carried out in the filtrate. Methanol, ethanol, and acetone are the most useful eluents, a fact which was discovered by Tswett. However, it is not necessary to use the pure eluent; the addition of the eluents, in amounts of 0.5 to 2.0%, to the original solvent used will result in the rapid solution of the adsorbed material.

However, a different procedure must be employed if there are several adsorption zones. This involves the removal of the adsorption column intact, and separation of the desired adsorption zone from the rest of the column; the eluent is then added to the adsorbent, which has been immediately crushed after being separated from the rest of the column. The pigment dissolves quickly and the adsorbent is then filtered off, leaving the solution of the desired pigment. In order that the column be removed intact, it should be slightly dried. This can be attained by holding the hand over the mouth of the tube and continuing suction for 15 seconds after the solvent has passed out of the tube. More recent tubes of uniform bore containing interchangeable glass connections at the lower end make it simple to force out the column with a plunger of appropriate size.

In order to obtain the sample for extraction, one should not simply cut the column at right angles to the long axis. Any unloaded portions of the column should first be rejected and the areas of adsorption should be carefully separated. An easy procedure is to take the portion of the column desired in the left hand and carefully trim off the white or slightly colored portions at an acute angle to the outside. This is advisable, since the area of penetration is usually deeper in the center and a considerable amount of

valuable pigment may be lost if one sections the column at a right angle to the periphery. According to Zechmeister,⁵¹⁷ in the preparation of the portion to be eluted: "With complicated columns one indulges almost in a form of sculpture!"

The nature of the adsorption medium is of the greatest importance in obtaining satisfactory results in chromatography. Theoretically, all substances in a finely divided form, or present as a powder, as well as in the form of fibers, can adsorb, provided, of course, that they are not soluble in the solvent employed. A satisfactory adsorbent must exert no chemical action on the adsorbed material, and must be capable of releasing it to an eluent if the procedure is one in which identification, purification, or preparation of a pigment is desired. However, in some cases in which the object is simply to remove a single component so that an examination of the filtrate can be carried out, a reaction between the adsorbent and the product to be removed may be desirable. Such a procedure is used in the determination⁵¹⁹ of vitamin A: the removal of all vitamin A and carotenoids is effected by passing their solution through a floridin column; the vitamin A reacts quantitatively with the floridin and cannot be eluted from it.

Another condition which may preclude the use of certain adsorbents is their color. If they are dark, it may be impossible to establish the sequence of layers. However, when it is desired to remove only one component, this objection is no longer valid. Strongly acid or strongly basic adsorbents are usually unsatisfactory.

In order to serve effectively, an adsorbent must be able to bind the adsorbed products with sufficient tenacity to hold them on the column, but in such a manner that differentiation may occur in the strength of such attraction for the various materials. It is also necessary that the adsorption affinity be of such a degree that elution can be accomplished when desired. The strong adsorbents are referred to as "active." In certain cases, it is possible to increase, activate, or deactivate adsorbents. Activation is frequently brought about by washing with water or acid, followed by heating of the material. When the solid phase acts too energetically, it binds all substances indiscriminately, and it has no value as an adsorbent in chromatography.

The optimum proportion of adsorbed material to adsorbent varies greatly with different combinations; it may vary between 1:10 to 1:100,000. The size of the particle of adsorbent, also, may result in a marked variation in its behavior. Finer particles ordinarily act extremely vigorously as adsorbing agents, although Koschura⁵¹⁵ reports that coarser particles of alumina may be much more active toward certain pigments than

⁵¹⁹ J. Awapara, F. H. Mattson, J. W. Mehl, and H. J. Deuel, Jr., *Science*, **101**, 602-604 (1946).

are the finely pulverized samples. The average range of particle size of certain adsorbents varies from 1.2 μ for light calcium carbonate and 1.5 μ for floridin, floridin XXF, and magnesium oxide, to 10 μ for acid clay (Java) and 10.5 μ for hydrated gypsum, while commercial alumina (produced in the laboratory) has an average granule size of 2 μ , and calcium hydroxide, also produced in the laboratory, averages 2.5 μ . The particles of the alumina standardized according to the Brockmann method⁵²⁰ have a mean size of 7 μ .⁵¹⁷ The type of adsorbent which is most useful depends upon whether it is used with an anhydrous solvent or with an aqueous medium. In the former case any moisture is generally harmful; in the latter instance, the adsorbent must not fix water or swell, for this will tend to form gaps in the column.

There are a number of different adsorbents which have been more or less frequently employed. These include powdered sucrose,^{509-511,521} lactose (Winterstein and Stein),⁵²² inulin (Spoehr),⁵²³ as well as various types of alumina such as "fibrous alumina" (Wislicenus),⁵²⁴ Merck's alumina, aluminum oxide-hydrate, and "Hydralo" as recommended by Strain.^{525,526} Magnesium oxide may be especially satisfactory, according to Euler and Gard,³²⁹ and Strain,^{92,525,527} while calcium hydroxide (Karrer and Walker),¹⁷¹ calcium sulfate (Karrer and Weber),⁵²³ and calcium carbonate (Tswett),⁵⁰⁹⁻⁵¹¹ are frequently employed. Bleaching earths, which are hydrated aluminum silicates having widely varying calcium, magnesium, and iron contents, include Frankonite KL (Koschara)⁵¹⁵ and floridins XXF and XS.^{515,519} Fuller's earth is sometimes used; it is designated as Lloyd reagent. Cerecedo and Hennessy⁵²⁹ have employed the Zeolite known as "Decalso." Less frequently used adsorbents are anhydrous sodium sulfate, lead sulfide, talc, kieselguhr, infusorial earths, kaolin, silica gel ("intermediate activated," Silica Gel Corporation, Baltimore, Md.) and also the Moosburg clays known as "Tonsil" and "Clarit." A number of charcoals are sometimes satisfactory, such as "Carboraffin" (produced by carbonization of pine wood with zinc chloride), "Norite," and various blood charcoals. "Amberlite" and similar resins are especially effective, but their deep color renders their use quite limited.

A number of types of apparatus are employed. For a further discussion

⁵²⁰ H. Brockmann and H. Schodder, *Ber.*, *74*, 73-78 (1941).

⁵²¹ H. H. Strain, *Science*, *83*, 241-242 (1936).

⁵²² A. Winterstein and G. Stein, *Z. physiol. Chem.*, *220*, 247-263, 263-277 (1933).

⁵²³ H. A. Spoehr, *Plant Physiol.*, *13*, 207-208 (1938).

⁵²⁴ H. Wislicenus, *Collegium*, 1906. Cited by L. Zechmeister and L. v. Cholnoky, *Progress in Chromatography, 1938-1947*, Chapman & Hall, London, 1950, p. 46.

⁵²⁵ H. H. Strain, *J. Biol. Chem.*, *105*, 523-535 (1934).

⁵²⁶ H. H. Strain, *J. Am. Chem. Soc.*, *57*, 758-761 (1935).

⁵²⁷ H. H. Strain, *Science*, *79*, 325-326 (1934).

⁵²⁸ P. Karrer and H. M. Weber, *Helv. Chim. Acta*, *19*, 1025-1027 (1936).

⁵²⁹ L. R. Cerecedo and D. J. Hennessy, *J. Am. Chem. Soc.*, *59*, 1617-1619 (1937).

of this subject, the reader is referred to the monographs of Zechmeister and Cholnoky.^{516,517}

d. Use of Chromatography in the Study of Carotenoids. The important contribution of chromatography to our knowledge of the carotenoids is to be ascribed to the great variability in adsorption affinities of various members of the group. These differences are directly related to the chemical composition of the particular member involved. The order in which the pigments will be deposited on the column (highest affinity at top to lowest adsorption at bottom or in filtrate) is governed by the following groups:

(a) *Presence of the Carbonyl Group Conjugated with a Double Bond.* Thus, capsanthin (1 —CO and 2 —OH) appears above zeaxanthin (2 —OH).

(b) *Those with the Highest Number of Alcohol Groups.* Thus, zeaxanthin (2 —OH) will appear above cryptoxanthin (1 —OH).

(c) *Presence of a Hydroxyl Group.* Thus, cryptoxanthin (1 —OH) is more easily adsorbed than is β -carotene (0 —OH).

(d) *Occurrence of the Double Bonds in Conjugated Form.* β -Carotene with 11 conjugated F is adsorbed higher than α -carotene (10 conjugated + 1 isolated F).

(e) *Presence of an Increased Number of Double F.* Lycopene with 11 conjugated and 2 isolated F appears above γ -carotene (11 conjugated + 1 isolated F).

The rules appear to apply, not only for the natural carotenoids, but also for their decomposition products. The effect of the hydroxyl group is quantitatively greater than is that of a conjugated double bond. Thus, lutein with only 10 F and 2 OH groups occupies a higher position on the adsorption column than does lycoxanthin with 11 F and only one OH group. Rhodoxanthin with 12 F and 2 ketones but no hydroxyls is not as well adsorbed as a polyene alcohol having only 11 F.

When the hydroxyl groups are esterified, the adsorption of the resulting ester is greatly depressed and compares in retention on the column with that of the poorest adsorbed carotenoids, namely, the hydrocarbons. Thus, physalien (zeaxanthin dipalmitate) is not only less easily adsorbed than the free alcohol (zeaxanthin) but even less readily than the corresponding monohydric carotenol, cryptoxanthin.

The order of adsorption of the various carotenoids as determined by Winterstein⁵¹² and more recently extended by Zechmeister and v. Cholnoky⁵¹⁶ is shown in Table 35.

As noted in the earlier section, the stereochemical isomers of any single carotenoid assume characteristic positions on the chromatographic column. In most sets of carotenoids, the natural (all-*trans*) form is present about halfway down the column. The better known stereoisomers of the all-*trans* form are located below it, *i.e.*, are adsorbed less readily, although in most cases some isomers which are more strongly adsorbed than is the all-*trans* compound are also present in the isomeric mixture (see page 622).

Strain⁵³⁰ has recently shown that the Winterstein arrangement is not an absolute one, since the order of adsorption depends upon the adsorbent and the solvent.^{328,531,532} Powdered sugar attracts the polar hydroxyl groups

TABLE 35
ORDER OF ADSORPTION OF CAROTENOIDS ON TSWETT COLUMN^a

Order of affinity ^b	Carotenoid	Formula	Constituent groups			
			Alc. groups	Double bonds		
				Total ^c	C-conjugated	Conj. with ketone
1	Fucoxanthin	C ₄₀ H ₅₆ O ₆	4-5	[13]	10	2
2	Capsorubin	C ₄₀ H ₆₀ O ₄	2	[12]	9	2
3	Capsanthin	C ₄₀ H ₅₈ O ₃	2	[11.5]	10	1
4	Violaxanthin	C ₄₀ H ₅₆ O ₄	4	10	—	—
5	Taraxanthin	C ₄₀ H ₅₆ O ₄	3-4	11	—	—
6	Antheraxanthin	C ₄₀ H ₅₆ O ₃	—	—	—	—
7	Petaloxanthin	C ₄₀ H ₅₆ O ₃	—	—	—	—
8	Flavoxanthin	C ₄₀ H ₅₆ O ₃	3	11	—	—
9	Lycophyll	C ₄₀ H ₅₆ O ₂	2	13	11	—
10	Zeaxanthin	C ₄₀ H ₅₆ O ₂	2	11	11	—
11	Lutein	C ₄₀ H ₅₆ O ₂	2	11	10	—
12	Lycoxanthin	C ₄₀ H ₅₆ O	1	13	11	—
13	Cryptoxanthin	C ₄₀ H ₅₆ O	1	11	11	—
	Rubixanthin	C ₄₀ H ₅₆ O	1	12	11	—
14	Rhodoxanthin ^d	C ₄₀ H ₅₆ O ₂	0	[15]	12	2
15	Physalien ^e	C ₇₂ H ₁₁₆ O ₄	(2)	11	11	—
16	Helenien ^e	C ₇₂ H ₁₁₆ O ₄	(2)	11	11	—
17	Lycopene	C ₄₀ H ₅₆	0	13	11	—
18	γ-Carotene	C ₄₀ H ₅₆	0	12	11	—
19	β-Carotene	C ₄₀ H ₅₆	0	11	11	—
20	α-Carotene	C ₄₀ H ₅₆	0	11	10	—

^a Adapted from A. Winterstein,⁵¹² p. 1414, by L. Zechmeister, *Die Carotinoide*, Springer, Berlin, 1934, p. 95.

^b Arranged in decreasing order of affinity. The substance with a lower number will appear higher on the column than any substance having a higher number.

^c Values in parentheses give equivalent conjugated bonds, assuming each double bond conjugated with a ketone has a value of 1.5 times that of the carbon double bond.

^d Zechmeister⁵¹⁷ gives this position as below cryptoxanthin, but the relation to physalien is not established.

^e Hydroxyl groups combined in ester linkage.

of the xanthophylls by preference, while it has little affinity for the unsaturated portions of the carotenoids. On the other hand, magnesia primarily attracts the unsaturated linkages of both the carotenes and the xanthophylls.

⁵³⁰ H. H. Strain, *J. Am. Chem. Soc.*, 70, 588-591 (1948).

⁵³¹ H. H. Strain, W. M. Manning, and G. Hardin, *Biol. Bull.*, 86, 169-191 (1944).

⁵³² H. H. Strain, *Ind. Eng. Chem., Anal. Ed.*, 18, 605-609 (1946).

7. Methods for the Preparation of Carotenoids

In the preparation of different carotenoids, the same general methods apply. If the source of the material is a substance which contains a mixture of different polyenes, the carotenoids are first extracted from the saponified mixture with petroleum ether or with a similar solvent. The carotenols can easily be separated from the hydrocarbons by extraction with methanol (95–90%). In the preparation of a hydrocarbon, the petroleum ether solution is washed several times to remove any dissolved alcohol; it is then dried over a suitable dehydrating agent such as sodium sulfate, after which the solution is chromatographed. The adsorption zone of the desired fraction is removed as described earlier, the carotenoid is eluted, the eluting agent is removed by washing with water, the solution is dried, and the petroleum ether solution is again chromatographed. This procedure may be repeated several times to obtain a product of satisfactory purity. After it is eluted, it is transferred to a suitable solvent, which is evaporated to a small volume. If the crystals are desired, it can be allowed to crystallize in the cold.

A similar procedure may be employed when the product belongs to the group of carotenols. However, the carotenoid must be transferred to a non-polar solvent such as petroleum ether or benzene before chromatography. This is readily accomplished by the addition of sufficient water to decrease the methanol to a 50% aqueous solution. It is then extracted with petroleum ether, and the petroleum ether is finally washed with water to remove any dissolved methanol. After drying, as described above, the solution is chromatographed. The product eluted from the adsorption zone may be purified from hydrocarbons by a new extraction of the petroleum ether solution on 95% methanol. This may be rechromatographed after being transferred to petroleum ether. Ultimate crystallization can be carried out as described for the carotenes. For specialized methods of preparation of the different carotenoids, the reader is referred to Zechmeister's monograph.²³

8. Related Compounds

(1) *Phytofluene*

One of the polyenes closely related to the carotenoids which is widely distributed in nature is a colorless compound first isolated by Zechmeister and Polgár.⁵³³ It was later proposed that this compound be designated as "phytofluene."⁵³⁴

a. Occurrence. Phytofluene has a distribution in plants which roughly

⁵³³ L. Zechmeister and A. Polgár, *Science*, 100, 317–318 (1944).

⁵³⁴ L. Zechmeister and A. Sandoval, *Arch. Biochem.*, 8, 425–430 (1945).

TABLE 36
 EXAMPLES OF OCCURRENCE OF PHYTOFLUENE IN PLANTS^a

Family	Plant	Common name	Phytofluene, mg./kg. fresh material
	Petals		
<i>Bignoniaceae</i>	<i>Clytostoma calistegioides</i> (<i>Bignonia speciosa</i>)	Argentine trumpet vine (clytostoma climber)	Present
	<i>Tecomaria capensis</i> (Thbg.) Fenzl	Cape honeysuckle	Present
<i>Cannaceae</i>	<i>Canna speciosa</i> (King Humbert var.)	Himalaya canna (purple-yellow)	0.2
<i>Compositae</i>	<i>Gazania rigens</i> R. Br.	Yellow gazania (treasure-flower)	32.5
	<i>Zinnia elegans</i> Jacq.	Double zinnia ("youth and old age")	3.6
<i>Loganiaceae</i>	<i>Gelsemium sempervirens</i> Art.	Carolina yellow jessamine	Present
<i>Papaveraceae</i>	<i>Eschscholtzia californica</i> Cham.	California poppy	5.0
	<i>Spartium junceum</i> L.	Weavers' broom	0.1
<i>Papilionatae</i> (<i>Leguminosae</i>)	<i>Mimulus longiflorus</i> Grant	Monkey-flower, pale yellow	27.8
<i>Scrophulariaceae</i>	<i>Photinia speciosa</i>	"Shining shrub" photinia	0.5
<i>Spiraeiadeae</i> (<i>Rosaceae</i>)	<i>Fremontia californica</i> Torr.	California fremontia (flannel-bush)	Present
<i>Sterculiaceae</i>			
	Fruits		
<i>Araceae</i>	<i>Zantedeschia aethiopica</i> (L.) Spreng.	Calla lily	1.1
<i>Cucurbitaceae</i>	<i>Citrullus vulgaris</i> L.	Watermelon, flesh	2.2
	<i>Cucumis melo</i> L.	Muskmelon, flesh	0.6
	<i>Cucumis melo</i> L.	Persian melon, flesh	Present
	<i>Cucurbita maxima</i> Duch.	Winter squash, flesh	Present
	<i>Zea mays</i> L.	Commercial yellow corn meal	0.6
<i>Gramineae</i>	<i>Eugenia uniflora</i> Linn.	Surinam cherry (<i>Pitanga eugenia</i>)	0.7
<i>Myrtaceae</i>	<i>Butia eriospatha</i> Becc.	Gum-tree, without seeds	0.3
<i>Palmae</i>	<i>Pyraecantha crenata-serrata</i> Schneid.	Chinese firethorn	0.4
<i>Rosaceae</i>	<i>Pyraecantha angustifolia</i> Schneid.	Narrow-leaf firethorn	22.1, 23.0, 14.7, 27.7
	<i>Rose canina</i> L.	Dog-rose, without seeds	1.8

Family	Plant	Common name	Phytofluene, mg./kg. fresh material
		Fruits (<i>continued</i>)	
	<i>Prunus domestica</i> L.	Plum, flesh	1.0
	<i>Prunus persica</i> Sieb. Zucc.	Peach, flesh	0.8
	<i>Citrus aurantium</i> Risso	Seville orange, juice	0.3
	<i>Citrus aurantium</i> Risso	Seville orange, pigmented rind	1.5
	<i>Citrus aurantium</i> Risso	Seville orange, white inner rind	2.3
	<i>Capsicum frutescens</i> L.	Red pepper, skin	4.6
	<i>Capsicum frutescens</i> L.	Orange pepper, skin	Present
	<i>Lycopersicon esculentum</i> . Mill.	Tomato, unripe, no seeds	2.0
	<i>Lycopersicon esculentum</i> Mill.	Tomato, ripened 19°, no seeds	10.6
	<i>Lycopersicon esculentum</i> Mill.	Tomato, "San Marzano"	6.0
	<i>Lycopersicon esculentum</i> Mill.	Tomato paste, commercial	19.0, 28.5, 16.5, 14.5, 21.5, 18.4
	<i>Diospyros kaki</i> L.	Japanese persimmon, flesh	1.0
	<i>Arbutus unedo</i> L.	Strawberry tree (madrone), unripe	0.8
	<i>Arbutus unedo</i> L.	Strawberry tree, ripe	1.5
		Stems and roots	
	<i>Cuscuta californica</i> Choisy	Chaparral dodder	2.3
	<i>Daucus carota</i> L.	Carrot	7.3, 8.3
<i>Convolvulaceae</i>			
<i>Umbelliferae</i>			

^a L. Zechmeister and A. Sandoval, *Arch. Biochem.*, 8, 425-430 (1945); *J. Am. Chem. Soc.*, 68, 197-201 (1946).

parallels that of the carotenoids. According to Zechmeister and Sandoval,⁵³⁴ this polyene is not present in chlorophyll-rich products such as grass, spinach, green leaves, or the needles of the deodar cedar (*Cedrus deodara* Lond.), nor was any phytofluene found at any stages in the leaves of the camphor tree (*Cinnamomum camphora* Nees), although tests were made on the young (pinkish) leaves, on the green leaves, and finally on the reddish autumnal leaves obtained before necrosis. Furthermore, this colorless polyene was absent from a group of plants which contained no chlorophyll or carotenoids, such as radishes, potatoes, apple flesh, whole wheat flour, and the petals of the white marguerite (*Chrysanthemum frutescens*).

On the other hand, phytofluene is present in a wide variety of plant organs which also produce carotenoid pigments.^{534,535} The petals of flowers, for instance, the yellow or treasure-flower gazania (*Gazania rigens* R. Br.), may contain as much as 30 mg. per kilogram, while a number of fruits are also good sources. A very practical source is tomato paste, in which it varies in amount from 15 to 30 mg. per kilogram. A biosynthesis of this polyene apparently takes place in the ripening of the berries of the *Pyracantha angustifolia* Schneid., since the unripe fruit contains 6.6 mg. per kilogram, while the ripe berries have a concentration of 14.7 mg. per kilogram. Phytofluene has not been observed in several cryptogams tested, which included baker's yeast and white toadstool, although it has been found in a red yeast (*Rhodotorula rubra*) and in some colored mutants, in the amount of 0.6 to 1.3 milligram per cent.⁵³⁶ Zechmeister and Haxo⁵³⁷ found that another cryptogam, the bread mold, *Neurospora* spp., contained a somewhat higher amount, namely 3 to 4.5 mg. per 100 grams of dry material. It is interesting that the phytofluene concentration did not vary for the cultures grown in light and in darkness, respectively, although the carotenoid content was greatly increased when the fungus was grown in light.

Zechmeister and Sandoval⁵³⁴ reported the absence of phytofluene in such animal products as egg-yolk, dried milk powder, pig liver, commercial ox gall concentrate, sardine meal, sardine oil, and dog-fish oil. The distribution of phytofluene in some plants is indicated in Table 36.

Phytofluene has an isoprenic structure, and it is closely related to carotene. Not only does it usually occur jointly with the carotenoids, but the molecular weight as determined by the Rast micromethod or the macro-cryoscopic procedure (520 to 500, average, 505) is only 6% lower than that of carotene. Moreover, it has been shown by analysis to contain approximately 5 methyl groups and 7 double bonds, all of which are in line with its

⁵³⁵ L. Zechmeister and A. Sandoval, *J. Am. Chem. Soc.*, **68**, 197-201 (1946).

⁵³⁶ J. Bonner, A. Sandoval, Y. W. Tang, and L. Zechmeister, *Arch. Biochem.*, **10**, 113-123 (1946).

⁵³⁷ L. Zechmeister and F. Haxo, *Arch. Biochem.*, **11**, 539-541 (1946).

isoprenic structure. The empirical formula is believed to be $C_{40}H_{64 \pm 2}$. Accordingly, phytofluene is the first naturally occurring representative of the C_{40} polyenes which is more saturated than are the common carotenoids.⁵³⁵

The most distinctive property of phytofluene is its spectral absorption in the ultraviolet. Three maxima are found: at 331, 348, and 367 $m\mu$.

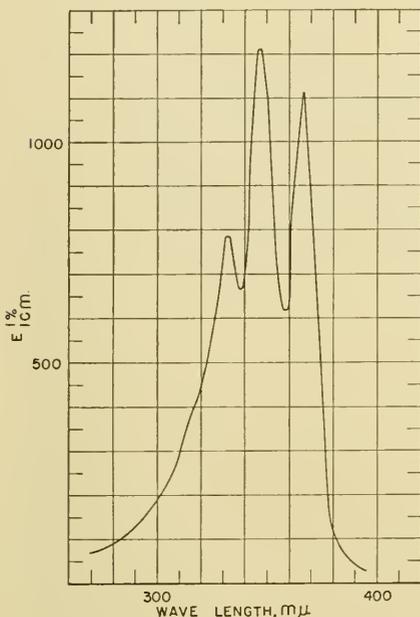


Fig. 30. Extinction curve of phytofluene in hexane.⁵³⁵

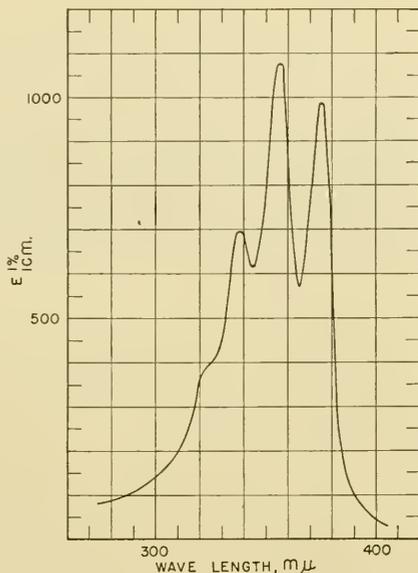


Fig. 31. Extinction curve of phytofluene in benzene.⁵³⁵

Because of the location of these maxima it seems highly improbable that more than 5 of the double bonds are conjugated. The extinction maximum at 348 $m\mu$ in hexane is almost identical with the maximum for vitamin A_2 at 345 $m\mu$. Karrer and Bretscher⁵³⁸ assume that vitamin A_2 has an entirely open structure which contains one isolated and 5 conjugated double bonds. A similar structure might be postulated for phytofluene, with the exception that most of the molecule is saturated. The absorption curves of phytofluene in hexane and benzene are included in Figures 30 and 31. The extinction values at the maxima and minima are included in Table 37.

Phytofluene has an unusual reaction with antimony trichloride. A transient blue color results which shows two bands (615 and 570 $m\mu$). In 30 seconds it changes to a purple color and then to one resembling perman-

⁵³⁸ P. Karrer and E. Bretscher, *Helv. Chim. Acta*, 26, 1758-1778 (1943).

TABLE 37
 EXTINCTION COEFFICIENTS OF PHYTOFLUENE AT MAXIMA AND MINIMA^a

Wave length, m μ	In hexane		Wave length, m μ	In benzene	
	Ext. coeff. $\times 10^{-3}$			Ext. coeff. $\times 10^{-3}$	
	Max.	Min.		Max.	Min.
332	0.78 (7)	—	338	0.69 (6)	—
338	—	0.66 (7)	344	—	0.61 (2)
348	1.21	—	355	1.06	—
358	—	0.61 (0)	365	—	0.57 (7)
367	1.13	—	374	0.97 (2)	—

^a L. Zechmeister and A. Sandoval, *J. Am. Chem. Soc.*, 68, 197-201 (1946).

ganate. The 615 m μ band disappears, while the value at 570 reaches about one-half its former intensity. Finally, the band moves to 585 m μ .

Zechmeister and Sandoval⁵³⁵ believe that phytofluene is connected with the biosynthesis of the carotenoid pigments. This polyene, however, possesses no vitamin A bioactivity.

DISTRIBUTION, PROPERTIES, AND CHEMISTRY OF THE VITAMINS A

I. Introduction and Historical Development

Although cod liver oil has for many years enjoyed a high reputation as a therapeutic product, the causative agent which is responsible for its efficacy was not recognized until well into the twentieth century. The discovery of the cause for its beneficial activity was complicated by the dual nature of the curative factors in this oil: (1) the factor which controls the antixerophthalmic behavior of vitamin A; and (2) the factor which exhibits the antirachitic potency of vitamin D.

The recognition of vitamin A as a component of cod liver oil was more difficult because of the relatively small concentration of this fat-soluble vitamin. Although the quantity of vitamin A present in this oil is entirely adequate for detection by biological tests or by the antimony trichloride reaction, it is too low to offer a satisfactory source of material for the isolation of this vitamin. Actually cod liver oil is a rather poor source of vitamin A. Samples of cod liver oil containing 800 International Units of vitamin A per gram are the ones usually obtained. This is only one two-hundred-fiftieth of the concentration frequently reported in the liver oil of the soupfin shark (*Galeorhinus zyopterus*). Since crystalline vitamin A possesses a biopotency of approximately 3,333,000 International Units per gram, it is evident that one can expect about a 0.02% concentration of this vitamin in cod liver oil.

Following the early recognition of the biological effect of deficiency of fat-soluble factors, by Osborne and Mendel,¹⁻³ and by McCollum and Davis,⁴⁻⁷ and the demonstration of the dual nature of the cod liver oil vitamins,⁸ chemical and physical methods for the estimation of vitamin A were necessary to replace the laborious and inaccurate bioassay test, before

¹ T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, **15**, 311-326 (1913).

² T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, **16**, 423-427 (1913).

³ T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, **17**, 401-408 (1914).

⁴ E. V. McCollum and M. Davis, *J. Biol. Chem.*, **15**, 167-175 (1913).

⁵ E. V. McCollum and M. Davis, *J. Biol. Chem.*, **19**, 245-250 (1914).

⁶ E. V. McCollum and M. Davis, *J. Biol. Chem.*, **20**, 641-658 (1915).

⁷ E. V. McCollum and M. Davis, *J. Biol. Chem.*, **23**, 231-246 (1915).

⁸ E. V. McCollum, N. Simmonds, J. E. Becker, and P. G. Shipley, *J. Biol. Chem.*, **53**, 293-312 (1922).

progress could be made in its isolation. The vitamin A content of cod liver oil proved to be a poor standard for comparison because of the considerable variations in potency which obtain between different samples.^{9,10}

One of the first attempts at a chemical evaluation of vitamin A was the suggestion of Steenbock and Boutwell¹¹ as to its relationship to the lipochromes. However, probably because of the minute amounts of the vitamin present, Rosenheim and Drummond¹² were unable to demonstrate any correlation between growth-promoting activity and the presence of any particular lipochromes

The Drummond Watson reaction,¹³ which involves the production of a transient purple color when sulfuric acid is mixed with such vitamin-A-rich sources as the liver oils of mammals, fish, and birds, is presumably related to the presence of vitamin A; however, the transitory nature of the color production precludes its use in quantitative measurements. On the other hand, Rosenheim and Drummond¹⁴ later proposed the use of a chloroform solution of arsenic trichloride for the determination of vitamin A, which is much more practical; arsenic trichloride produces an intense blue color with vitamin A, which persists for a sufficiently long period to enable one to make quantitative measurements of its intensity. The reliability of this technic for the colorimetric determination of vitamin A is attested to by the fact that it gives parallel results with growth-promoting activity; furthermore, the reaction ceases when vitamin A is destroyed by oxidation. The extreme sensitivity of this method has rendered it most useful in the quantitative determination of small amounts of vitamin A.

The Carr-Price method¹⁵ in current use today is an adaptation of the arsenic chloride reaction. The latter investigators replaced arsenic chloride with antimony trichloride, which they found considerably more stable. A more exact evaluation was made possible by the use of a tintometer for comparison, but this has been replaced by colorimeters or spectrophotometers in the more modern technics in which the color intensity at specific wave lengths is employed as a standard of comparison.

Although it was soon recognized that carotenes also responded to the antimony trichloride test,¹⁴ the absorption maxima were shown by Euler, Euler, and Hellström^{16,17} to differ, being 590 m μ for carotene and 617 m μ for

⁹ J. C. Drummond and S. S. Zilva, *J. Soc. Chem. Ind.*, 41, 280-284T (1922).

¹⁰ S. S. Zilva and J. C. Drummond, *J. Soc. Chem. Ind.*, 42, 185-188T (1923).

¹¹ H. Steenbock and P. W. Boutwell, *J. Biol. Chem.*, 41, 81-96 (1920).

¹² O. Rosenheim and J. C. Drummond, *Lancet*, 1920, I, 862-864.

¹³ J. C. Drummond and A. F. Watson, *Analyst*, 47, 341-349 (1922).

¹⁴ O. Rosenheim and J. C. Drummond, *Biochem. J.*, 19, 753-756 (1925).

¹⁵ F. H. Carr and E. A. Price, *Biochem. J.*, 20, 497-500 (1926).

¹⁶ B. v. Euler, H. v. Euler, and H. Hellström, *Biochem. Z.*, 203, 370-384 (1928).

¹⁷ B. v. Euler, H. v. Euler, and H. Hellström, *Svensk Kem. Tid.*, 40, 256-262 (1928); *Chem. Abst.*, 23, 3013 (1929).

vitamin A. Gillam and Morton¹⁸ reported that the higher maxima for the blue color with cod liver oil were at 606 $m\mu$ before saponification and 620 $m\mu$ after this treatment. However, such carotenoids as lycopene, bixin, capsanthin, and fucoxanthin also develop a blue color with antimony trichloride,¹⁹ although they are completely devoid of provitamin A activity. They are not present in fish liver oils or in most animal sources of vitamin A.

The investigation of the nature of vitamin A has been markedly aided by the use of the property of absorption in the ultraviolet portion of the spectrum. It was first reported by Takahashi and his co-workers²⁰ that a relationship obtains between growth-promoting activity and selective absorption in the region of 320 $m\mu$. Morton and Heilbron²¹ demonstrated that oils rich in vitamin A, as determined biologically, possess a wide area of absorption between 300 and 350 $m\mu$, with the maximum at 328 $m\mu$. It was further demonstrated that this property is also retained in the non-saponifiable residue of such vitamin-A-rich oils; moreover, it is proportional to the intensity of the antimony trichloride reaction, and disappears at the same rate as in the colorimetric test when the oils are aerated or irradiated.^{22,23} Further proof of the quantitative relationship between the results of biological estimation and those of ultraviolet absorption are given by Drummond and Morton²⁴ and by Morton, Heilbron, and Spring²⁵; such data were found to be reliable if the value at 328 $m\mu$ was not increased by the presence of biologically inert vitamin A decomposition products.²⁶ The importance of spectroscopy in relation to the determination of vitamin A is discussed on pages 728 to 733.

Further stimulus to the isolation of pure vitamin A preparations was given by the demonstration that it could be concentrated by high-vacuum distillation. Although the results were at first unsatisfactory, due to the extensive decomposition, even with pressures as low as 0.01 mm.,^{27,28} Carr and Jewell²⁹ succeeded in preparing an excellent product with a minimum decomposition by distillation in a specially constructed molecular still at pressures below 10^{-3} mm. The fraction boiling at 137–138°C. had a considerably increased potency.

¹⁸ A. E. Gillam and R. A. Morton, *Biochem. J.*, **25**, 1346–1351 (1931).

¹⁹ H. v. Euler, P. Karrer, and M. Rydbom, *Ber.*, **62**, 2445–2451 (1929).

²⁰ K. Takahashi, Z. Nakamiya, K. Kawakami, and T. Kitasato, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **3**, 81–148 (1925).

²¹ R. A. Morton and I. M. Heilbron, *Biochem. J.*, **22**, 987–996 (1928).

²² P. R. Peacock, *Lancet*, 1926, **II**, 328–330.

²³ S. G. Willimott and F. Wokes, *Pharm. J.*, **118**, 217–218 (1927); *Chem. Abst.*, **21**, 3220 (1927).

²⁴ J. C. Drummond and R. A. Morton, *Biochem. J.*, **23**, 785–802 (1929).

²⁵ R. A. Morton, I. M. Heilbron, and F. S. Spring, *Biochem. J.*, **24**, 136–140 (1930).

²⁶ A. Chevallier and P. Chabre, *Bull. soc. chim. biol.*, **16**, 1451–1478 (1934).

²⁷ I. M. Heilbron, R. N. Heslop, R. A. Morton, E. T. Webster, J. L. Rea, and J. C. Drummond, *Biochem. J.*, **26**, 1178–1193 (1932).

²⁸ J. C. Drummond and L. C. Baker, *Biochem. J.*, **23**, 274–291 (1929).

²⁹ F. H. Carr and W. Jewell, *Nature*, **131**, 92 (1933).

The best preparation of Carr and Jewell had an extinction value, E (1%, 1 cm.), of 1600, contrasted with the figures obtained by Heilbron *et al.*,²⁷ *i.e.*, 1370 for a halibut distillate, 1330 for a distillate of sturgeon liver oil, and 1350 and 1250 for two samples prepared by Karrer.³⁰

Holmes and Corbet³¹ were the first to prepare vitamin A in crystalline form. They accomplished this by prolonged cooling of a vitamin A concentrate dissolved in methyl alcohol containing small amounts of water at temperatures below -50°C . The concentrates were prepared from the liver oils of the Japanese ishinagi (*Stereolepis ishinagi*) and of the mackerel (*Scomber scombrus*). The vitamin A separated in the form of yellow rosettes with a melting point of $7.5\text{--}8.0^{\circ}\text{C}$.; it had an extinction maximum of 2100 and a biological potency of 3,000,000 International Units per gram. The results as regards melting point and biological potency were later confirmed by Mead.³² However, Baxter and Robeson³³ demonstrated that the crystals isolated from methanolic solution contained approximately 10% of methyl alcohol of crystallization. The latter workers prepared vitamin A samples having identical extinction maxima of 1750, and melting points of $63\text{--}64^{\circ}\text{C}$., from shark liver, ling cod liver (*Molva vulgaris*), black sea bass or California jewfish liver (*Stereolepis gigas*), and halibut (*Hippoglossus hippoglossus*) visceral oils, which originally had such varied extinction coefficients as 100, 129, 317, and 72, respectively. They employed vacuum distillation, saponification, and crystallization from ethyl formate at low temperatures.

Further progress in determining the nature of vitamin A resulted from the preparation of crystalline derivatives. In 1935 Hamano^{34,35} first succeeded in preparing a β -naphthoate and an anthraquinone- β -carboxylate from several fish liver oil concentrates. Mead³² later confirmed these results, while Baxter and Robeson^{36,37} subsequently synthesized a number of additional esters. The nature and properties of these crystalline products are included in Table 1. The crystalline structure of the vitamin A alcohol, both from ethyl formate and from methyl alcohol, is shown in Plate 1. Photographs of the acetate and succinate esters are found in Plate 2, while those of the palmitate and β -naphthoate are given in Plate 3.

³⁰ I. M. Heilbron, W. E. Jones, and A. L. Bacharach, *Vitamins and Hormones*, 2, 155-213 (1944).

³¹ H. N. Holmes and R. E. Corbet, *J. Am. Chem. Soc.*, 59, 2042-2047 (1939).

³² T. H. Mead, S. W. F. Underhill, and K. H. Coward, *Biochem. J.*, 33, 589-600 (1939).

³³ J. G. Baxter and C. D. Robeson, *J. Am. Chem. Soc.*, 64, 2411-2416 (1942).

³⁴ S. Hamano, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 23, 69-73 (1935).

³⁵ S. Hamano, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, 32, 44-49 (1937).

³⁶ J. G. Baxter and C. D. Robeson, *J. Am. Chem. Soc.*, 64, 2407-2410 (1942).

³⁷ J. G. Baxter and C. D. Robeson, *Science*, 92, 203-204 (1940).

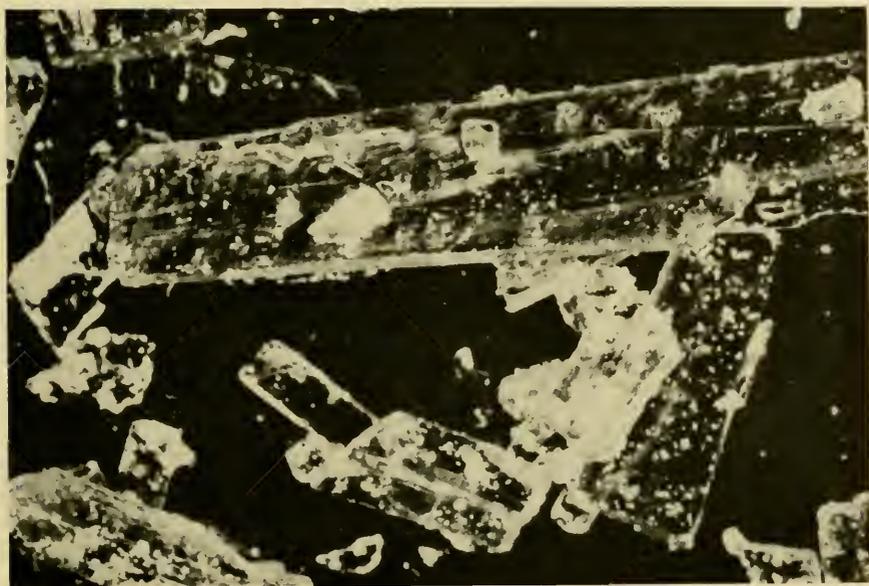


Plate 1. (Top) Crystalline vitamin A, m.p. 63–64°, $\times 14$. (Bottom) Crystals of vitamin A (MeOH), m.p. 7–10°, $\times 18$.²² Courtesy Distillation Products Industries.

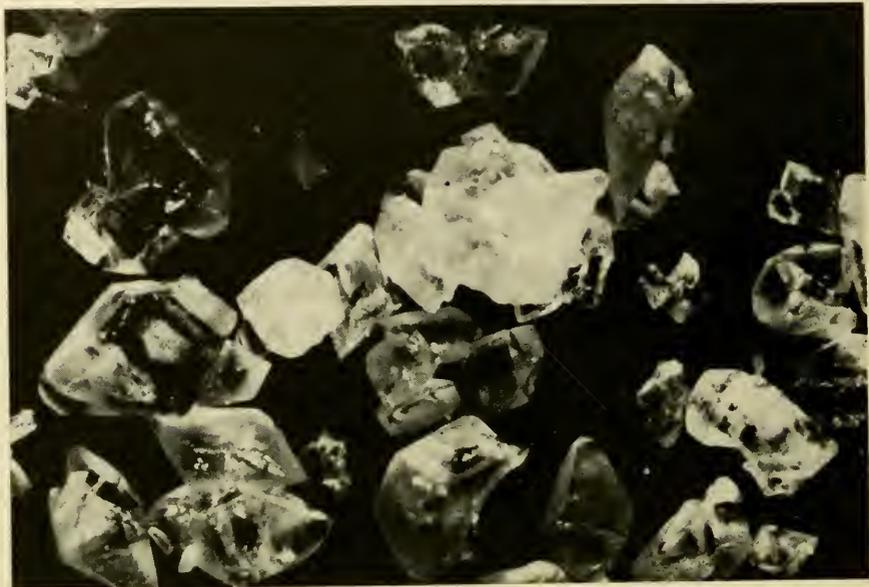


Plate 2. (Top) Vitamin A acetate, $\times 30$. (Bottom) Divitamin A succinate, $\times 30$.³⁶
Courtesy Distillation Products Industries.

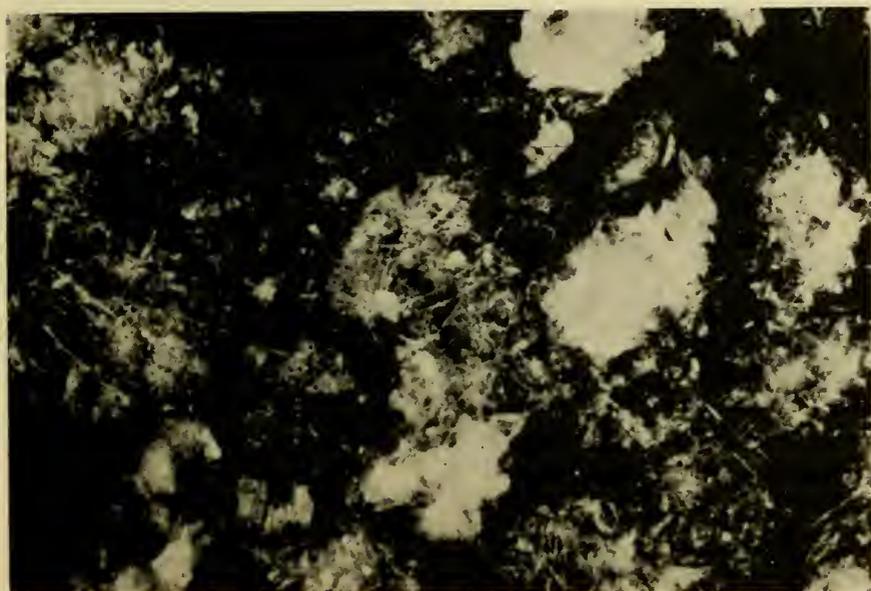
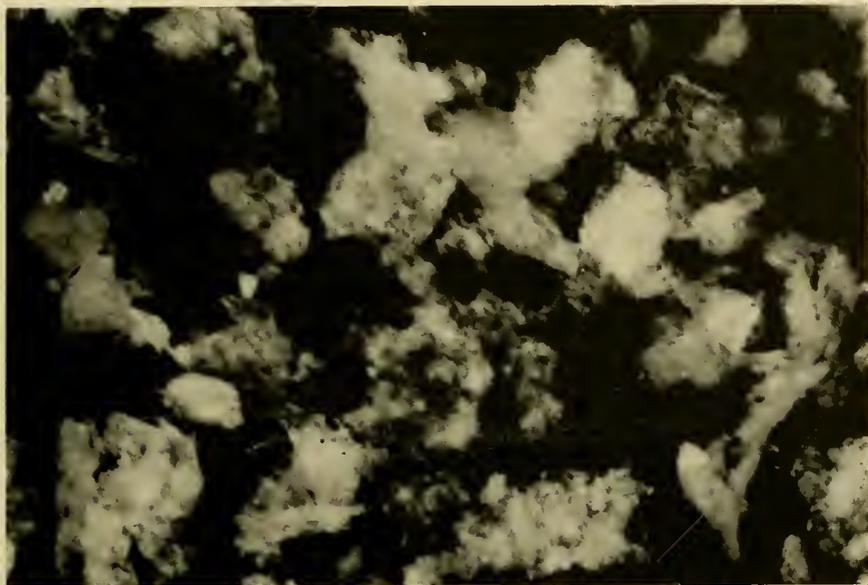


Plate 3. (Top) Vitamin A palmitate, $\times 40$. (Bottom) Vitamin A β -naphthoate, $\times 24$.⁵⁶
Courtesy Distillation Products Industries.

TABLE I
PROPERTIES OF SEVERAL CRYSTALLINE VITAMIN A ESTERS

Vitamin A compound	Nature of crystals	Crystn. medium	M.p., °C.	E (1%, 1 cm.)	Ref.
Alcohol	Prisms	Ethyl formate	63-64	1750	<i>a</i>
Acetate	Pale yellow prisms	Methanol	57-58	1510	<i>b</i>
Anthraquinone-2-carboxylate	Yellow prisms ^d	Methanol ^d	124	—	<i>c,d</i>
β -Naphthoate	Yellow prisms	Ethanol	74-76	1090	<i>b,c</i>
Palmitate	Yellow prisms	Propylene oxide	27-28	940	<i>b</i>
Succinate	Yellow prisms	Ethyl formate	76-77	1240	<i>b</i>

^a J. G. Baxter and C. D. Robeson, *J. Am. Chem. Soc.*, *64*, 2411-2416 (1942).

^b J. G. Baxter and C. D. Robeson, *J. Am. Chem. Soc.*, *64*, 2407-2410 (1942).

^c S. Hamano, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, *28*, 69-73 (1935); *32*, 44-49 (1937).

^d T. H. Mead, *Biochem. J.*, *33*, 589-600 (1939).

Other crystalline derivatives include the dimaleic adduct of acetylated vitamin A (m.p., 261-262°C.).³⁸ and the corresponding benzoylated compound (m.p., 263-264°C.). Hamano^{35,39} prepared the dimaleic adduct of vitamin A palmitate (m.p., 220°C.) by using the liver oils themselves. It was later demonstrated by Tischer⁴⁰ that this same compound was produced in cod liver oil after treatment with maleic anhydride; it was separated from the mixture by molecular distillation.

The structure of vitamin A was first correctly postulated by Karrer, Morf, and Schöpp.⁴¹ This structure was confirmed by the synthesis of perhydrovitamin A,⁴² which was shown to be identical with the saturated alcohol prepared from the natural product. The many syntheses for vitamin A now in current use are all based upon the Karrer structure.⁴³

The confusion regarding the chemical nature of vitamin A has been largely cleared up by the recognition that several types of isomers are possible. In addition to ordinary vitamin A (A₁), which is formed in marine forms as well as in most mammals, a second type, probably including an additional double bond, occurs in the storage depots of fresh-water fishes. This is called vitamin A₂. Isomers of another type have also recently been demonstrated. These are the stereoisomers. Ordinary vitamin A presumably represents the all-*trans* form; only 2 of the 5 double bonds in the molecule are able to assume a *cis* configuration, while the others are prevented from doing so because of steric hindrance.⁴⁴ Zechmeister⁴⁵ has in-

³⁸ K. Kawakami, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, *26*, 77-81 (1935).

³⁹ S. Hamano, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, *26*, 87-90 (1935).

⁴⁰ A. O. Tischer, *J. Biol. Chem.*, *125*, 475-477 (1938).

⁴¹ P. Karrer, R. Morf, and K. Schöpp, *Helv. Chim. Acta*, *14*, 1431-1436 (1931).

⁴² P. Karrer and R. Morf, *Helv. Chim. Acta*, *16*, 625-641 (1933).

⁴³ N. A. Milas, *Vitamins and Hormones*, *5*, 1-38 (1947).

⁴⁴ L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, *3*, 203-235 (1939).

⁴⁵ L. Zechmeister, *Vitamins and Hormones*, *7*, 57-81 (1949).

licated the possibility of 3 stereoisomers of all-*trans*-vitamin A₁: 3-*cis*-vitamin A, 5-*cis*-vitamin A, and 3,5-di-*cis*-vitamin A. Robeson and Baxter^{46,47} isolated a stereoisomeric form from some fish liver oils; this is probably the 5-*cis* form.⁴⁶

2. Occurrence of the Vitamins A

Vitamin A occurs only in the animal organism, and it is never found in plants. This is in marked contrast to β -carotene, which occurs primarily in plants but which may be found in animal tissues under some conditions. In the latter case, it is believed that the tissue carotenoids are derived from the food; there is no absolute proof, however, that some animal tissues may not be capable of synthesizing carotenoids from appropriate precursors. The colored yeasts and other fungi are known to contain β -carotene and other carotenoids, but no vitamin A.^{48,49} Vitamin A is present in most animals; the largest quantity is usually concentrated in the liver.

(1) Vitamin A in Fishes

Fish livers constitute the chief natural source of vitamin A, but extreme differences in the concentrations are observed in various species. The distribution of a number of such oils is given in Table 2. The amount of vitamin A present varies with the type of food consumed, with sex, the season of the year, and with general living conditions. Schmidt-Nielsen and Schmidt-Nielsen⁵⁰ showed that the ratio of vitamin A in some fish liver oils, as determined from the antimony trichloride blue values, was as follows:

Halibut (<i>Hippoglossus hippoglossus</i>).....	700
Mackerel (<i>Scomber scombrus</i>).....	570
North Atlantic salmon (<i>Salmo salar</i>).....	250
Red perch or Norway haddock (<i>Sebastes marinus</i>).....	70
Porbeagle (<i>Lamna cornubica</i>) (mackerel shark).....	6

It was also noted by these workers that the liver oils which could be isolated only by solvent extraction invariably had a higher vitamin A content than did those in which the oils could be separated by steaming.

Von Euler and Karrer,⁵¹ as well as Lovern,⁵² have pointed out that the liver oil of the halibut (*Hippoglossus hippoglossus*) is 50 to 100 times richer in vitamin A than is a good grade of cod liver oil, although the authors

⁴⁶ C. D. Robeson and J. G. Baxter, *Nature*, 155, 300 (1945).

⁴⁷ C. D. Robeson and J. G. Baxter, *J. Am. Chem. Soc.*, 69, 136-141 (1947).

⁴⁸ E. Lederer, *Les Caroténoïdes des Plantes*, Paris, 1934. Cited by R. J. Williams, *Vitamins and Hormones*, 1, 230 (1943).

⁴⁹ A. Scheunert and J. Reschke, *Deut. med. Wochschr.*, 57, 349-351 (1931).

⁵⁰ S. Schmidt-Nielsen and S. Schmidt-Nielsen, *Biochem. J.*, 23, 1153-1157 (1929).

⁵¹ H. v. Euler and P. Karrer, *Naturwissenschaften*, 19, 676 (1931).

⁵² J. A. Lovern, *Nature*, 129, 726 (1932).

noted that wide variations in the content of vitamin A obtained. Ling cod liver oil (*Molva vulgaris*), on the other hand, may be even more potent in vitamin A than is halibut liver oil.⁵³ The wide variation in vitamin A content in different fish liver oils is illustrated by the recent calculation of the vitamin A content of fish liver oils, expressed as per cent of the oil, which has been made by Jewell, Mead, and Phipps.⁵⁴ Whereas cod liver oil (*Gadus morrhua*) contains only 0.01% of vitamin A, the conger eel (*Conger vulgaris*) has 20 times the amount (0.2%), the school shark (*Galeorhinus australis*) 70 times the quantity (0.7%), the halibut (*Hippoglossus hippoglossus*) 170 times the concentration (1.7%), and the tunny, *Thunnus thynnus* (*vulgaris*), 470 times the figure for the cod (4.7%). The vitamin A contents of 25 varieties of Chinese fishes have been reported by Mar.⁵⁵ Basu and Rai Sircar⁵⁶ studied the vitamin A distribution in the livers of a number of Bengal fishes.

TABLE 2
DISTRIBUTION OF VITAMIN A IN VARIOUS FISH LIVER OILS^a

Source of oil	Zoological name	Potency, I.U./g.
Haddock	<i>Gadus aeglefinus</i>	65
Cod	<i>Gadus morrhua</i>	600
Striped bass	<i>Roccus lineatus</i>	4,500
Jack smelt (silversides)	<i>Atherinopsis californiensis</i>	10,000
Albacore	<i>Germo alalunga</i>	18,000
Red grouper	<i>Epinephelus morio</i>	25,000
Boston mackerel	<i>Scomber scombrus</i>	30,000
Pacific barracuda	<i>Sphyræna argentea</i>	40,000
Skipjack tuna (bonito)	<i>Katsuwonus pelamis</i>	40,000
California yellowtail	<i>Seriola dorsalis</i>	50,000
White sea-bass	<i>Cynoscion nobilis</i>	50,000
Red snapper	<i>Lutianus campechanus</i>	60,000
Totuava (California weakfish)	<i>Ericcion macdonaldi</i>	60,000
Bluefin tuna (tunny)	<i>Thunnus thynnus</i>	60,000
Pacific yellowfin tuna	<i>Neothunnus macropterus</i>	70,000
Pacific mackerel	<i>Scomber (Pneumatophorus) diego</i>	80,000
Pacific bonito	<i>Sarda chilensis</i>	120,000
Spotted cabrilla	<i>Epinephelus analogus</i>	170,000
Broadbill swordfish	<i>Xiphias gladius</i>	250,000
Ishinagi	<i>Stereolepis ishinagi</i>	300,000
Black sea-bass	<i>Stereolepis gigas</i>	600,000

^a H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1942.

⁵³ L. B. Pett, M. Lipkind, and G. A. Le Page, *Nature*, 144, 634 (1939).

⁵⁴ W. Jewell, T. H. Mead, and J. W. Phipps, *J. Soc. Chem. Ind.*, 58, 56-64T (1939).

⁵⁵ P. G. Mar, *Chinese J. Physiol.*, 16, 67-72 (1941).

⁵⁶ K. P. Basu and B. C. Rai Sircar, *Indian J. Med. Research*, 27, 721-729 (1940).

Other visceral organs of some fishes have been found to be richer sources of vitamin A than is the liver.⁵⁷⁻⁵⁹ In the case of the halibut, large deposits of vitamin A, almost exclusively in the form of the ester, occur in the intestine, where they are localized in the mucosa and in particular in the tunica propria.⁶⁰⁻⁶³

(2) Vitamin A in Animals Other Than Fishes

The liver is also the chief site of storage of vitamin A in animals other than fishes. Jensen and With⁶⁴ demonstrated the presence of this fat-soluble vitamin in two species of reptiles, 36 species of birds, and 22 species of mammals, including man. The concentration is usually highest in fish livers, next in the livers of birds and reptiles, and lowest in the livers of mammals. In the last group, the concentration in the liver of guinea pigs has been found to be unusually low.⁶⁴ Large amounts, approaching that present in the fish livers, were reported in one mammal, the great blue whale (*Balaenoptera musculus*).⁶⁵

Strangely enough, on the basis of a survey of the distribution of vitamin A, Karrer, von Euler, and Schöpp⁶⁶ reported that the livers of the following mammals were devoid of vitamin A: Bengal tiger (*Felis tigris*), adult and young male lion (*Felis leo*), crab-eating raccoon (*Procyon cancrivorus*), harbor seal (*Phoca vitulina*), cormorant (*Phalacrocorax carbo*), salamander (axolotl) (*Ambystoma* spp.), and one species of reptile, the leopard adder (*Coluber quadrilineatus*). It is not known whether the absence of vitamin A is the result of the previous diet or whether vitamin A is readily destroyed or is not stored in these animals. In such cases, one might suspect that it is not a necessary nutrient. Other species which apparently do not require vitamin A are the cockroach or Croton bug, *Blattella* (*Phyllodromia*) *germanica*,⁶⁷ and the clothes moth (*Tineola bisselliella*).⁶⁸ Bowers and McCay⁶⁹ were able to raise the cockroach to maturity on a vitamin-A-free diet. The fact that vitamin A was not synthesized *de novo* is indicated by its ab-

⁵⁷ J. A. Lovern, J. R. Edisbury, and R. A. Morton, *Nature*, 140, 276 (1937).

⁵⁸ J. R. Edisbury, J. A. Lovern, and R. A. Morton, *Biochem. J.*, 31, 416-423 (1937).

⁵⁹ J. R. Edisbury, R. A. Morton, G. W. Simpkins, and J. A. Lovern, *Biochem. J.*, 32, 118-140 (1938).

⁶⁰ J. A. Lovern, T. H. Mead, and R. A. Morton, *Chemistry & Industry*, 58, 147 (1939).

⁶¹ J. A. Lovern and R. A. Morton, *Chemistry & Industry*, 58, 147 (1939).

⁶² J. A. Lovern and R. A. Morton, *Biochem. J.*, 33, 330-337 (1939).

⁶³ J. Glover and R. A. Morton, *Biochem. J.*, 42, lxiii-lxiv (1948).

⁶⁴ H. B. Jensen and T. K. With, *Biochem. J.*, 33, 1771-1786 (1939).

⁶⁵ S. Schmidt-Nielsen and S. Schmidt-Nielsen, *Kgl. Norske Videnskab. Selskabs Förel.*, 3, No. 46, 177-180 (1930); *Chem. Abst.*, 26, 1642 (1932).

⁶⁶ P. Karrer, H. v. Euler, and K. Schöpp, *Helv. Chim. Acta*, 15, 493-495 (1932).

⁶⁷ C. M. McCay, *Physiol. Zoöl.*, 11, 89-103 (1938).

⁶⁸ M. F. Crowell and C. M. McCay, *Physiol. Zoöl.*, 10, 368-372 (1937).

⁶⁹ R. E. Bowers and C. M. McCay, *Science*, 92, 291 (1940).

sence as shown by tests of 150 g. of oil obtained from the bodies of a large number of cockroaches. There has been a conviction in some quarters that the invertebrates lack vitamin A. This theory has been supported by the failure to identify vitamin A in any tissue. Moreover, large hauls of zooplankton, consisting principally of invertebrate material, failed in a number of instances to show even traces of vitamin A.^{70,71} However, it has now been proven that high concentrations of vitamin A are present in the retinas not only of the vertebrates but also of the invertebrates. In the earlier studies on invertebrates, the presence of vitamin A had been overlooked because the retinas constituted such a relatively insignificant proportion of the total body tissues.

(3) *Distribution of Vitamins A₁ and A₂*

Although the usual form of vitamin A (vitamin A₁) is the predominant type in which this compound occurs naturally, a second form, vitamin A₂, has been found in the livers of fresh-water fishes. The presence of this additional kind of vitamin A was first suspected as a result of variations in the reaction of certain fish liver oils to antimony trichloride. This phenomenon was first observed by Heilbron, Gillam, and Morton⁷² as early as 1931. These workers found that absorption bands appeared at 690–700 $m\mu$ and 635 to 655 $m\mu$ when some fish liver oils were treated with antimony trichloride, as well as in the usual area of 615–620 $m\mu$. They postulated that one or more chromogenic substances, in addition to vitamin A, were present, but they did not realize that another form of vitamin A was responsible for the anomalous reaction.

Edisbury, Morton, and Simpkins⁷³ found that an absorption band only rarely developed at 693 $m\mu$ when cod liver oil was treated with the Carr-Price reagent, and never occurred when whale oil was similarly treated, but that a marked positive response usually obtained in the case of halibut liver oils, as well as of the visceral oils of this species. The ratios of absorption at 693:620 $m\mu$ were usually approximately 1:6 in the case of the hepatic fat, and 1:10 in the body fat of the halibut. However, in the eyes of the goldfish (*Carassius auratus*), the intensity of the 693 $m\mu$ band was 1.5 greater than that at 620 $m\mu$.⁷³ In the brown trout, only the 693 $m\mu$ band could be detected. This led Edisbury, Morton, and Simpkins⁷³ to suggest that the chromogen responsible for the 693 $m\mu$ band should be regarded as vitamin A₂, because its distribution suggested that it could replace vitamin A₁ in the fresh-water fish. Almost simultaneously, Lederer and Rosan-

⁷⁰ J. C. Drummond and E. R. Gunther, *J. Exptl. Biol.*, *11*, 203–209 (1934).

⁷¹ A. E. Gillam, M. S. El Ridi, and R. S. Wimpenny, *J. Exptl. Biol.*, *16*, 71–88 (1939).

⁷² I. M. Heilbron, A. E. Gillam, and R. A. Morton, *Biochem. J.*, *25*, 1352–1366 (1931).

⁷³ J. R. Edisbury, R. A. Morton, and G. W. Simpkins, *Nature*, *140*, 234 (1937).

ova⁷⁴ discovered that the liver oils from certain Russian fresh-water fishes gave a stronger response at 690 m μ with an antimony trichloride reagent than at the conventional 620 m μ position. When the non-saponifiable fractions of liver oils of fresh-water fishes were dissolved in alcohol, the maximum absorption in the ultraviolet area was found at 340–350 m μ .⁷⁵ A new band at 280–285 m μ was also noted. The high ratios between extinction values at 693 m μ and 620 m μ in antimony trichloride tests were always associated with the liver oils of fresh-water fishes in which the maximum absorption of the untreated product was 340–345 m μ . Edisbury *et al.*⁵⁹ found that the goldfish eye preparations also had absorption maxima at 350 and 288 m μ .

In a further study of the Carr-Price reaction with the liver oils of fresh-water fishes, Gillam *et al.*⁷⁶ reported that the 693:620 m μ ratio was usually in the neighborhood of 2:1, contrasted with a ratio of 0.15:1 for the liver oils of marine fishes. Morton⁷⁷ reports that, although the proportions of A₁ and A₂ in the liver oils of fresh-water fishes are highly variable, certain statements can be made. For example, $E_{693m\mu}:E_{620m\mu}$ is highest between 2 and 3 in the carnivorous fishes such as the pike (*Esox lucius*), the pike perch or sander (*Lucioperca sandra*), the fresh-water perch (*Perca fluviatilis*), and the wels, or European catfish (*Silurus glanis*). In the case of omnivorous fishes, the ratio was found to be considerably lower, which indicates a much lower proportion of vitamin A₂ and considerable amounts of vitamin A₁. Fishes which are included in this category are the bream (*Abramis brama*), the carp (*Cyprinus carpio*), and the tench (*Tinca tinca*). The liver oils of migratory fishes also showed the lower $E_{693m\mu}:E_{620m\mu}$ ratio. This was the case with the North Atlantic salmon (*Salmo salar*), the sturgeon (*Acipenser sturio*), the eel (*Anguilla vulgaris*), and the brown brook trout (*Salmo fario*) and rainbow trout (*Salmo irideus*). It was found that the ratio of vitamins A₂:A₁ is fairly constant in any given species; it is also independent of sex and age. It shows little seasonal variation and is not related to the geographic source of the fishes.⁷⁷ The ratio of $E_{693m\mu}:E_{620m\mu}$ is usually highest in the liver oils, pyloric caeca, and other absorbing surfaces of the gut. However, according to Morton,⁷⁷ Lederer found that, in carp, a ratio of 2.3:3.5 obtained in the retina, as against 0.5:0.9 in the liver. The vitamin A₂ content of the pike perch is given in Table 3, which follows on page 680.

⁷⁴ E. Lederer and V. A. Rosanova, *Biokhimiya*, 2, 293–303 (1937); *Chem. Abst.*, 31, 5105 (1937). Cited by R. A. Morton, *The Application of Absorption Spectra to the Study of Vitamins, Hormones, and Coenzymes*, 2nd ed., Jarrell, Ash, Boston, 1942, p. 76.

⁷⁵ E. Lederer, V. Rosanova, A. E. Gillam, and I. M. Heilbron, *Nature*, 140, 233 (1937).

⁷⁶ A. E. Gillam, I. M. Heilbron, W. E. Jones, E. Lederer, and V. Rosanova, *Biochem. J.*, 32, 405–416 (1938).

⁷⁷ R. A. Morton, *The Application of Absorption Spectra to the Study of Vitamins, Hormones, and Coenzymes*, 2nd ed., Jarrell, Ash, Boston, 1942.

TABLE 3
DISTRIBUTION OF VITAMIN A₂ IN THE PIKE PERCH (*Lucioperca sandra*)

Organ	<i>E</i> (693 m μ)	Vitamin A, p.p.m. in fresh organs	Vitamin A, mg./fish
	<i>E</i> (620 m μ)		
Liver	2.4	60	0.4
Stomach	1.0	1.4	0.01
Pyloric caeca	2.25	165	1.1
Pericaecal fat	1.0	6	0.09
Caecum wall	2.4	125	—
Caecum contents	2.1	34	—

Adapted from E. Lederer and F. H. Rathmann, *Biochem. J.*, *32*, 1252-1261 (1938); by R. A. Morton, *The Application of Absorption Spectra to the Study of Vitamins, Hormones, and Coenzymes*, 2nd ed., Jarrell, Ash, Boston, 1942, p. 79.

(4) Vitamin-A-Like Compounds in the Retina

The retina has long been recognized as a concentrated source of a pigment resembling vitamin A. This pigment has been known as visual purple or rhodopsin; apparently it is a conjugated protein in which the vitamin-A-like product serves as the prosthetic group. Rhodopsin has frequently been referred to in the literature as a "carotenoid" protein. Inasmuch as recent findings have indicated that the prosthetic group is vitamin A aldehyde,⁷⁸⁻⁸¹ rather than one of the C₄₀ polyenes, this terminology would appear to be somewhat misleading.

On being bleached by light, the retinas are changed from the pinkish color of rhodopsin to a light yellow color. This occurs as a result of a series of photochemical and thermal reactions. Wald⁸² was able to prepare the vitamin-A-like product so formed by a petroleum ether extract of illuminated retinas. He called it *retinene* (now subdivided into retinene₁ and retinene₂). Wald reported that this product was not identical with vitamin A, since it had an absorption maximum at 365 m μ in petroleum ether (instead of 325 m μ), and the absorption maximum in chloroform was displaced to 385 m μ . When treated with antimony trichloride, the retinene preparation displayed a bluish color similar to that exhibited by a number of carotenoids, but the absorption maximum was at 664 m μ instead of at 615-620 m μ , as is the case with vitamin A. Although retinene and the specific protein revert to rhodopsin in the dark, in the intact retina, retinene is to some extent converted to vitamin A. In the case of retinene₁, vitamin A₁ can be identified by its typical absorption band in chloroform (328

⁷⁸ R. A. Morton, *Nature*, *153*, 69-71 (1944).

⁷⁹ R. A. Morton and T. W. Goodwin, *Nature*, *153*, 405-406 (1944).

⁸⁰ E. G. E. Hawkins and R. F. Hunter, *J. Chem. Soc.*, 1944, 411.

⁸¹ S. Ball, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, *42*, 516-523 (1948).

⁸² G. Wald, *J. Gen. Physiol.*, *19*, 351-371, 781-795 (1935-1936).

$m\mu$) and by the position of the absorption maximum of the antimony trichloride reaction product at 615–620 $m\mu$.

A second type of retinal pigment closely related to rhodopsin occurs in the fresh-water fishes. This has been called porphyropsin. Wald^{83–85} succeeded in preparing a retinene from porphyropsin which differed from the previously described retinene; it was therefore referred to as retinene₂, while the original retinene isolated from illuminated rhodopsin is now known as retinene₁. Since the discovery of vitamin A₂ in fresh-water fishes, it has become evident that retinene₂ bears the same relationship to vitamin A₂ that retinene₁ does to vitamin A₁. Like retinene₁, retinene₂ reverts to the conjugate protein (porphyropsin) in the retina; some vitamin A₂ is also formed as a by-product, just as vitamin A₁ is a by-product in the formation of rhodopsin. Retinene₂ has a maximum absorption of 390 $m\mu$ in petroleum ether and of 405 $m\mu$ in chloroform; these are distinctly higher than those of retinene₁. Moreover, the chromogenic product formed when it is reacted with antimony trichloride has an absorption maximum somewhat greater than 705 $m\mu$. Finally, the vitamin A₂ formed from retinene₂ has the typical absorption maxima of 345–350 $m\mu$ in cyclohexane, 355 $m\mu$ in chloroform, and 693 $m\mu$ for the reaction product with antimony trichloride.

According to Wald,⁸⁶ rhodopsin or porphyropsin has never been found in any tissue other than the retina. In the case of invertebrates, large amounts of potential vitamin A may be stored in the retina and very little or none at all in the liver.⁸⁶ Retinene₁ and vitamin A₁ have been demonstrated in the squid (*Loligo peali*). In a number of crustacea, such as the fiddler crab (*Carcinus maenas* and *Uca pugnax*) and the lobster (*Homarus* spp.),⁸⁷ vitamin A₁ occurs in the retina, but no trace of retinene₁ has been found.⁸⁷ In a fresh-water crustacean, the crayfish (*Cambarus virilis*), three components appear in the retina: vitamin A₁, astaxanthin, and retinene₁.

Although the retinas of land vertebrates contain vitamin A₁ aldehyde exclusively (except after feeding high concentrations of vitamin A₂), the nature of the pigment in the retinas of fishes is dependent upon their normal habitat. Marine fishes have the rhodopsin-vitamin A₁ system, while the fresh-water fishes possess the porphyropsin-vitamin A₂ system. Where fishes live both in fresh- and in salt-water environs, the chief pigment in the retinas is the type which is present when they are in the environment in which they breed. Thus, the anadromous fishes, such as the salmon (*Salmo salar*), which spawn in fresh water, possess both the rhodopsin and the porphyropsin systems, but chiefly the latter, along with vitamin A₂. The retinas of three genera of this group, the brook or fountain trout

⁸³ G. Wald, *J. Gen. Physiol.*, 22, 391–415 (1938–1939).

⁸⁴ G. Wald, *Nature*, 140, 545–546 (1937).

⁸⁵ G. Wald, *J. Gen. Physiol.*, 22, 775–794 (1938–1939).

⁸⁶ G. Wald, *Vitamins and Hormones*, 1, 197–227 (1943).

⁸⁷ G. Wald, *Am. J. Physiol.*, 133, 479–480 (1941).

(*Salvelinus fontinalis*), the rainbow trout (*Salmo irideus*), and the king or quinnat salmon (*Oncorhynchus tshawytscha*), have all been shown to follow this pattern. In the case of the so-called catadromous fishes such as the American "fresh-water" eel (*Anguilla rostrata*) and the killifish (*Fundulus heteroclitus*), which are fresh-water fishes but which spawn in salt water, the retinas likewise contain both rhodopsin and porphyropsin; however, in line with the salt-water varieties of fishes, the chief pigment gives rise to retinene₁ and vitamin A₁. Some anadromous fishes such as the white perch (*Morone americana*) and the alewife (*Pomolobus pseudoharengus*) contain exclusively the porphyropsin (vitamin A₂) system.^{83,88}

Among the amphibia, the common newt (*Triturus viridescens*) follows the pattern of the anadromous fishes and possesses only porphyropsin.⁸⁹ The frog (*Rana* spp.), on the other hand, has only the typical rhodopsin system. Since Wald⁸⁶ considers the retinal use of vitamin A₂ as an extremely ancient one, the variation in composition of the retinal pigments in two such closely related species of amphibia is difficult to explain.

Rhodopsin has been shown to be the photosensitive compound in the eyes of such birds as the owl⁹⁰ and the chicken.⁸⁴ In the case of the chicken, it has been demonstrated that the filter pigments in the retina also contain astaxanthin,⁹¹ a mixture of xanthophylls, and an unidentified carotenoid resembling sarcinene, which is a pigment related to the air-borne coccus *Sarcina lutea*.⁸⁹ Astacene is found in the retina only in the case of the chicken, and appears to be synthesized, since it is absent from the embryonic retina and does not occur in the yolk.⁹²

The retinas of rats, rabbits, cattle, sheep, and pigs,^{93,94} as well as of the monkey, cat, and dog,⁹⁰ and also of man,^{86,95} possess the rhodopsin-vitamin A₁ system. The vitamin A₂-porphyropsin system can replace the usual rhodopsin system in the retina of the rat when vitamin A₂ is administered over a 12-week period.⁹⁶

The interrelations of the types of vitamins A and carotenoids of importance in retinal vision are represented diagrammatically in Figure 1.

Although the vitamins A are of such fundamental importance in vision, their content in the retina may actually contribute only an extremely small

⁸⁸ G. Wald, *J. Gen. Physiol.*, 25, 235-245 (1941-1942).

⁸⁹ G. Wald, *Biol. Sympos.*, 7, 43-71 (1942).

⁹⁰ E. Köttgen and G. Abelsdorff, *Z. Psychol. Physiol. Sinnesorgane*, 12, 161-184 (1896).

⁹¹ R. Kuhn, J. Stone, and N. A. Sørensen, *Ber.*, 72, 1688-1701 (1939).

⁹² G. Wald and H. Zussman, *J. Biol. Chem.*, 122, 449-460 (1938).

⁹³ G. Wald, *J. Gen. Physiol.*, 18, 905-915 (1934-1935).

⁹⁴ G. Wald, *J. Gen. Physiol.*, 21, 795-832 (1937-1938).

⁹⁵ A. König and E. Köttgen, *Sitzber. kgl. preuss. Akad. Wiss.*, 1894, I, 577-598.

⁹⁶ E. M. Shantz, N. D. Embree, H. Carpenter, and J. H. Wills, Jr., *J. Biol. Chem.*, 163, 455-464 (1946).

proportion of the total vitamin A reserves in the body. For example, out of a calculated total of 300 mg. of vitamin A in the tissues of the dog, only 4 μ g. or approximately 0.001% of the total occur in the retinal pigments.⁹⁷

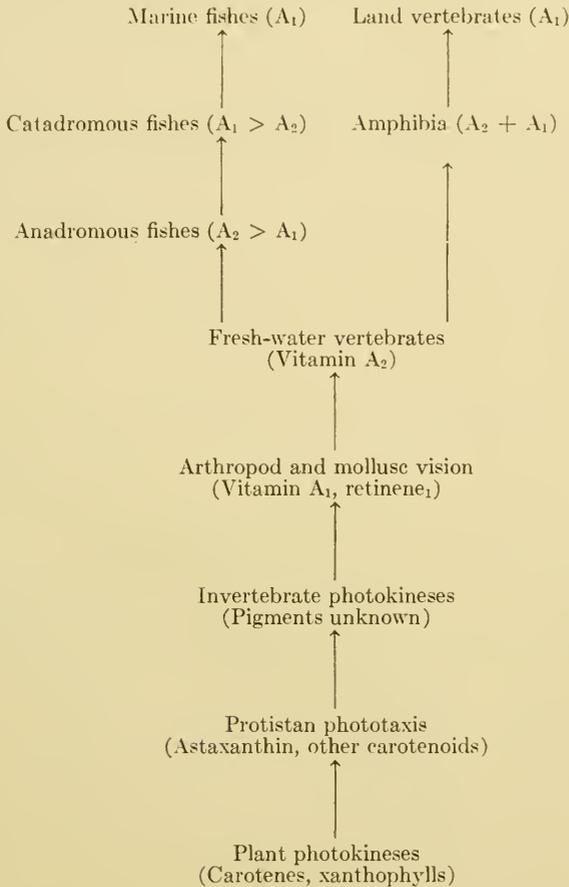


Fig. 1. The retinal pigments as related to phylogenetic development.⁹⁶ The vitamin A components are now believed to be vitamin A aldehydes.

On the other hand, the vitamin A content of the retinas of some species of gold fish is almost as great as the total vitamin A in the rest of the body.⁹⁹ In addition to the article of Wald⁹⁶ on carotenoids and vitamins A in photo-reception, Granit⁹⁸ has written an excellent review on this subject.

⁹⁷ S. W. Clausen and A. B. McCoord, *Unpublished results*. Cited by R. J. Williams, *Vitamins and Hormones*, 1, 236, 240 (1943).

⁹⁸ R. Granit, *Sensory Mechanisms of the Retina*, Oxford Univ. Press, 1947.

(5) *Vitamin A in Mammalian Tissues Other Than the Retina*

Vitamin A also occurs in a number of other tissues, such as corpora lutea, lungs, and kidneys,⁹⁹ as well as in the retina. Williams¹⁰⁰ has summarized data on the vitamin A content of a number of tissues of normal rats and men (Table 4).

TABLE 4

VITAMIN A CONTENT OF TISSUES OBTAINED FROM NORMAL RATS AND NORMAL MAN^a

Tissue	Vitamin A, I.U./g. moist tissue		Tissue	Vitamin A, I.U./g. moist tissue	
	Man	Rat		Man	Rat
Adrenal	6.0	49	Liver	156	7.6
Brain	0.5	0.0	Lung	1.2	3.3
Colon	0.8	—	Muscle	0.5	0.7
Heart	1.4	0.2	Skin	1.3	—
Ileum	1.3	—	Spleen	1.0	—
Kidney	2.7	2.7	Stomach	1.3	—

^a Adapted from R. J. Williams, *Vitamins and Hormones*, 1, 229-247 (1943), pp. 236, 237.

TABLE 5

VITAMIN A CONTENT OF DIFFERENT SPECIES^a

Species	Vitamin A, I.U./kg. carcass	Per cent of total Vitamin A in liver
Dog	7,925; 21,000; 27,500	19; 16; 43
Cat	2,100; 56,400	24; 92
Rat	24,000	97
Gopher	11,800	67
Rabbit	1,840; 2,500	35; 21
Guinea pig	29,500; 2,040	92; 10
Monkey	6,150	67
Chicken	4,400	50
Man	5,500; 4,680	69; 85

^a R. J. Williams, *Vitamins and Hormones*, 1, 229-247 (1943), p. 240.

There is a marked variation in the vitamin A content of different mammals.¹⁰¹ However, because of the wide variations noted in different animals of the same species, it is difficult to ascribe significance to small differences in the values. The results of Clausen and McCoord cited by Williams¹⁰⁰ are summarized in Table 5. These workers reported that in some species certain tissues were devoid of vitamin A. These include lung

⁹⁹ T. Moore, *Biochem. J.*, 25, 275-286 (1931).

¹⁰⁰ R. J. Williams, *Vitamins and Hormones*, 1, 229-247 (1943).

¹⁰¹ S. W. Clausen, *Harvey Lectures*, 38, 199-226 (1942-1943).

(dog, guinea pig), small intestine (rabbit), large intestine (cat), stomach (guinea pig, cat), heart (guinea pig), spleen (rabbit, guinea pig), pancreas (rabbit), ovaries (rabbit), thyroid gland (rabbit), and adrenal (gopher). Further studies in this field to determine the relationship between vitamin A content and function are obviously most desirable.

Another site for the storage of vitamin A, in addition to the liver, may be the depot fat. Such storage depends upon the species as well as upon the vitamin A intake. Certain mammals such as cattle, sheep, and pigs do not contain vitamin A in these fat depots; on the other hand, the depot fats of the dog may contain as much vitamin A as the liver, or even more.^{97,100} In some cases, the intestinal fats of fishes may contain 60–70% of the total vitamin A.⁵⁹ An average value of 32.3 I.U. per gram of storage fat has been reported in the general body fat of the chicken, although, after supplementation with massive doses of vitamin A, this value was increased about 8-fold (222.6 I.U.).¹⁰²

(6) *Vitamin A and Carotene in Milk*

One of the principal natural sources in which vitamin A is found is butter fat. Vitamin A is present in this product not only as such but also in the form of the provitamin A, β -carotene. In a recent nation-wide survey¹⁰³ on the role of butter as a source of vitamin A in the diet of the people of the United States, it was found that the vitamin A content of summer butter approximated 15,000 International Units per pound, or 33 units per gram. In the case of winter butter, the total vitamin A averaged about 50% of the summer level (7500 I.U. per pound or 16.5 I.U. per gram), when the herds were fed on U. S. No. 2 field-cured alfalfa hay or average quality corn silage; when an excellent quality of alfalfa silage was used, the summer levels of vitamin A were largely maintained.

A number of factors other than season can influence the total amount of vitamin A in milk, as well as the proportion of carotene and vitamin A. One of the most important of these is the breed of the cow. It is well known that, in the case of the Holstein cow, the carotene level is relatively lower than in the Guernsey breed; on the other hand, the amount of preformed vitamin A in the former case may be higher, so that the total vitamin A may approximate the same values in the two species. The total vitamin A and the carotene fraction are dependent upon the feed, being highest when the consumption of carotene-rich green grass is abundant, and lower in the winter when only dry feed is available. The vitamin A in milk

¹⁰² H. J. Deuel, Jr., M. C. Hrubetz, F. H. Mattson, M. G. Morehouse, and A. Richardson, *J. Nutrition*, **26**, 673–685 (1943).

¹⁰³ U. S. Dept. Agr., *Misc. Bull.*, No. 636, 1–47 (1937).

can be markedly increased by feeding massive doses of vitamin A to the cows.^{104,105} These results are illustrated in Table 6.

TABLE 6
EFFECT OF SUPPLEMENTARY VITAMIN A ON THE COMPOSITION OF MILK FAT OF COWS

Daily vitamin A supplement, I.U.	Length of supplement, weeks	Carotene and vitamin A/g. fat		
		β -Carotene, μ g.	Vitamin A, I.U.	Total vit. A, I.U.
Holstein				
0	0	4.23	24.4	31.4
700,000	8	2.88	66.0	70.8
2,100,000	5	2.77	128.2	132.8
2,800,000	3	2.23	167.9	171.6
4,200,000	2	2.15	280.4	284.0
Guernsey				
0	0	9.79	25.5	41.8
2,650,000	3	4.99	223.2	231.5
5,300,000	11	2.28	178.4	182.2

TABLE 7
CAROTENE AND VITAMIN A IN COLOSTRUM FROM VARIOUS BREEDS OF CATTLE^a

(The first sample in each case represents the colostrum; the second sample is the mature milk)

Breed	Date of sample	Carotene, mg. % fat	Vitamin A, mg. % fat
Friesian	Oct. 20	3.40	1.24
	Nov. 20	0.38	0.52
Ayrshire	Oct. 22	4.60	2.96
	Nov. 22	0.18	0.37
Guernsey	Oct. 28	3.61	1.13
	Nov. 28	0.91	0.67
Shorthorn	Oct. 13	2.90	3.51
	Nov. 13	0.28	0.48

^a I. M. Heilbron, W. E. Jones, and A. L. Bacharach, *Vitamins and Hormones*, 2, 155-213 (1944), p. 195. Data from A. E. Gillam, I. M. Heilbron, W. S. Ferguson, and S. J. Watson, *Biochem. J.*, 30, 1728-1734 (1936), p. 1733.

One interesting relationship which is unexplained is the suppressing action of oral doses of vitamin A on the level of milk carotene. The effect develops slowly and continues for a number of weeks after the vitamin A

¹⁰⁴ H. J. Deuel, Jr., N. Halliday, L. F. Hallman, C. Johnston, and A. J. Miller, *J. Nutrition*, 22, 303-313 (1941).

¹⁰⁵ H. J. Deuel, Jr., L. F. Hallman, C. Johnston, and F. Mattson, *J. Nutrition*, 23, 567-579 (1942).

feeding has been suspended. A similar suppression of carotenoid pigments in hen eggs occurs when massive doses of vitamin A are given to the laying hen.¹⁰²

Colostrum, also, has a considerably higher content of vitamin A than has mature milk. The higher content is the result of the augmented levels of both carotene and vitamin A. These results are beautifully illustrated in the data of Gillam *et al.*,¹⁰⁶ given in Table 7. These results confirm the earlier findings of Dann¹⁰⁷ and of Semb *et al.*¹⁰⁸

The vitamin A content of human milk varies with the period during lactation; it can also be considerably augmented by vitamin A supplementation. Hrubetz *et al.*¹⁰⁹ reported values ranging between 331 and 170 I.U. per 100 milliliters at varying times during the progression of the lactation cycle. After the ingestion of 100,000 or 200,000 I.U. daily, the average was markedly increased. The maximum value found was 2160 I.U. per 100 milliliters.

(7) Vitamin A in Egg Yolk

Another common source of vitamin A is the hen egg. In this case the vitamin A is present entirely in the yolk. The amount also varies with diet. Sherman¹¹⁰ reported values of 2,500 to 5,000 I.U. per 100 grams of yolk; this amounts to 400–800 I.U. per 16 grams, which is the weight of the yolk of the average-sized egg. Russell and Taylor¹¹¹ give the value as 500–800 I.U. per yolk, while Deuel *et al.*¹⁰² report values of 800–900 I.U. of total vitamin A per 18 grams of yolk for all groups of birds given supplemental vitamins A up to levels of 15,000 I.U. of vitamin A per pound of ration fed. When the diet contained 200,000 I.U. per pound of total food, the average total vitamin A content of the yolks was reported as 2,170 I.U. per egg. Thus, increased dietary vitamin A can produce an increase in the vitamin A content of the egg.

(8) Vitamin A in the Free and the Esterified Form

Although vitamin A occurs in nature chiefly in the form of its esters, it may likewise be present as the free alcohol.^{112, 113} Vitamin A palmitate has

¹⁰⁶ A. E. Gillam, I. M. Heilbron, W. S. Ferguson, and S. J. Watson, *Biochem. J.*, **30**, 1728–1734 (1936).

¹⁰⁷ W. J. Dann, *Biochem. J.*, **27**, 1998–2005 (1933).

¹⁰⁸ J. Semb, C. A. Baumann, and H. Steenbock, *J. Biol. Chem.*, **107**, 697–703 (1934).

¹⁰⁹ M. C. Hrubetz, H. J. Deuel, Jr., and B. J. Hanley, *J. Nutrition*, **29**, 245–254 (1945).

¹¹⁰ H. C. Sherman, *Chemistry of Food and Nutrition*, 7th ed., Macmillan, New York, 1947, p. 634.

¹¹¹ W. C. Russell and M. W. Taylor, *J. Nutrition*, **10**, 613–623 (1935).

¹¹² A. L. Bacharach and E. L. Smith, *Quart. J. Pharm.*, **1**, 539–545 (1928).

¹¹³ L. Reti, *Compt. rend. soc. biol.*, **120**, 577–580 (1935).

been separated from the liver oil of the Japanese ishinagi (*Stercolepis ishinagi*)^{38,39} and of the cod (*Gadus morrhua*)^{40,114} by the formation of an addition product with maleic anhydride or by distillation in a molecular still. A number of other methods have been employed for the demonstration of the nature of vitamin A in such sources. Bacharach and Smith¹¹² found that vitamin A in cod liver oil was largely insoluble in 92% ethanol; they considered this as proof that, in cod liver oil, vitamin A is largely in the ester form. Reti¹¹³ employed the method of partition between petroleum ether and ethyl or methyl alcohol for demonstrating the presence of the ester; in this manner he obtained positive results with the oils of a number of fishes, chickens, and various mammals. The above investigator also states that vitamin A is never found free under such conditions before saponification of the liver oils. In 1937, Hickman,^{114,115} using molecular distillation and the technic of the elimination curve, concluded that the vitamin A in both cod and halibut liver oils is largely esterified. In an extension of this work, Gray, Hickman, and Brown¹¹⁶ were able to show that rat livers contained vitamin A almost entirely in the form of the ester, irrespective of whether U.S.P. reference oil, vitamin A caproate, vitamin A stearate, distilled ester concentrate, or β -carotene was fed. In all cases there was a small amount of free vitamin A alcohol. Reed and co-workers,¹¹⁷ by the use of an entirely independent technic—chromatographic adsorption—obtained results leading to the conclusion that, in halibut oil, 94% of the vitamin A appears as the ester, while in the commercial distilled palmitate 98% occurs as the ester.

However, Sobotka, Kann, and Winternitz,¹¹⁸ who used the fluorescence technic, came to conclusions, as regards the proportion of vitamin A occurring as an ester, which diverged somewhat from those reported in the earlier investigations. Their estimations of the proportion of ester and alcohol were based upon the fact that the esters of vitamin A show a strong fluorescence in ethanol, while the vitamin A alcohol gives only a weak fluorescence. It was reported that, when this method is applied to U.S.P. reference oil, halibut liver oil and oleum percomorphum, only 60% of the total vitamin A appears to be in ester form, while 80% is present in combined form in the commercial distilled vitamin A ester.

The whole subject has been thoroughly re-examined by Kascher and Baxter¹¹⁹ by the use of several procedures. One of the most effective was a

¹¹⁴ K. C. D. Hickman, *Ind. Eng. Chem.*, *29*, 1107–1111 (1937).

¹¹⁵ K. C. D. Hickman, *Ind. Eng. Chem.*, *29*, 968–975 (1937).

¹¹⁶ E. Le B. Gray, K. C. D. Hickman, and E. F. Brown, *J. Nutrition*, *19*, 39–46 (1940).

¹¹⁷ G. Reed, E. C. Wise, and R. J. L. Frundt, *Ind. Eng. Chem., Anal. Ed.*, *16*, 509–510 (1944).

¹¹⁸ H. Sobotka, S. Kann, and W. Winternitz, *J. Biol. Chem.*, *152*, 635–639 (1944).

¹¹⁹ H. M. Kascher and J. G. Baxter, *Ind. Eng. Chem., Anal. Ed.*, *17*, 499–503 (1945).

new method of Embree and Kuhrt¹²⁰ in which the ester and free alcohol are separated by distribution between petroleum ether and 83% aqueous ethanol. The results of their studies are recorded in Table 8.

TABLE 8
VITAMIN A ESTER ANALYSES AS DETERMINED BY PETROLEUM ETHER:83% ETHYL ALCOHOL DISTRIBUTION^a

Liver oil	Per cent total vit. A added as cryst. vit. A alcohol	Potency, units/g.	Per cent esters	
			Found	Calcd.
Fish liver oils				
Halibut No. 2 (<i>Hippoglossus hippoglossus</i>)	—	62,800	98	—
Halibut No. 2 (<i>Hippoglossus hippoglossus</i>)	52	129,500	50	48
Oleum percomorphum	—	68,400	100	—
U.S.P. reference	—	1,700	100	—
Ling cod (<i>Ophiodon elongatus</i>)	—	57,400	99	—
Soupin shark (<i>Galeorhinus zyopterus</i>)	—	95,400	100	—
Great barracuda (<i>Sphyræna barracuda</i>)	—	28,600	99	—
Mexican shark (<i>Pleurotremi</i> spp.)	—	64,000	99	—
Whale (<i>Cetaceæ</i> spp.)	—	6,000	100	—
Hake (<i>Merluccius merluccius</i>)	—	6,700	100	—
Green pollack (coalfish) (<i>Pollachius virens</i>)	—	3,500	100	—
Tuna (<i>Thunnus</i> spp.)	—	6,750	96	—
Spiny dogfish (<i>Squalus suckleyi</i> , Pacific)	—	13,000	97	—
Distilled concentrates (examined by Kascher and Baxter)				
1	—	192,000	100	—
1 + cryst. vit. A alcohol	10	211,000	90	90
2	—	534,000	99	—
3	—	59,200	100	—

^a H. M. Kascher and J. G. Baxter, *Ind. Eng. Chem., Anal. Ed.*, 17, 499-503 (1945), p. 499.

Kascher and Baxter¹¹⁹ have demonstrated not only the high proportion of ester in a wide variety of fish liver oils and distilled concentrates, but also the high accuracy of their technic, by obtaining a quantitative recovery of vitamin A alcohol when added to the fish liver oils or vitamin A concentrates. The reason for the aberrant results obtained by the fluorescence technic is ascribed to the fact that this method cannot be employed satisfactorily with fish liver oils or vitamin A concentrates.

Vitamin A may also be combined with protein under certain conditions so that it becomes unextractable with ordinary fat solvents until it is hydrolyzed. Although simple extraction methods are ample for removing this vitamin from most tissues,^{121,122} Lovern *et al.*⁵⁷ have reported that in fish eyes vitamin A is not directly extractable, and that it can be removed only after its separation from the protein. These protein-vitamin A com-

¹²⁰ N. D. Embree and N. H. Kuhrt, *Unpublished results*, reported by H. M. Kascher and J. G. Baxter, *Ind. Eng. Chem., Anal. Ed.*, 17, 499-503 (1945).

¹²¹ A. W. Davies, *Biochem. J.*, 27, 1770-1774 (1933).

¹²² L. I. Pugsley, *J. Biol. Chem.*, 128, lxxx (1939).

binations in the retina are now recognized as the compounds rhodopsin and porphyropsin, although it is believed that vitamin A aldehyde, rather than the alcohol, is the component concerned.

3. Structure of the Vitamins A

(1) Vitamin A (A_1)

Although carotene possesses vitamin A activity, it has already been indicated that the provitamins A and vitamins A are different substances. In the first place, all evidence points to the fact that a marked divergency in molecular weight obtains between these compounds. Bruins, Overhoff, and Wolff¹²³ found a molecular weight of 536 for carotene compared with one of 330–333 for vitamin A. These workers made a direct comparison of the diffusion constants of these products prepared from sheep liver fat. A number of other workers have confirmed the fact that the molecular weight of vitamin A approximates 300. Karrer, Morf, and Schöpp⁴¹ obtained values varying between 300 and 320 by the Rast method, while Heilbron and collaborators²⁷ reported a mean value of 327 for a series of determinations in which the cryoscopic method was employed. These same authors,²⁷ using the Smith and Young¹²⁴ modification of the Rast micromethod, found the apparent molecular weight of vitamin A to be 312.

A second obvious difference between carotene and vitamin A is the fact that the latter is an alcohol while the former is a hydrocarbon. Drummond, Channon, and Coward¹²⁵ suspected that vitamin A might be an alcohol, although evidence was inconclusive; this hypothesis was confirmed by Karrer, Morf, and Schöpp.⁴¹ The latter workers were able to prepare an acetate ester as well as a *p*-nitrobenzoate from a concentrate made from the liver oil of the Atlantic saury (*Scomberesox saurus*); the free vitamin A alcohol could be regenerated from these products. The most probable molecular formula for vitamin A alcohol was determined as $C_{26}H_{30}O$ or $C_{22}H_{22}O$, on the basis of analyses of the two above-mentioned vitamin A esters and of the alcohol regenerated from the acetate. We now know the first empirical formula to be correct; this would be in accordance with a molecular weight of 286.

The further structure of vitamin A was elucidated by degradation reactions similar to those used for establishing the structure of the carotenoids. The presence of a β -ionone ring was established by the isolation of geronic acid after treatment with ozone.¹²⁶ The presence of the methyl groups in the side chain and in the whole molecule was shown to be equivalent to

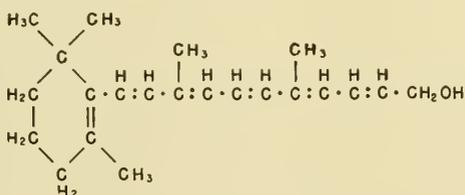
¹²³ H. R. Bruins, J. Overhoff, and L. K. Wolff, *Biochem. J.*, **25**, 430–438 (1931).

¹²⁴ J. H. C. Smith and W. G. Young, *J. Biol. Chem.*, **75**, 289–298 (1927).

¹²⁵ J. C. Drummond, H. J. Channon, and K. H. Coward, *Biochem. J.*, **19**, 1047–1067 (1925).

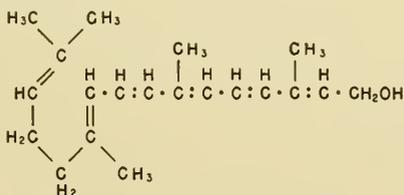
¹²⁶ P. Karrer, R. Morf, and K. Schöpp, *Helv. Chim. Acta*, **14**, 1036–1040 (1931).

Several possible formulas have been suggested. The first proposed structure was that of Gillam *et al.*,⁷⁶ which differs from that of vitamin A₁ in having an extra vinylene group on the aliphatic chain, and therefore possesses a C₂₂ composition.

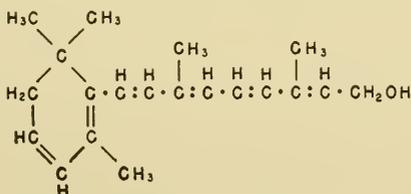


Formula I for vitamin A₂ proposed
by Gillam *et al.*⁷⁶

A second suggested formula for vitamin A₂ is that of Karrer, Geiger, and Bretscher,¹³² which contains an open β -ionone ring and bears the same relation to vitamin A₁ as lycopene does to β -carotene. Morton, Salah, and Stubbs¹³³ have proposed a third formula for vitamin A₂ which contains a dehydroionone ring.



Formula II for vitamin A₂ proposed
by Karrer *et al.*¹³²



Formula III for vitamin A₂ proposed
by Morton *et al.*¹³³

The strongest evidence for the Gillam formula (Formula I) is that, on oxidation of vitamin A₂ with aluminum *tert*-butoxide in the presence of acetone, as described by Batty *et al.*¹³⁴ for vitamin A₁, a ketone is produced with an empirical composition of C₂₅H₃₄O as determined from the crystalline *p*-chlorobenzoylhydrazone derivative (m.p., 142–143°C.).⁷⁶ Vitamin

¹³² P. Karrer, A. Geiger, and E. Bretscher, *Helv. Chim. Acta*, **24**, 161E–172E (1941).

¹³³ R. A. Morton, M. K. Salah, and A. L. Stubbs, *Nature*, **159**, 744 (1947).

¹³⁴ J. W. Batty, A. Burawoy, S. H. Harper, I. M. Heilbron, and W. E. Jones, *J. Chem. Soc.*, 1938, 175–179.

A₁, under similar circumstances, gives a ketone with 23 carbons. This would seem to be almost absolute proof that the C₂₂ formula (I) is the correct one. The ketone of vitamin A so prepared has an absorption maximum of 401 m μ (in alcohol), while the ketone prepared from vitamin A₂ has a maximum absorption at 411 m μ .

However, several reactions on the part of vitamin A₂ are difficult to explain if one accepts the Gillam formula (I). The main difficulty lies in explaining the proximity of the elimination maxima of vitamins A₁ and A₂ when these are subjected to molecular distillation. Gray,¹³⁵ as well as Lovern and collaborators,¹³⁶ found that the elimination maximum of vitamin A₂ is only 3°C. higher than that of vitamin A₁; this would indicate that the two vitamins have the same number of carbons but that vitamin A₂ has an additional unsaturated linkage. This conviction was further substantiated in later work of Gray and Cawley,¹³⁷ who showed that the elimination maximum was lowered 2°C. by each increase in a non-conjugated double bond, and to the extent of 3°C. by each additional conjugated double bond, while it was raised 5°C. by each added CH₂ group. Karrer, Rügger, and Geiger¹³⁸ synthesized a material which presumably had the structure given in Formula I, except for an additional methyl group. This substance failed to show the color reactions for vitamin A₂, although it had previously been demonstrated that alkyl groups located in such positions would not interfere in the color reactions of the carotenoids. Last, the spectral absorption characteristics predicated for β -apo-5-carotenol (C₂₂) by Karrer *et al.*^{138,139} differ from those actually found for vitamin A₂, and so it is not believed that the C₂₂ formula applies to vitamin A₂. Embree and Shantz¹⁴⁰ have demonstrated that the products formed on cyclization of vitamins A₁ and A₂ with alcoholic hydrochloric acid have very similar absorption spectra. This may be considered as support of a C₂₀ rather than a C₂₂ formula.

In 1941 Karrer and his collaborators¹³² proposed the open-chain formula (II), largely because of the production of acetone when vitamin A₂ was treated with ozone. Another finding which supported the hypothetical structure was the fact that the absorption maximum was at 345 m μ . The lycopene structure now appears less probable, inasmuch as Karrer and Bretscher¹⁴¹ were later unable to prove the presence of acetone on the basis of the formation of its *p*-nitrophenylhydrazone, although the iodoform reaction indicated the presence of acetone. However, these authors mention the possibility that an unidentified substance other than acetone may be

¹³⁵ E. Le B. Gray, *J. Biol. Chem.*, **131**, 317-326 (1939).

¹³⁶ J. A. Lovern, T. H. Mead, and R. A. Morton, *Biochem. J.*, **33**, 338-343 (1939).

¹³⁷ E. Le B. Gray and J. D. Cawley, *J. Biol. Chem.*, **134**, 397-401 (1940).

¹³⁸ P. Karrer, A. Rügger, and A. Geiger, *Helv. Chim. Acta*, **21**, 1171-1174 (1938).

¹³⁹ H. v. Euler, P. Karrer, and U. Solmsen, *Helv. Chim. Acta*, **21**, 211-222 (1938).

¹⁴⁰ N. D. Embree and E. M. Shantz, *J. Biol. Chem.*, **132**, 619-626 (1949).

¹⁴¹ P. Karrer and E. Bretscher, *Helv. Chim. Acta*, **25**, 1650-1653 (1942).

responsible for the positive iodoform test. The principal argument against Formula II is the fact that lycopene, unlike β -carotene, fails to function as a provitamin A.¹⁹ No active provitamin A is known which does not contain one intact unsubstituted β -ionone ring. The evidence would appear to be preponderantly opposed to the Karrer open-chain structure (II).

The third formula proposed by Morton and collaborators¹³³ is based upon the claim that the "C₂₀ aldehyde"¹⁴² obtained when vitamin A₂ is oxidized with aluminum *tert*-butoxide in benzene using diethyl ketone as a hydrogen acceptor (the so-called Oppenauer reaction) is, in fact, an aldehyde of vitamin A₂. Moreover, the aldehyde corresponding to Formula II is very different from retinene₂, which is closely related to vitamin A₂ alcohol. Finally, Morton and Creed¹⁴³ have shown that β -carotene serves as a provitamin A for both vitamin A₂ and vitamin A₁. This would seem to preclude the open-chain formula or the C₂₂ formula and to support a C₂₀ structure. However, one bit of evidence speaks against the Morton formula. On ozonolysis, no α,α' -dimethylsuccinic acid could be demonstrated. Such a degradation product would surely have been expected if a double bond had existed between carbons 3 and 4. In spite of this discrepancy, Formula III would appear to be the most probable one of the three proposed.

Although the conflicting data leave the question as to the structure of vitamin A₂ still undecided, a definite answer should soon be forthcoming, on the basis of the pure crystalline product recently isolated by Shantz.¹⁴⁴

Gillam and his associates⁷⁶ consider that vitamin A₂ has biological activity for rats, while Karrer, Geiger, and Bretscher,¹³² on the other hand, claim that it possesses no such activity. However, more recently Jensen, Shantz, Embree, Cawley, and Harris,¹⁴⁵ using a liver oil concentrate obtained from the northern pike (*Esox lucius*), which contained only impure vitamin A₂ and was uncontaminated with vitamin A₁, concluded that vitamin A₂ possesses a minimum biopotency of 47,500 U.S.P. units per gram. Shantz and Brinkman¹⁴⁶ have revised this figure still further to 1,300,000 units per gram, using as a comparison the vitamin A acetate reference oil. This would indicate that vitamin A₂ is only about 40% as effective as crystalline vitamin A₁, which has a biopotency originally reported to be 4,300,000 U.S.P. units per gram,³³ but more recently revised to a value of 3,333,000 U.S.P. units per gram on the basis of the new U.S.P. standard.

¹⁴² E. Hawarth, I. M. Heilbron, W. E. Jones, A. L. Morrison, and J. B. Polya, *J. Chem. Soc.*, 1939, 128-132.

¹⁴³ R. A. Morton, and R. H. Creed, *Biochem. J.*, 33, 318-324 (1939).

¹⁴⁴ E. M. Shantz, *Science*, 108, 417-419 (1948).

¹⁴⁵ J. L. Jensen, E. M. Shantz, N. D. Embree, J. D. Cawley, and P. L. Harris, *J. Biol. Chem.*, 149, 473-477 (1943).

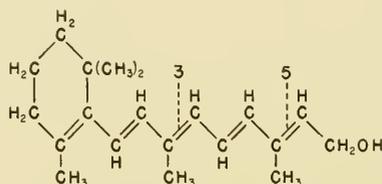
¹⁴⁶ E. M. Shantz, and J. H. Brinkman, Report of 114th Meeting of the American Chemical Society, Washington, D. C., Aug. 30 to Sept. 3, 1948, Abstract No. 24, p. 17c; *J. Biol. Chem.*, 183, 467-471 (1950).

The provitamin A activity of vitamin A₂ is further substantiated by the work of Lederer and Rathmann¹⁴⁷ who found that the livers of rats and frogs had vitamin A activity after vitamin A₂ had been fed.

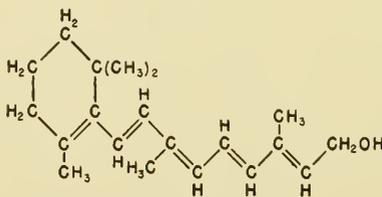
Although a number of other homologues of vitamin A are known—the apocarotenols (see chapter VI, pages 531 and 532)—no other types of vitamin A are found as natural products. However, stereoisomeric forms of vitamin A are found as natural products. However, stereoisomeric forms of vitamin A have been discovered in fish liver oils, differing from vitamin A only in the *trans* or *cis* configuration of the double bonds.

(3) Stereoisomeric Forms of Vitamin A₁

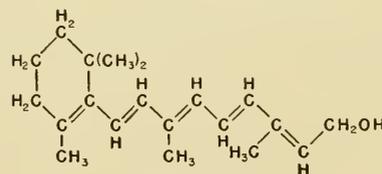
The possibility that stereoisomeric forms of vitamin A may exist has been recognized only recently. Zechmeister⁴⁵ has pointed out that, in addition to all-*trans*-vitamin A, which is the usual form, three other isomers are possible: 3-*cis*-, 5-*cis*-, and 3,5-di-*cis*-vitamin A. The structure of these possible isomers is illustrated here.



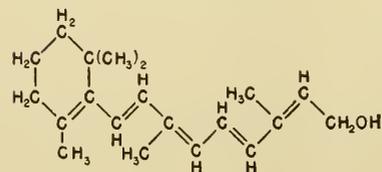
All-*trans*-vitamin A



3-*cis*-Vitamin A



5-*cis*-Vitamin A



3,5-Di-*cis*-vitamin A

¹⁴⁷ E. Lederer and F. H. Rathmann, *Biochem. J.*, **32**, 1252-1261 (1938).

The formation of stereoisomers of vitamin A could be expected to occur when sources of natural vitamin A are exposed to ultraviolet light, iodine, or other forms of catalysis which are known to bring about such *trans* → *cis* rearrangements. Smith and his collaborators¹⁴⁸ found that, when vitamin A concentrates were exposed to ultraviolet light, a marked decrease in the absorption maximum at 328 m μ obtained, but that these readings tended to revert to the original values when the samples were allowed to stand in the dark. However, the more pronounced the lowering in absorption maximum at 328 m μ , the less efficient was the subsequent recovery of the absorption when the samples were again placed in the dark. These authors ascribed "these irregularities [as being] probably due to differences in proportion of *cis-trans* isomers of vitamin A." Thus, ultraviolet radiation produces not only a reversible photochemical isomerization, but also a concomitant photochemical destruction. The presence of variable proportions of such stereoisomers of vitamin A in natural oils was suggested by Smith *et al.*¹⁴⁹ as an explanation for the variations in absorption maxima of different vitamin A preparations in several solvents. Morton¹⁵⁰ reached the same conclusions on the basis of fluctuations in the *E* (1%, 1 cm.)(328:620 m μ) (antimony trichloride) for vitamin A in various mineral oils. Baxter *et al.*,¹⁵¹ in 1941, reported on a non-crystallizable vitamin A obtained from the liver oil of the ling cod (*Ophiodon elongatus*) which appeared to be identical with crystalline vitamin A but proved refractory to crystallization. The first recognition of a stereoisomeric form of vitamin A was the isolation of "neovitamin A" from natural sources by Robeson and Baxter.^{46,47} In the case of some fish liver oils, the neo form constituted as much as 35–39% of the total vitamin A. The neovitamin A which was prepared as a crystalline product proved to be identical with "non-crystallizable vitamin A." Recently, Cawley *et al.*¹⁵² found that synthetic vitamin A preparations contained neovitamin A to the extent of about one-third of the total; this would constitute a ratio comparable to that found in some natural fats. It is believed that a catalytic conversion, vitamin A \rightleftharpoons neovitamin A, may take place both *in vitro* and *in vivo*.

Robeson and Baxter⁴⁷ have found that marked variations in properties and reactions obtain between vitamin A and neovitamin A. These are listed in Table 9.

¹⁴⁸ E. L. Smith, F. A. Robinson, B. E. Stern, and F. E. Young, *Biochem. J.*, **33**, 207–212 (1939).

¹⁴⁹ E. L. Smith, B. E. Stern, and F. E. Young, *Nature*, **141**, 551–552 (1938).

¹⁵⁰ R. A. Morton, *Nature*, **141**, 552 (1938).

¹⁵¹ J. G. Baxter, P. L. Harris, K. C. D. Hickman, and C. D. Robeson, *J. Biol. Chem.*, **141**, 991–992 (1941).

¹⁵² J. D. Cawley, C. D. Robeson, L. Weisler, E. M. Shantz, N. D. Embree, and J. G. Baxter, *Science*, **107**, 346 (1948).

TABLE 9
COMPARISON OF THE PROPERTIES OF VITAMIN A AND OF NEOVITAMIN A^a

Physical property or reaction	Vitamin A	Neovitamin A
Crystal form.....	Yellow prisms	Yellow needles
Melting point, °C.....	62-64	58-60
Melting point of phenylazobenzoate, °C.....	79-80	94-96
Melting point of anthraquinone β -carboxylate, °C....	121-122	134-136 (red)
λ max., $m\mu$	324-325	328
E (1%, 1 cm.).....	1740 (325 $m\mu$)	1645 (328 $m\mu$)
Resistance to atmospheric oxidation.....	Less	More
Rate of maleic anhydride addition.....	Rapid	Slow
Rate of dehydration with alcoholic HCl.....	Rapid	Slow

^a Adapted from C. D. Robeson and J. G. Baxter, *J. Am. Chem. Soc.*, **69**, 136-141 (1947).

In the case of the natural fish liver oils, the neovitamin A is believed to be a *cis* isomer. This conclusion is based upon the fact that *cis* isomers have lower melting points than those of the corresponding *trans* compounds,¹⁵³ that they have lower extinction coefficients at their absorption maxima,¹⁵⁴ and, finally, that they add maleic anhydride more slowly.¹⁵⁵ The *cis* bond is assigned to the 5 position, since the only other double bond which can undergo steric rearrangement is *trans* in all the commonly occurring carotenoids. The crystals of neovitamin A *p*-phenylazobenzoate contrasted with vitamin A *p*-phenylazobenzoate are shown in Plate 4, while the crystals of neovitamin A and vitamin A are compared in Plate 5.

Neovitamin A and all-*trans*-vitamin A have been considered to possess approximately the same biological potency.⁴⁷ However, the recent investigations of Harris *et al.*^{155a} have given a figure of 85.3% for neovitamin A, as compared with the all-*trans* isomer, when the calculations were based upon the U.S.P. XIII growth method; the value as determined by the liver storage test was 75.6%. Since the E (1%, 1 cm.) of pure all-*trans*-vitamin A at 325 $m\mu$ is 1740, while that of the neo form is 1645 at 328 $m\mu$, the biopotencies obtained by these methods would be revised to 80.7 and 71.5% on the molar basis. Harris, Ames, and Brinkman^{155a} calculated that, in terms of U.S.P. units, which are based upon the rat-growth method, the biopotency of neovitamin A is 2,690,000 U.S.P. units per gram. The interconversion *in vivo* of one form to the other was likewise demonstrated by these workers.^{155a}

¹⁵³ L. Zechmeister, *Chem. Revs.*, **34**, 267-344 (1944).

¹⁵⁴ H. P. Koch, *Chemistry & Industry*, **20**, 273-275 (1942).

¹⁵⁵ R. S. Morrell, T. R. Bolam, W. R. Davis, S. Marks, E. O. Phillips, and W. S. Sim, *Trans. Faraday Soc.*, **38**, 362-366 (1942).

^{155a} P. L. Harris, S. R. Ames, and J. H. Brinkman, *J. Am. Chem. Soc.*, **73**, 1252-1254 (1951).



Plate 4. The crystals of neovitamin A *p*-phenylazobenzoate (top), and of vitamin A *p*-phenylazobenzoate (bottom), $\times 15$.⁴⁷ Courtesy Distillation Products Industries.

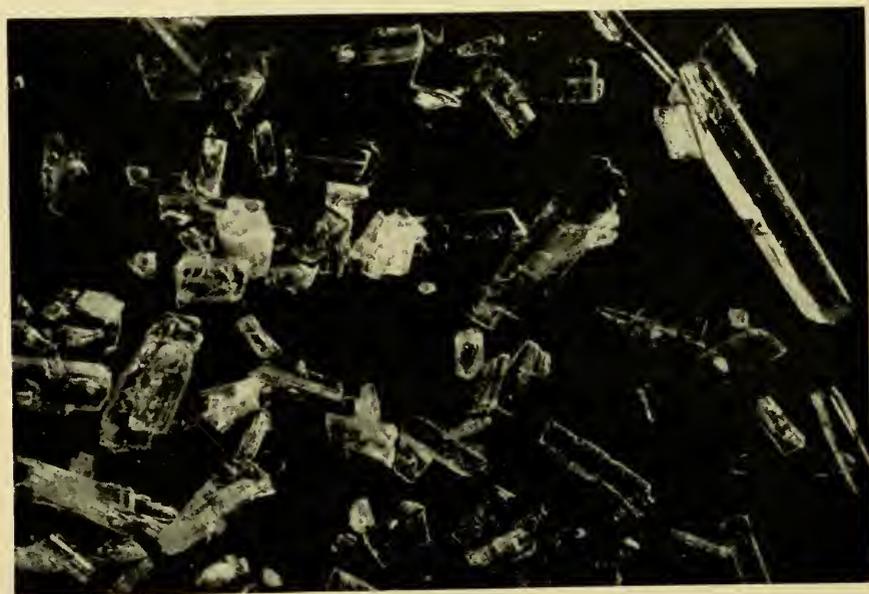


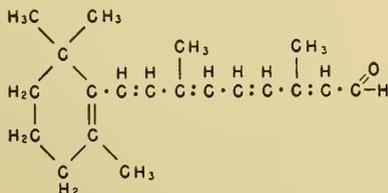
Plate 5. The crystals of neovitamin A (top), $\times 15$, and of vitamin A (bottom), $\times 10$.⁴⁷
Courtesy Distillation Products Industries.

4. Occurrence and Structure of Compounds Related to Vitamins A

(1) Vitamin A Aldehyde

The importance of vitamin A aldehyde as an intermediate in vitamin A metabolism has recently been emphasized by the demonstration that retinene₁ is vitamin A₁ aldehyde, while retinene₂ is vitamin A₂ aldehyde.^{81, 82, 156-158} Morton,⁷⁸ in 1944, first suggested that retinene₁ must be the vitamin A aldehyde; later he confirmed this finding.¹⁵⁹ This was the most plausible explanation for the displacement of the absorption maximum from 326 m μ to 369 m μ in retinene₁ when compared with vitamin A₁. Such a marked alteration in the absorption spectrum can best be explained by an increase in the number of conjugated bonds from 5 to 6; if the terminal —CH₂OH group of vitamin A is replaced by a —CHO group, this would provide the sixth conjugated bond.

The close relationship between vitamin A aldehyde and vitamin A is emphasized by Glover, Goodwin, and Morton,¹⁶⁰ whose work showed that retinene₁, when administered orally, subcutaneously, or intraperitoneally, is rapidly converted to vitamin A₁. The conversion of the aldehyde to the vitamin A alcohol is a reduction which occurs in the gut or in the subcutaneous tissues. Although Ball *et al.*¹⁶¹ had suggested earlier that the liver could effect the conversion of vitamin A aldehyde to vitamin A alcohol, it was later stated by some of the same authors¹⁶⁰ that no direct evidence exists to substantiate such a change in the liver. It is believed that retinene₁ may be an intermediate product in the transformation of β -carotene to vitamin A₁ *in vivo*.

Vitamin A₁ aldehyde

Vitamin A aldehyde has been prepared by a number of workers. Although it was expected that direct oxidation of vitamin A alcohol would result in an attack not only on the terminal alcohol group but also on the double bonds, Morton and Goodwin⁷⁹ apparently were able to prepare some of the aldehyde by shaking vitamin A concentrates dissolved in light

¹⁵⁶ M. K. Salah and R. A. Morton, *Repts. Biochem. Soc.*, Dec. 4, 1948, pp. 6-7.

¹⁵⁷ G. Wald, *J. Gen. Physiol.*, 20, 45-56 (1936-1937).

¹⁵⁸ R. A. Morton, M. K. Salah, and A. L. Stubbs, *Biochem. J.*, 40, lix-lx (1946).

¹⁵⁹ R. A. Morton, M. K. Salah, and A. L. Stubbs, *Biochem. J.*, 41, xxiv (1947).

¹⁶⁰ J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, 43, 109-114 (1948).

¹⁶¹ S. Ball, J. Glover, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, 41, xxiv (1947).

petroleum ether with dilute aqueous potassium permanganate containing sulfuric acid. On chromatographic separation, small amounts of material were always present with an absorption maximum at 365–370 $m\mu$ in saturated hydrocarbon solvents, and 385 $m\mu$ in chloroform. These workers found that the material obtained by chromatographic separation also reacted with antimony trichloride; the maximum band appeared at 665 $m\mu$. Presumably the compound at hand was vitamin A₁ aldehyde.

Hawkins and Hunter,⁸⁰ likewise, synthesized vitamin A₁ aldehyde from vitamin A₁ by the Oppenauer reaction; however, the preparation was apparently impure, as it showed an absorption maximum not only at 368 $m\mu$ but also at 350 $m\mu$. The authors reported a maximum absorption at 657 $m\mu$ when it was reacted with antimony trichloride. However, when the preparation was examined by Ball, Goodwin, and Morton,⁸¹ it was found that the variation from the earlier color maximum of 664 $m\mu$ was a result of spectroscopic technic only. Hunter and Williams¹⁶² obtained the aldehyde by the oxidation of β -carotene. This is of great interest, as it provides chemical proof of the conversion of the provitamin A, β -carotene, to vitamin A.

Van Dorp and Arens¹⁶³ synthesized the vitamin A₁ aldehyde in the course of a synthesis of vitamin A alcohol (see Section 5). The most satisfactory procedure for the preparation of vitamin A₁ aldehyde has been recently reported by Ball, Goodwin, and Morton.⁸¹ Vitamin A₁ was oxidized in light petroleum with manganese dioxide for 6 to 10 days in the dark; yields of the aldehyde as high as 80% of the theoretical were obtained. The vitamin A₁ aldehyde was separated chromatographically on an alumina (Brockmann) column; the slow-travelling brown zone proved to be the vitamin A₁ aldehyde. After removal of some impurities which crystallized from petroleum ether at -72°C ., the mother liquor was concentrated to one-fourth volume. A brown semisolid mass separated after 24 hours at -72°C . After recrystallization of this mass from petroleum ether at -72°C . for 10 days, reddish brown crystals were obtained, which melted at 56.5 to 58 $^{\circ}\text{C}$., and which showed a single maximum in cyclohexane at 373 $m\mu$. After a final recrystallization from petroleum ether, orange-red crystals were obtained; these were large clusters, predominantly needle-like, which melted at 61–62 $^{\circ}\text{C}$.

Ball *et al.*⁸¹ note the following values for maximum absorption of vitamin A aldehyde: light petroleum ether (40–60 $^{\circ}$), 369.5 $m\mu$; cyclohexane, 373 $m\mu$; ethanol, 385.5 $m\mu$; chloroform, 389 $m\mu$. The extinction values, E (1%, 1 cm.), in these various solvents at the maximum absorption are as follows: light petroleum ether (40–60 $^{\circ}$), 1685; cyclohexane, 1548; etha-

¹⁶² R. F. Hunter and N. E. Williams, *J. Chem. Soc.*, 1945, 554–556.

¹⁶³ D. A. van Dorp and J. F. Arens, *Nature*, 160, 189 (1947).

anol, 1400; chloroform, 1303. Curves for these absorption spectra are included in Figure 2.

When crystalline vitamin A₁ aldehyde reacts with antimony trichloride, it is found that the absorption maximum is at 664 m μ , and the E (1%, 1 cm.) value amounts to 3400. A number of derivatives were prepared, including the 2:4-dinitrophenylhydrazone (reddish brown needles, m.p., 207–208°C.) and the semicarbazone, m.p., 161–164°C., $\lambda_{(\text{CHCl}_3)} = 385 \text{ m}\mu$, E (1%, 1 cm.) = 1748. Two hydrazones were obtained, one melting at

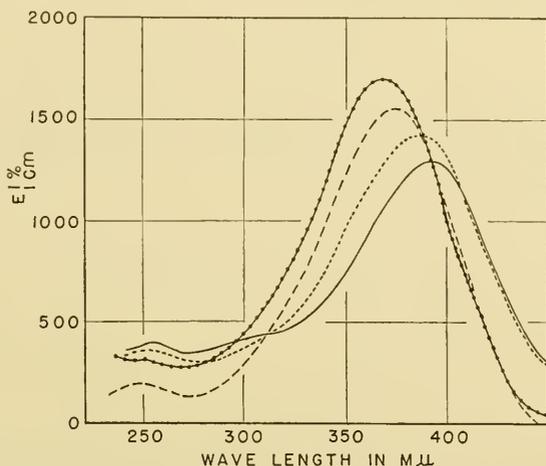


Fig. 2. Absorption spectra of vitamin A aldehyde in various solvents: (—●—●) light petroleum ether; (—) cyclohexane; (--) ethanol; (—) chloroform.⁸¹

108–109°C. and the second at 135–145°C. However, with pure retinene₁, the semicarbazone had a melting point of 193–195°C. and E (1%, 1 cm., 385 m μ) = 2062. The hydrazone obtained from the pure retinene melted at 177°C. and had two maxima, E (1%, 1 cm., 462 m μ) = 1930 and E (1%, 1 cm., 297 m μ) = 334.

As noted earlier, vitamin A₂ aldehyde has been shown to be the same as retinene₂. The retinal pigment was first obtained in crystalline form by Salah and Morton.¹⁵⁶ Wald,¹⁵⁷ who prepared it first from the retinas of frogs,¹⁶⁴ characterized it as a deeply yellow substance soluble in petroleum ether.¹⁵⁷ According to Morton, Salah, and Stubbs¹⁵⁸ it has a maximum absorption at 405 m μ in chloroform, and reacts with antimony chloride to give a color with a maximum at 702–706 m μ . It has also been prepared

¹⁶⁴ G. Wald, *Nature*, 134, 65 (1934).

Vitamin A acid has a biological potency in the same range as that of vitamin A itself. When it was administered orally in peanut oil, as the free acid, to vitamin-A-deficient rats, 4 μg . proved to be equivalent to one International unit of vitamin A. However, when an aqueous solution of the

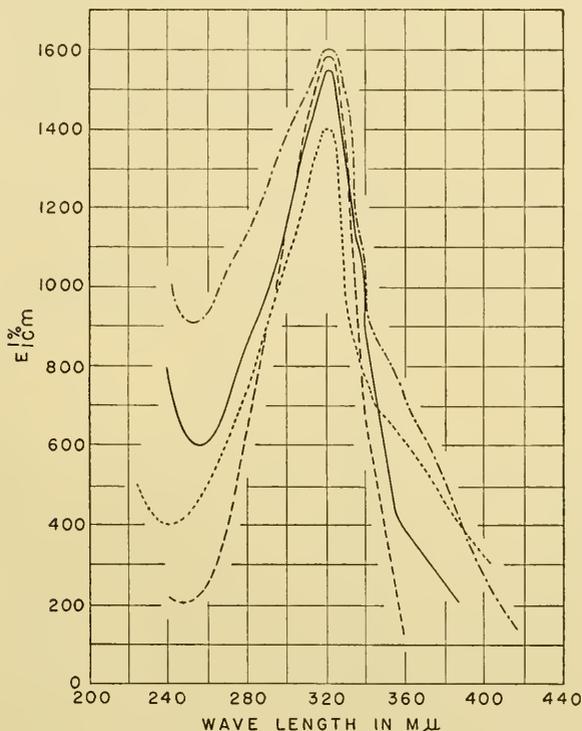


Fig. 3. The absorption spectra in ethanol of the following compounds: (—) methyl ether of vitamin A; (---) ethyl ether of vitamin A; (- - -) 5-dehydromethyl ether of vitamin A; (- · - ·) 5-dehydroethyl ether of vitamin A.⁴³

sodium salt was injected subcutaneously, it was found that the biopotency was approximately 50% of that of vitamin A alcohol, *i.e.*, 0.6 μg . was found to be equivalent to one International unit.⁴³

(3) Vitamin A Ethers and Homologues

A number of vitamin A ethers have recently been synthesized and have been shown to possess considerable biological activity. Milas and co-workers¹⁷²⁻¹⁷⁴ were the first to prepare pure samples of the methyl and

¹⁷² N. A. Milas, *U. S. Patent* Nos. 2,369,156 to 2,369,168 (Feb. 13, 1945).

¹⁷³ N. A. Milas, *U. S. Patent* Nos. 2,382,085 and 2,382,086 (Aug. 14, 1945).

¹⁷⁴ N. A. Milas, E. Sakal, J. T. Plati, J. T. Rivers, J. K. Gladding, F. X. Grossi, Z. Weiss, M. A. Campbell, and H. F. Wright, *J. Am. Chem. Soc.*, **70**, 1597-1607 (1948)

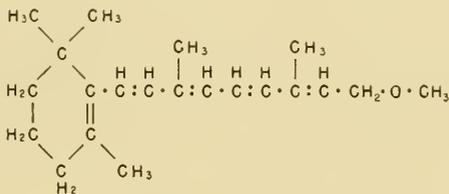
TABLE 10
SUMMARY OF ULTRAVIOLET ABSORPTION SPECTRA AND BIOLOGICAL ACTIVITY OF VITAMIN A ETHERS AND RELATED COMPOUNDS^a

Compound	Method of preparation	λ (max.), m μ	E (1%, 1 cm.)	$\epsilon_{\text{mol.}}$	log $\epsilon_{\text{mol.}}$	Biological activity
Vitamin A methyl ether	Natural from crystalline alcohol ¹⁷⁵	326	1660	49,800	4.70	+
	From 5-dehydrovitamin A methyl ether	323	1560	46,800	4.67	+
Vitamin A ethyl ether	By dehydrohalogenation of carbinol By dehydrobromination of carbinol or glycol methyl ether By reduction (Zn + alkali) of 5-dehydrovitamin A ethyl ether By dehydration of carbinol with <i>p</i> -toluenesulfonic acid	(285-290)	1415	42,450	4.63	+
		325-328	1090	—	—	+
		325	1590	49,926	4.70	+
		325-327	1500	47,100	4.67	+
<i>Allo</i> form	By dehydrobromination of glycol ethyl ether or carbinol	{ 330	1690	53,066	4.70	+
		{ 348	1830	57,462	4.76	
5-Dehydrovitamin A methyl ether	From acetyleneglycol of vitamin A methyl ether	{ 367	1520	47,728	4.68	+
5-Dehydrovitamin A ethyl ether	From acetyleneglycol of vitamin A ethyl ether	{ 322	1600	47,680	4.68	
Homovitamin A ethyl ether	From dehydration of acetyleneglycol of vitamin A ethyl ether or carbinol By dehydration of corresponding tetraenol ethyl ether	321	—	41,250	4.62	+
5-Dehydrohomovitamin A ethyl ether	By dehydrobromination of corresponding glycol	328	—	56,750	4.75	+
		(367)	—	30,000	4.48	
5-Dehydrohomovitamin A ethyl ether	By dehydration of corresponding carbinol By dehydration of corresponding glycol	321	—	52,000	4.72	+
		321	—	42,500	4.63	

^a Data recorded here are adapted from N. A. Milas, *Vitamins and Hormones*, 5, 1-38 (1947).

ethyl ethers, while isopropyl and the *tert*-butyl ethers were obtained only in impure form.

a. Vitamin A Methyl Ether. In 1946, Hanze and co-workers¹⁷⁵ prepared the pure methyl ether of vitamin A from the crystalline vitamin A alcohol; they reported that, like other vitamin A ethers, it is a light yellow solid, melting at 33–34°C., which has an extinction value, E (1%, 1 cm.) of 1600 at the $\lambda_{(\max.)}$ of 326 $m\mu$. The biological potency was given as 3,000,000 U.S.P. units per gram. The formula and absorption spectra are shown here.



Vitamin A methyl ether

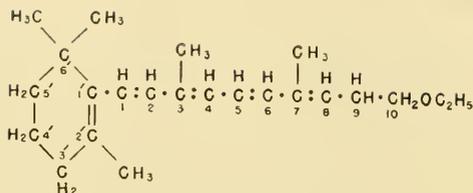
The extinction coefficient of vitamin A methyl ether is 1660, which approximates that of vitamin A. The spectrophotometric absorption also appears to be quite similar to that of the parent free alcohol. The methyl ether of vitamin A produces a blue color with antimony trichloride, which has two maxima. One maximum is at 580 $m\mu$, while the second one is at 618–620 $m\mu$. In one preparation in which the extinction coefficient, E (1%, 1 cm., 325–328 $m\mu$), was 1415, the corresponding extinction for the colored product with antimony trichloride, E (1%, 1 cm., 620 $m\mu$), was found to be 1660. The properties of some vitamin A ethers and related compounds are summarized in Table 10.

b. Homovitamin A and 5-Dehydrohomovitamin A Ethers. Two important synthetic compounds which possess a considerable degree of vitamin A activity are homovitamin A ethyl ether and 5-dehydrohomovitamin A ethyl ether. Each of these compounds has one additional carbon atom on the aliphatic chain.

Homovitamin A ethyl ether ($C_{21}H_{31}OC_2H_5$) is described chemically as [1-(2',6',6'-trimethylcyclohexen-1'-yl)-3,7-dimethyldeca-1,3,5,7-tetraenyl]-10-ethyl ether.¹⁷⁶ The structural formula, including the key for the revised numbering system, is given here.

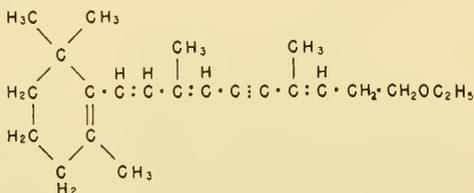
¹⁷⁵ A. R. Hanze, T. W. Conger, E. C. Wise, and D. I. Weisblat, *J. Am. Chem. Soc.*, **68**, 1389 (1946).

¹⁷⁶ N. A. Milas, S. W. Lee, C. Schuerch, Jr., R. O. Edgerton, J. T. Plati, F. X. Grossi, Z. Weiss, and M. A. Campbell, *J. Am. Chem. Soc.*, **70**, 1591–1596 (1948).



Homovitamin A ethyl ether
(including Milas's numbering system)

5-Dehydrohomovitamin A ethyl ether ($C_{21}H_{29}OC_2H_5$) is a closely related compound which is readily changed to the corresponding homovitamin A by reduction with palladium in the presence of calcium carbonate. Chemically, the 5-dehydrovitamin A ether is known as [1-(2',6',6'-trimethylcyclohexen-1'-yl)-3,7-dimethyldeca-1,3,7-trien-5-ynyl]-10-ethyl ether. The structural formula is given here.



5-Dehydrovitamin A ethyl ether

The homologues of the vitamin A ethers show properties almost indistinguishable from those of the true vitamin A ethers themselves. Thus, the 5-dehydrohomovitamin A ethyl ether has a single absorption band with a maximum ($\lambda_{max.}$) = 321 $m\mu$) in the ultraviolet only slightly lower than that of the vitamin A ether; in the Carr-Price reaction, the purplish blue color produced has two maxima practically identical with those of true vitamin A ether, *i.e.*, 580 and 622 $m\mu$. Homovitamin A ethyl ether prepared by different methods gave somewhat different absorption maxima; in one case, the single maximum absorption was at 321 $m\mu$, while in the other case the main absorption maximum was at 328 $m\mu$, with a slight inflection at 367 $m\mu$. Other properties are listed in Table 10.

Both of the homologues possess slight biological activity. Harris¹⁷⁷ reported that 5-dehydrohomovitamin A ethyl ether cured xerophthalmia and caused an average weight gain, in rats, of 32 g. over a 28-day period, when administered in doses of 96 mg. daily. In the case of the homovitamin A ethyl ester, it was found that 60 mg. were active, but Milas⁴³ noted that the compound was highly unstable. The absorption spectra of the vitamin A homologues are given in Figure 4.

¹⁷⁷ R. S. Harris, cited by N. A. Milas, *Vitamins and Hormones*, 5, 5 (1947).

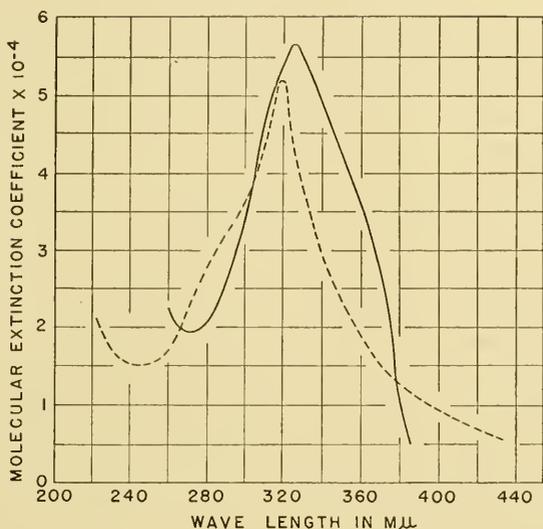
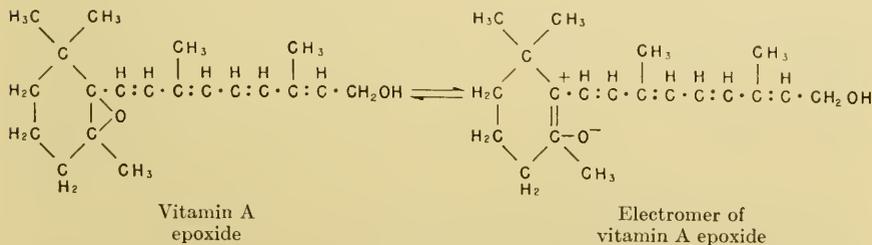


Fig. 4. The absorption spectra in ethanol of the following compounds: (---) 5-dehydrohomovitamin A ethyl ether obtained from carbinol; (—) homovitamin A ethyl ether obtained by dehydrobromination of partially hydrogenated glycol.⁴³

(4) Vitamin A Epoxide

According to Karrer and Jucker,¹⁷⁸ vitamin A forms an epoxide by a method analogous to that of the carotenoids. It is believed that vitamin A epoxide co-exists with vitamin A in fish liver oils and animal fats, in which it originates by the autoxidation of the latter.¹⁷⁸ Karrer¹⁷⁹ suggested the formula shown here for vitamin A epoxide. This author also assumed that it was in equilibrium with its electromer.



Vitamin A epoxide can be synthesized by treating vitamin A with phthalic acid peracid.⁴³ A similar procedure has also been used for the prepara-

¹⁷⁸ P. Karrer and E. Jucker, *Helv. Chim. Acta*, 28, 717-722 (1945).

¹⁷⁹ P. Karrer, *Helv. Chim. Acta*, 28, 474-475 (1945).

tion of the epoxides of β - and α -carotenes.^{180,181} According to Swern,¹⁸² peracids attack a tertiary-tertiary double bond, like the $\Delta^{1,2}$ bond in the β -ionone ring, in preference to that of any other double bond. This would explain the ready production of epoxides when vitamin A or the carotenes are treated with peracids.

The characteristic absorption band of vitamin A epoxide is at 272 $m\mu$ (chloroform), while with antimony trichloride the absorption maxima is at 580 $m\mu$. Milas⁴³ believes that vitamin A epoxide is simultaneously produced, in the synthesis of vitamin A methyl ester, through the dehydrohalogenation method, or from the carbinol. This author also casts some doubt upon the accuracy of the location of the band in the far ultraviolet at 272 $m\mu$, as suggested by Karrer and Jucker.¹⁷⁸ Since there are 4 conjugate double bonds in the molecule, there should be absorption in the region of 285–290 $m\mu$. Support for such an hypothesis is afforded by the demonstration of a single band at 285 $m\mu$ in an autoxidized sample of pure natural vitamin A¹⁷⁴ kept in methanol under nitrogen for 8 years,⁴³ which presumably contained this epoxide. The epoxide of the synthetic methyl ether of vitamin A has a similar band. Moreover, in view of the similarity in the spectrum, there would seem to be some indication that the vitamin A epoxide is identical with the 580 $m\mu$ chromogen of van Eekelen *et al.*,¹⁸³ as well as with the subvitamin A of Embree and Shantz¹⁸⁴ and of Hawkins and Hunter.¹⁸⁵

(5) Kitol

Embree and Shantz¹⁸⁶ were the first to separate a vitamin-A-like product from whale oil which they called kitol (from a modification of the Greek word for whale, $\kappa\eta\tau\sigma\sigma$). Although whale oil is the chief source of kitol, the latter does occur in fish liver oils; dog-fish liver oil was found to contain 0.06%, shark liver oil 0.8%.¹⁸⁷ Its presence in the liver oil of a fresh-water species of fish, the northern pike, was also established.¹⁸⁶ In addition, kitol has been reported in the liver oils of such terrestrial mammals as oxen and sheep.^{186,188}

Kitol is a dihydric alcohol which has a molecular weight about twice that of vitamin A. It occurs in whale liver oil as the ester. The empirical

¹⁸⁰ P. Karrer and E. Jucker, *Helv. Chim. Acta*, **28**, 427–436 (1945).

¹⁸¹ P. Karrer and E. Jucker, *Helv. Chim. Acta*, **28**, 471–473 (1945).

¹⁸² D. Swern, *J. Am. Chem. Soc.*, **69**, 1692–1698 (1947).

¹⁸³ M. van Eekelen, A. Emmerie, H. W. Julius, and L. K. Wolff, *Nature*, **132**, 171 (1933).

¹⁸⁴ N. D. Embree and E. M. Shantz, *J. Am. Chem. Soc.*, **65**, 906–909 (1943).

¹⁸⁵ E. G. E. Hawkins and R. F. Hunter, *Biochem. J.*, **38**, 34–37 (1944).

¹⁸⁶ N. D. Embree and E. M. Shantz, *J. Am. Chem. Soc.*, **65**, 910–913 (1943).

¹⁸⁷ F. B. Clough, H. M. Kascher, C. D. Robeson, and J. G. Baxter, *Science*, **105**, 436 (1947).

¹⁸⁸ K. Hickman, *Ann. Rev. Biochem.*, **12**, 353–396 (1943).

formula which has been assigned to it is $C_{40}H_{58}(OH)_2$. This corresponds to a molecular weight of 572; actually the observed value is 575. It was shown by microhydrogenation studies that this substance has 8 double bonds (7.85 found). The assumption that kitol is a dihydric alcohol is based upon the fact that it forms a dinitrobenzoate. It also contains at least one asymmetric carbon atom, as it displays optical activity ($[\alpha]_{546.1}^{25} = -1.35^\circ$ in chloroform).

Kitol possesses a number of distinctive properties. It can be crystallized from methyl alcohol in the form of elongated prisms which melt at 88–90°C. It is quite stable toward atmospheric oxidation; no change was observed in the absorption curve after 53 hours at room temperature in the dark. However, in daylight, a 56% destruction occurred after 46 hours under similar temperature conditions. Kitol was found to be relatively stable in ethanol, inasmuch as it was shown that the extinction coefficient had dropped only 7% after storage for 10 days in the dark at room temperature.¹⁸⁷

Kitol gives a maximum absorption at 290 $m\mu$, at which wave length the extinction coefficient, E (1%, 1 cm.), has been shown to be 707.¹⁸⁵ The presence of a peak in this area of the ultraviolet region in whale oil was noted a number of years ago by Pritchard *et al.*¹⁸⁹ and by Willstäedt and Jensen.¹⁹⁰ Inasmuch as the absorption is considerable at 328 $m\mu$, the presence of kitol will mask the presence of vitamin A. Although kitol reacts with antimony trichloride, the maximum absorption band is at 570 $m\mu$; the amount of chromogen produced at 620 $m\mu$ in this reaction can be used for the determination of the amount of vitamin A in the presence of kitol. The kitol present in the northern pike has the absorption band at 310 $m\mu$ instead of at 290 $m\mu$. With antimony trichloride, it reaches a peak at 510 $m\mu$. When it is subjected to distillation, the resulting product shows a maximum absorption with antimony trichloride at 690 $m\mu$. The authors believe that this indicates that it is vitamin A₂. The kitol from fresh-water fishes is therefore referred to as kitol₂.

The most important property of kitol is its conversion by heat to vitamin A. According to the results of Embree and Shantz¹⁸⁶ which are given in Table 11, one molecule of vitamin A is produced for each molecule of kitol destroyed. The destruction of kitol was based upon the decrease of the E (1%, 1 cm., 286 $m\mu$) value, while the amount of vitamin A produced was judged by the increase of the E (1%, 1 cm., 620 $m\mu$) after antimony trichloride was added. On the basis of later work, Clough *et al.*¹⁸⁷ reported that, on distillation of a peanut oil solution of kitol palmitate in the molecular still, 0.67 to 0.75 molecule of vitamin A originated for each molecule of the kitol ester destroyed. Kitol palmitate was found to have an extinction coefficient of 379 at the maximum absorption (290 $m\mu$).

¹⁸⁹ H. Pritchard, H. Wilkinson, J. R. Edisbury, and R. A. Morton, *Biochem. J.*, **31**, 258–265 (1937).

¹⁹⁰ H. Willstäedt and H. B. Jensen, *Nature*, **143**, 474 (1939).

TABLE 11
 THERMAL DECOMPOSITION OF KITOL^a

Time (min.) at 220°C.	<i>E</i> (620 m μ) (SbCl ₃)	<i>E</i> (286 m μ)	Loss <i>E</i> (286 m μ)		Molecules/g. $\times 10^{-6}$		Ratio, molecules vit. A to kitol
			Appar.	Corr.	Kitol lost	Vit. A gained	
0	0.0	33.0	—	—	—	—	—
1	3.1	32.5	0.5	0.87	2.61	2.26	0.9
2	6.8	32.2	0.8	1.62	4.86	4.97	1.0
3	12.3	31.8	1.2	2.7	8.1	9.00	1.1
4	15.1	31.5	1.5	3.3	9.9	11.0	1.1
8	26.1	30.7	2.3	5.4	16.2	19.1	1.2
16	33.4	30.0	3.0	7.0	21.0	24.4	1.2

^a N. D. Embree and E. M. Shantz, *J. Am. Chem. Soc.*, 65, 910-913 (1943).

Kitol is resistant to treatment with *N*/30 alcoholic hydrogen chloride; after neutralization, the recovered material yields vitamin A on pyrolysis. On the other hand, no vitamin A can be obtained from the reaction product of kitol and antimony trichloride. Kitol can be readily separated from vitamin A because it is more strongly adsorbed by alumina on the chromatographic column than is vitamin A.

Hickman¹⁸⁸ has suggested that kitol may be a detoxication product of vitamin A. It has therefore been suggested that it should be considered as a post- rather than as a provitamin A. Since it is well known that most mammals are unable to tolerate as large quantities of vitamin A as can most fishes, it was suggested that a mammal such as the whale, finding itself embarrassed by the large intake of vitamin A in its marine diet, develops a method for changing it into the non-toxic product kitol.

However, this earlier suggestion that kitol is a detoxicant now appears open to question. The presence of kitol in the liver oils of sheep and oxen, in spite of their relatively low vitamin A intake, would seem to preclude this explanation. Moreover, the rat does not form kitol even if given excessive amounts of the vitamin.¹⁹¹ Finally, the fact that such fishes as the dog-fish and shark, which are considered relatively immune to vitamin A toxicity, also form kitol would appear to render the detoxication theory untenable.

The following procedures¹⁸⁷ have been used for the preparation of crystalline kitol: a whale liver oil containing 0.8% kitol and 21,000 I.U. vitamin A per gram was used as the starting material. The preliminary concentration was effected by saponification, after which the sterols and other contaminants were removed from the non-saponifiable extract by low-temperature crystallization from acetone. The resulting oil was then mixed with a constant yield oil, and the mixture was distilled in a molecular still accord-

¹⁹¹ E. M. Shantz, *Personal communication*, Sept. 29, 1948.

ing to the Hickman^{114,115} procedure. The fraction distilling at 190–260°C. was rich in kitol. This was stripped at 160°C., after a constant-yield oil and residue oil had been added. The distilland was rich in kitol, and was practically free from vitamin A. The unsaponifiable extract of the distilland was treated with succinic anhydride to form the acid succinate, which was neutralized in 83% ethanol. The non-hydroxylic materials could be separated by extraction with petroleum ether. On saponification, the kitol was sufficiently pure to form a crystalline di-*p*-phenylazobenzoate ester. Crystalline kitol was subsequently prepared by crystallization of the non-saponifiable matter from the phenylazobenzoate with methyl alcohol. The yield amounted to only 10% of the original amount present in the whale-liver oil.

A number of synthetic esters of kitol have been prepared. These include the di-*p*-phenylazobenzoate ester, which exists in two forms; the lower one melts at 125–126°C., while the higher one melts at 149–150°C.¹⁸⁷ It is considered that these may be geometric isomers of the carbon-carbon type, although the possibility that a nitrogen-nitrogen stereoisomerism may be responsible is not excluded. A crystalline anthraquinone carboxylate ester melting at 195–197°C. has also been prepared. It apparently exists in a lower melting form as well, but this ester cannot be purified. The dinitrophthalate ester was also prepared by Clough and associates,¹⁸⁷ but it could not be obtained in crystalline form.

5. Synthesis of Vitamin A and Related Compounds

(1) Introduction

Extensive investigations to develop practical methods for the synthesis of vitamin A were pursued at an increased pace during World War II; they have now reached the point where vitamin A is being produced in considerable amounts synthetically for commercial use; facilities are at present being developed which will render possible the further replacement of the diminishing natural supply of vitamin A by the synthetic product. The earlier methods of synthesis are reviewed by Bogert,¹⁹² Sobotka and Bloch,¹⁹³ and Heilbron, Jones, and Bacharach,³⁰ while current procedures are admirably treated in a more recent comprehensive article by Milas.⁴³ The subject will be briefly reviewed here, but the reader is referred to Milas⁴³ and to the original sources for a more complete treatise on the subject.

After the elucidation of the structure of vitamin A by Karrer, Morf, and Schöpp^{41,126} in 1931, and its confirmation and extension by other workers

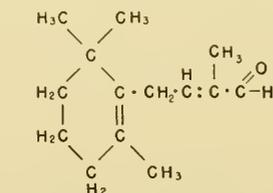
¹⁹² M. T. Bogert, "Carotenoids. The Polyene Pigments of Plants and Animals," in H. Gilman, *Organic Chemistry*, Vol. II, Wiley, New York, 1938, pp. 1138–1219.

¹⁹³ H. Sobotka and E. Bloch, *Chem. Revs.*, *34*, 435–460 (1944).

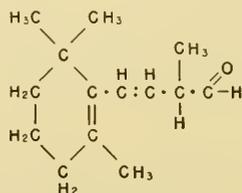
ionone and no aldehyde when this distillation was carried out, while Karrer and Rügger¹⁶⁸ reported only β -ionone. The synthesis of this key intermediate by the modifications in method introduced by Krauze and Slobodin,²⁰¹ who obtained a 64% yield, could not be repeated by Milas and Edgerton,²⁰² who also obtained only β -ionone. It is evident that until reproducible methods have been developed for the synthesis of β -ionylideneacetaldehyde in the pure state, the Kuhn-Morris method will not offer a practical solution for the synthesis of vitamin A.

(3) Milas Syntheses

Several different syntheses have recently been described by Milas^{43, 172, 173} which involve the preparation of vitamin A ethers or vitamin A esters. Although the products prepared by these reactions have the correct chemical composition, a satisfactory absorption spectrum, and the typical reaction with antimony trichloride, the order of potency obtained by bioassay was only 50,000 to 100,000 U.S.P. units per gram. However, the reactions do offer practicable methods for such syntheses, in spite of the low potency of the product. The key substance which is used as a starting material in both types of synthesis is the aldehyde obtained on decarboxylation of glycidic acid.²⁰³ The ester of glycidic acid is formed when β -ionone is condensed with ethyl chloroacetate at -30 to -60°C . in anhydrous ether or toluene, using alcohol-free sodium ethoxide or methoxide as a condensing agent. After hydrolysis, the free acid so produced can be decarboxylated by several methods, but the best results were obtained when it was distilled at reduced pressure in the presence of pyridine. Heilbron *et al.*²⁰⁴ proposed an alternate structure (IV) for the aldehyde formed on decarboxylation of glycidic acid, but Milas, Lee, *et al.*²⁰⁵ believe that formula V is the correct one, since geronic acid, rather than 3,3-dimethyloctan-2,7-dione, originates on ozonolysis.



(IV) Formula according to Heilbron *et al.*²⁰⁴

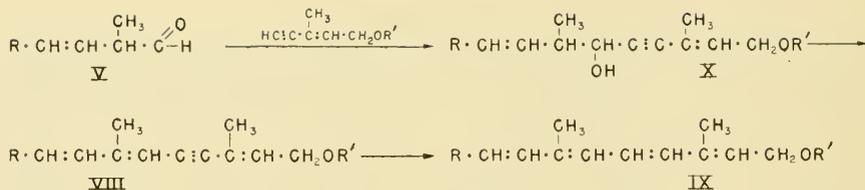


(V) Formula according to Milas *et al.*²⁰⁵

²⁰³ S. Ishikawa and T. Matsuura, *Sci. Repts. Tokyo Bunrika Daigaku*, A3, No. 60, 173-179 (1937); *Chem. Zentr.*, 1937, II, 3452-3453; *Chem. Abst.*, 31, 7851 (1937). Cited by I. M. Heilbron, *et al.*, *J. Chem. Soc.*, 1942, 727-733.

²⁰⁴ I. M. Heilbron, A. W. Johnson, E. R. H. Jones, and A. Spinks, *J. Chem. Soc.*, 1942, 727-733.

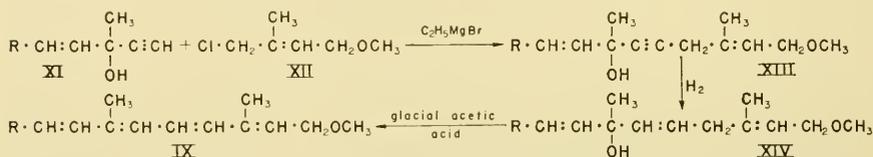
²⁰⁵ N. A. Milas, S. W. Lee, E. Sakal, H. C. Wohlers, N. S. McDonald, F. X. Grossi, and H. F. Wright, *J. Am. Chem. Soc.*, 70, 1584-1591 (1948).



Synthesis of Vitamin A (Milas "B" Method and Procedure of Isler *et al.*)

(4) Oroshnik Synthesis

The method of Oroshnik^{43,209} is quite similar to the Milas method and the procedure of Isler *et al.*, except that it differs in the starting material. The acetylenecarbinol (XI) is treated with ethyl magnesium bromide (Grignard reagent); the resulting Grignard acetylenecarbinol is reacted with 2-methyl-4-hydroxy-1-chloro-2-butene (XII) to form methoxyacetylenecarbinol (XIII), which is then catalytically reduced to the methoxycarbinol (XIV). The latter compound can then be dehydrated in glacial acetic acid with *p*-toluenesulfonic acid to form vitamin A methyl ether.



Oroshnik Synthesis of Vitamin A Methyl Ether

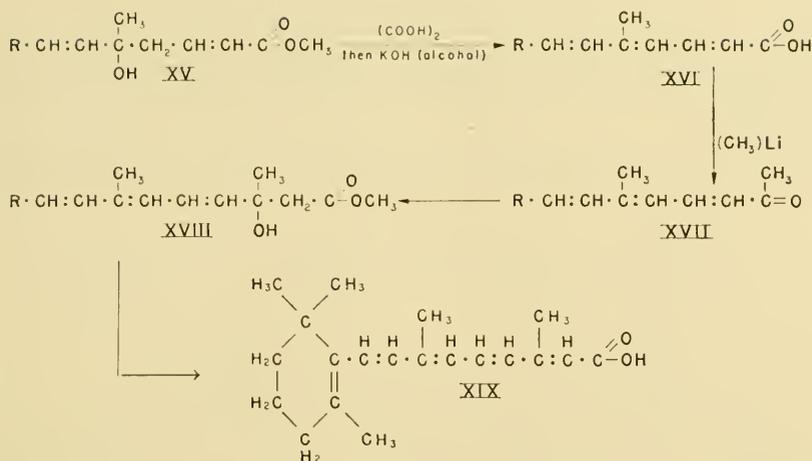
Milas⁴³ is of the opinion that vitamin A methyl ether prepared by the Oroshnik procedure is quite impure.

(5) Synthesis of Arens and van Dorp

a. Synthesis 1. Two different syntheses of vitamin A have been discovered by Arens and van Dorp. The first of these procedures involves the synthesis of vitamin A acid,^{169,170} which seems to be about as potent as vitamin A itself.²¹⁰ In this synthesis, β -ionone and γ -bromocrotonate are condensed by the Reformatsky reaction to produce an hydroxy ester (XV). After dehydration and saponification of the ester, the resulting acid (XVI) is treated with methyl lithium to yield a methyl ketone (XVII). After another Reformatsky reaction using bromoacetic ester, an hydroxy ester (XVIII) is formed which, on dehydration with oxalic acid and saponification, gives rise to an orange oil from which the acid corresponding to vitamin A (XIX) can be crystallized.

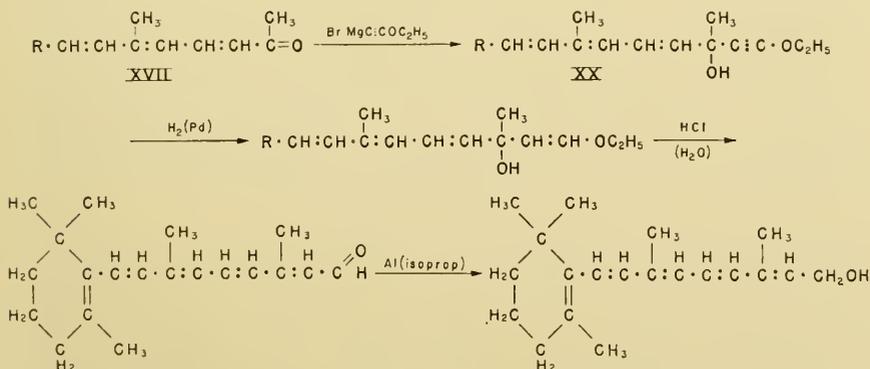
²⁰⁹ W. Oroshnik, *J. Am. Chem. Soc.*, **67**, 1627-1628 (1945).

²¹⁰ D. A. van Dorp and J. F. Arens, *Nature*, **153**, 60 (1946).



Arens and van Dorp Synthesis of Vitamin A Acid

b. Synthesis 2. The second procedure of van Dorp and Arens¹⁶³ which results in the formation of vitamin A alcohol, is identical with Method 1 up to the formation of the ketone (XVII). This compound is treated with $\text{BrMgC}:\text{COC}_2\text{H}_5$ to produce an acetylenic hydroxy ether of vitamin A (XX). Following a controlled reduction and treatment with hydrochloric acid, vitamin A aldehyde is formed; this can be reduced to vitamin A alcohol by means of aluminum isopropoxide.

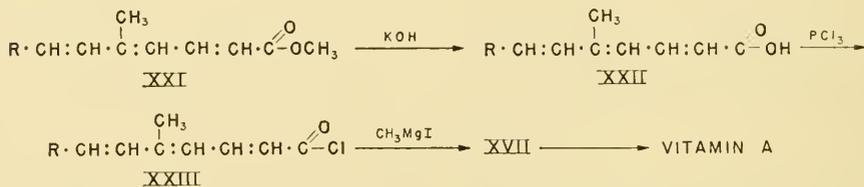


Synthesis of Vitamin A Alcohol by the Procedure of van Dorp and Arens

(6) Synthesis of Karrer, Jucker, and Schick

Karrer *et al.*¹⁷¹ independently proposed a synthesis of vitamin A somewhat similar to the method given in the Arens and van Dorp report.^{169,170} Bromocrotonic acid was reacted with β -ionone to form the dehydrated ester

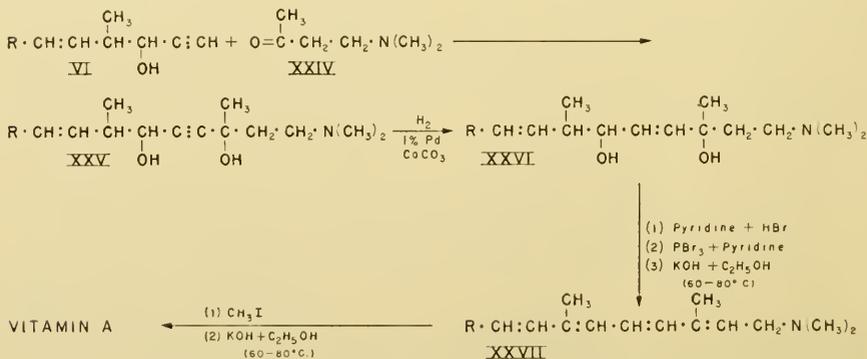
(XXI). The methyl ketone (XVII) was prepared by treating the acid chloride (XXIII) prepared from the dehydrated acid (XXII) with methyl zinc iodide. The remaining reactions were similar to those of Arens and van Dorp.



Synthesis of Vitamin A by the Method of Karrer *et al.*

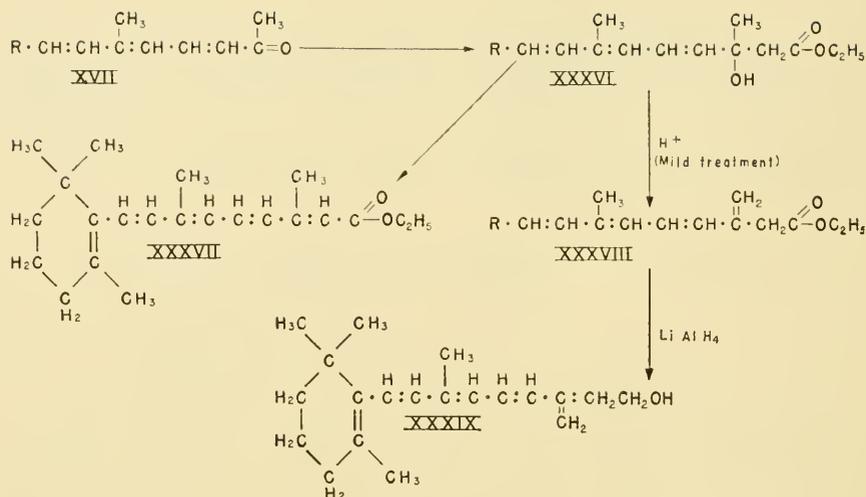
(7) *Synthesis of Vitamin A from Dimethylaminovitamin A*

Vitamin A has also been synthesized by Milas,^{43,211} using exhaustive methylation of dimethylaminovitamin A. This reaction would be expected to proceed satisfactorily, since it is known that, when heat is applied to trimethylalkyl quaternary salts or bases in which the alkyl group contains no loosely bound hydrogen atoms on the carbon combined with the quaternary nitrogen, decomposition results, trimethylamine and alcohol being the chief end products. In this procedure acetylenic carbinol (VI) is condensed with 4-dimethylaminobutan-2-one (XXIV) by means of the Grignard reaction, to give the dimethylaminoacetylenic glycol (XXV) in 30% yields. On selective hydrogenation of XXV, the glycol XXVI is formed which, in turn, is converted to the hydrobromide by pyridine hydrobromide. The dimethylaminovitamin A is prepared from this by treatment of the hydrobromide with phosphorus tribromide followed by debromination of the dibromide with alcoholic potash. The dimethylaminovitamin A (XXVII) can be converted into vitamin A by forming its methiodide, converting the latter into the hydroxide with alcoholic potash and heating the hydroxide to 60–70°C. These reactions are given in detail below:



Synthesis of Vitamin A from Dimethylaminovitamin A

²¹¹ N. A. Milas, *U. S. Patent No. 2,415,834*, Feb. 18, 1947.



Synthesis of Isomeric Vitamin A Alcohol

If the hydroxy ester (XXXVI) is treated with suitable catalysts such as acid or iodine, it is dehydrated to form a new compound, which is called "vitamin A₃" acid ethyl ester (XXXVIII). The latter compound can be changed to the "vitamin A₃" alcohol (XXXIX) by treatment with lithium aluminum hydride. The new vitamin A₃ possesses a marked biological potency, approximately 1,000,000 U.S.P. units per gram. It cannot be converted to vitamin A₁ by chemical means, and it is not believed that the biopotency can be traced to the presence of vitamin A as an impurity. No differences in the various biological indices of synthetic and of natural vitamin A, respectively, can be noted.²¹⁴

(10) Synthesis of Vitamin A Ethers

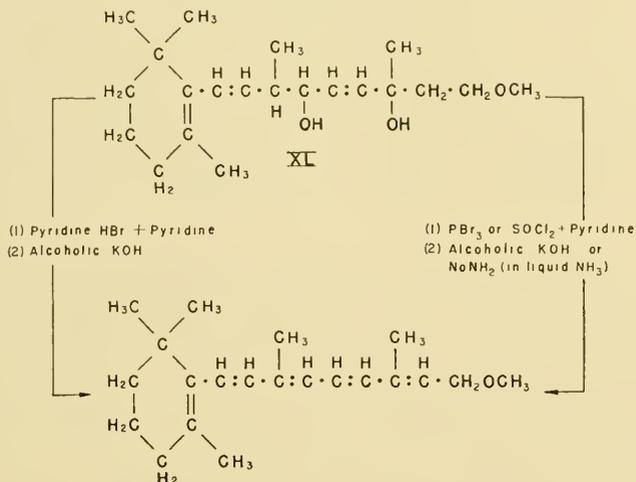
Several of the principal methods for synthesis of vitamin A already described, including those of Milas,²⁰⁶ Isler *et al.*,^{208,215} and Oroshnik²⁰⁹ involve the formation of the vitamin A ethers. Additional procedures are listed below.

a. Milas Synthesis from Glycol Methyl Ether. The glycol methyl ether (XL) can be readily prepared from the glycol (VII, see page 717) used in the preparation of vitamin A by the Milas "A" method, employing 1% palladium deposited on calcium carbonate as a catalyst. The glycol methyl ether can be changed to vitamin A methyl ether by any one of

²¹⁴ H. M. Wuest and N. Ercoli. Presented before the 115th Meeting of the American Chemical Society in San Francisco, March 27-Apr. 1, 1949, No. 21, pp. 12c, 13c.

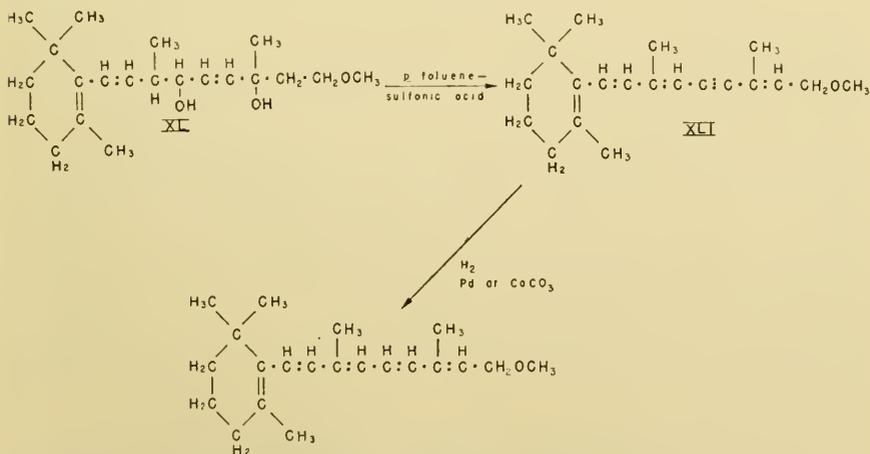
²¹⁵ O. Isler, W. Huber, A. Ronco, and M. Kofler, 1946, *Jubilee Volume for Emil C. Barrell*, p. 31, Hoffmann-LaRoche, Basle. Cited by N. A. Milas, *Vitamins and Hormones*, 5, 20 (1947).

several procedures. The ethyl, isopropyl, or *tert*-butyl ethers can be produced by using the corresponding glycol ethers.



Milas Synthesis of Vitamin A Methyl Ether

b. Milas Synthesis from 5-Dehydrovitamin A Methyl Ether. Another synthesis of the vitamin A methyl ether, by Milas *et al.*,^{43,174} is by the selective hydrogenation of 5-dehydrovitamin A methyl ether (XLI). The latter compound was prepared by the dehydration of the glycol with catalytic quantities of *p*-toluenesulfonic acid.



Second Milas Synthesis of Vitamin A Methyl Ether

The corresponding 5-dehydrovitamin A ethyl ether was also readily prepared by dehydration of the glycol ethyl ether or of the carbinol in the presence of *p*-toluenesulfonic acid. However, the preparation of the vitamin A ethyl ether could not be brought about by selective dehydrogenation.

(11) Other Syntheses of Vitamin A

Cawley *et al.*²¹⁶ have prepared crystalline vitamin A by methods which the authors state differ from any heretofore described in the literature. Vitamin A acetate and vitamin A anthraquinone carboxylate have been so synthesized, and are reported to be identical with similar esters prepared from the natural product. Such methods are presumably the basis for the procedures employed in the large-scale synthesis of vitamin A now being developed commercially. Details of the synthesis have not as yet been published.

Hunter and Williams¹⁶² have succeeded in preparing vitamin A from β -carotene by chemical methods, by oxidative fission of the central double bond of β -carotene with hydrogen peroxide to yield vitamin A aldehyde, and subsequent reduction to vitamin A alcohol by the Pondorff method. The maximum yield obtained was only 0.4 to 0.5%. Vitamin A was identified by the antimony trichloride reaction and by its transformation to cyclized vitamin A with *N*/30 alcoholic hydrogen chloride.

No synthesis has been successful so far for vitamin A₂, and none can be expected until its structure is proved. One *cis* stereoisomer of vitamin A has been prepared from fish liver oils by Robeson and Baxter.^{46,47} Presumably, this and other *cis* compounds of vitamin A can be synthesized by catalysts such as iodine, employed by Zechmeister.¹⁵³

(12) Syntheses of Homologues of Vitamin A Ethers

Several homologues of vitamin A have been synthesized which possess a rather high degree of biopotency. The principal compounds which have been investigated in this study are 5-dehydrohomovitamin A ethyl ether (XLVI) and homovitamin A ethyl ether (XLVII). Each of these compounds has one additional carbon atom at the end of the side chain.

Both of these homologues are readily prepared by two quite similar procedures. In both cases the aldehyde formed by decarboxylation of glycidic acid (V) is the starting point. The methods which have been worked out by Milas,¹⁷² and by Milas, Lee, *et al.*,¹⁷⁶ involve the condensation of the aldehyde with either 3-methyl-6-ethoxyhexa-1-yn-3-ol (XLII) or 3-methyl-6-ethoxyhexa-3-en-1-yne-1 (XLIII). In the first case the glycol (XLIV) is formed as the intermediate, while in the second instance the carbinol (XLV) is formed. These syntheses are diagrammed on page 725.

²¹⁶ J. D. Cawley, C. D. Robeson, L. Weisler, E. M. Shantz, N. D. Embree, and J. G. Baxter. Presented before the American Chemical Society Meeting in New York City, Sept., 1947, p. 26c.

6. Properties of Vitamins A and Related Compounds

(1) General Physical Properties

The several forms of vitamin A are very soluble in practically all fat solvents and are insoluble in water. It is impossible to separate vitamin A from vitamin A₂, or from β -carotene, on the basis of differential solubility alone. The same is true for the stereoisomers of vitamin A. However, a partial separation of carotene from vitamin A can be effected by precipitating the carotenoid from a mixture in a methanol solution at the temperature of solid carbon dioxide.

Vitamin A₁ alcohol crystallizes from methanol in the form of pale yellow platelets which melt at 7.5 to 8°C.³¹ and which contain methyl alcohol of crystallization. When the crystallization occurs from ethyl formate or propylene glycol solutions of vitamin A, pure vitamin A crystals result which melt at 63–64°C.³⁷

In addition to the reaction with antimony trichloride, which is discussed on page 732, vitamin A develops a blue color with acid earths when dissolved in non-polar solvents. This was originally discovered by Meunier.²¹⁷ The explanation for the reaction which Meunier proposed was that some acid earths possess incomplete electronic octets which enable them to give rise to an intensely blue color when they come in contact with vitamin A in a non-polar solvent. When unshared electrons are donated to such adsorbents, the vitamin molecule undergoes polarization and forms strongly charged resonating structures. Lowman,²¹⁸ independently, recommended this method for the detection of vitamin A and for its rough quantitative estimation. The latter worker used a non-polar solvent and the commercial adsorbent Super-Filtrol. Kreider²¹⁹ confirmed the results of Lowman. Zechmeister and Sandoval²²⁰ have pointed out that this reaction is not specific for vitamin A but that it is also given by carotene (as noted by the earlier workers), and also by a new fluorescent polyene which has been identified as phytofluene (see Chapter VI). Moreover, a positive reaction can be obtained on purified Super-Filtrol with a benzene solution of diphenyloctatetraene, $C_6H_5(CH:CH)_4 \cdot C_6H_5$.

Because of its alcohol group, vitamin A readily forms esters, not only with fatty acids but also with other organic acids. Such esters occur naturally in fish liver oils, as well as in the blood and tissues of higher animals. Some pure crystalline vitamin A esters have recently been prepared by Baxter and Robeson³⁶ (see Table 1). Vitamin A cannot be precipitated by digi-

²¹⁷ P. Meunier, *Compt. rend.*, 215, 470–473 (1942).

²¹⁸ A. Lowman, *Science*, 101, 183–184 (1945).

²¹⁹ H. R. Kreider, *Science*, 101, 377 (1945).

²²⁰ L. Zechmeister and A. Sandoval, *Science*, 101, 585 (1945).

tonin; this fact offers a satisfactory method for its separation from cholesterol, which can be quantitatively precipitated as the digtonide.

(2) Distillation

Heilbron *et al.*²⁷ and Carr and Jewell²⁹ state that vitamin A alcohol distills at 136–137°C. at a pressure of 10^{-7} mm. However, Hickman^{114,115} reported the distillation temperature as 120–125°C. under 5×10^{-3} mm. pressure. On the other hand, the natural vitamin A esters distill at much higher temperatures, *i.e.*, 200–240°C. under 10^{-3} mm. Distillation thus offers a simple method for the separation of the esters from the free vitamin A alcohol. Hickman²²¹ notes the following temperatures as elimination maxima (in °C.) for various free and esterified natural and synthetic

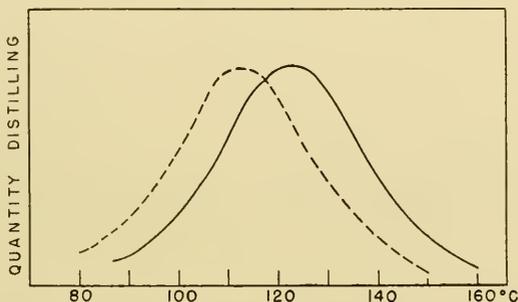


Fig. 5. The comparative elimination curves of anhydrovitamin A (---) and vitamin A alcohol (—) when subjected to molecular distillation.²²¹

vitamin A preparations: crystalline vitamin A alcohol, 123; saponified fish liver oil, commercial concentrates, 123; crystalline vitamin A acetate, 132; crystalline vitamin A palmitate, 208; halibut liver oil, 212; U.S.P. Reference Oil II, 214; and distilled vitamin A esters, commercial concentrates, 214. A typical elimination curve for vitamin A alcohol is given in Figure 5.

The separation of the vitamin A by distillation of an unsaponified fish liver oil is illustrated in Figure 6. By the passage of the oil through the molecular still with carefully controlled rates of distillation, it is possible to concentrate the vitamin A esters into a single distillate, while the undesirable acids and odors are separated in a lighter fraction. The bulk of the esters of the other non-saponifiable factors and the parent oil are left undistilled and substantially unchanged.

²²¹ K. C. D. Hickman, *Chem. Revs.*, 34, 51–106 (1944).

In addition to the review of Hickman²²¹ on molecular distillation, an earlier one by Embree²²² is also excellent.

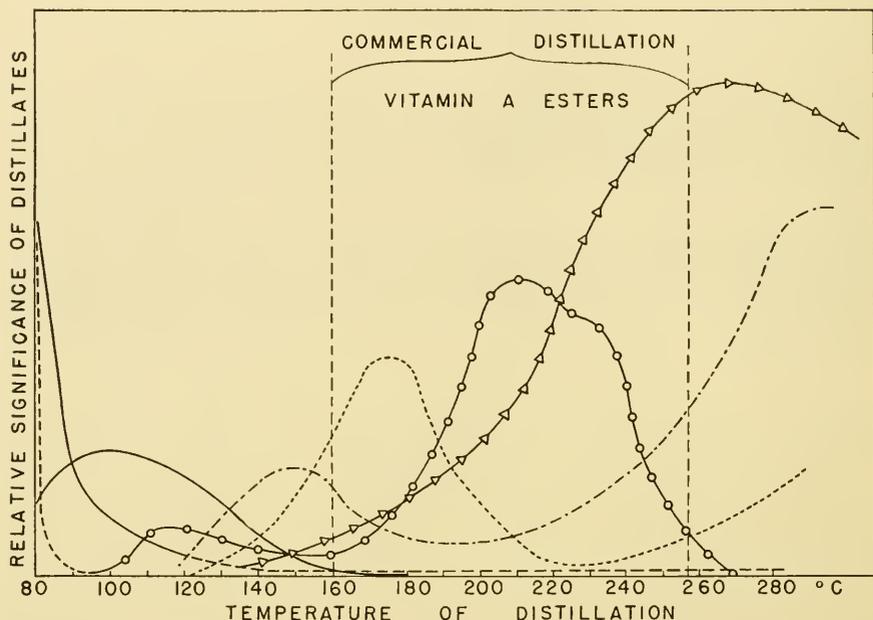


Fig. 6. Distillation map of a fish liver oil: (---) residual protein odor; (—) rancidity and reversion odor; (—) free fatty acids; (—) sterols and vitamin D, glyceride ethers, and their esters; (---) preservatives, tocopherols, and their esters; (—○—○) vitamin A and esters; (△—△) glyceride fats.²²¹

(3) Absorption Spectra

One of the most useful properties for the identification and quantitative determination of vitamin A is the absorption of light in the ultraviolet portion of the spectrum. Vitamin A₁ absorbs a broad band in which the maximum effect is obtained at 328 m μ (in chloroform),^{31,36,223} while the maximum absorption in the case of vitamin A₂ has been reported at 345–350 m μ .^{59,73} The absorption maximum of vitamin A₃, which has been obtained only as a fraction from liver oils, has been given as 285–290 m μ .¹⁸⁹ Typical curves for crystalline vitamin A₁, and for neovitamin A are given in Figure 7.

The wave length at which maximum absorption of vitamin A occurs varies to some extent with the solvent employed. The molecular extinction values for vitamin A in a number of solvents are recorded in Table 12.

²²² N. D. Embree, *Chem. Revs.*, 29, 317–332 (1941).

²²³ F. P. Zscheile and R. L. Henry, *Ind. Eng. Chem., Anal. Ed.*, 14, 422–425 (1942).

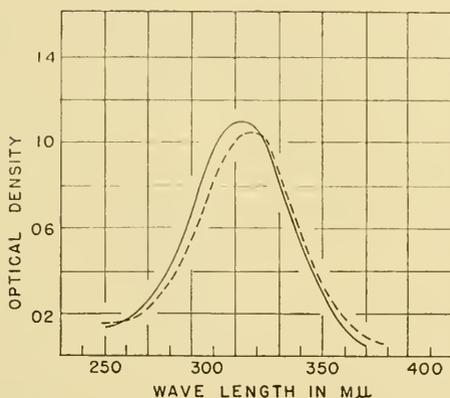


Fig. 7. The absorption spectra in ethanol: (—) vitamin A₁; (---) neovitamin A.^{44,224}

Although solutions of purified vitamin A preparations may be very exactly determined spectrophotometrically at their extinction maxima, there are a number of interfering substances in extracts of natural products which likewise absorb light in the 324–332 $m\mu$ area. The presence of appreciable amounts of such contaminants may therefore completely vitiate the determination if corrections are not made for such irrelevant absorption. The chief substances causing the irrelevant absorption are the carotenoids. Although the correction factor for these compounds may be a minor one if the extracts have not been heated, it is markedly increased by saponification or by any procedure which causes the compound to undergo stereoisomerism. Such interfering substances can ordinarily not be removed by saponification.

There are several methods which can be used to confirm the fact that the material absorbing at 328 $m\mu$ is vitamin A. According to Loofbourow,²²⁴ the "normality" of the vitamin A can be established: (1) by the demonstration of the absence of a fine structure in the absorption spectrum indicative of cyclized vitamin A, which possesses no biological activity; and (2) by proof that a well-defined maximum results at 617 $m\mu$ when the antimony trichloride test is applied. Moreover, the *E* (1%, 1 cm., 617 $m\mu$) should be approximately twice that obtained at 580 $m\mu$.

A second procedure for the confirmation of vitamin A as the material causing the absorption at 328 $m\mu$ is the demonstration that the absorption curves are typical for vitamin A. This test was employed by the War Food Administration for purchases of vitamin A during World War II. When the ratios of *E* (1%, 1 cm., 300 $m\mu$) and *E* (1%, 1 cm., 328 $m\mu$) were not more than 0.73, and the ratio of *E* (1%, 1 cm., 350 $m\mu$) to *E* (1%, 1 cm., 328 $m\mu$) did not exceed 0.65, the samples were considered to be satisfactory,

²²⁴ J. R. Loofbourow, *Vitamins and Hormones*, 1, 109–155 (1943).

TABLE 12
 ABSORPTION MAXIMA AND EXTINCTION COEFFICIENTS OF VITAMIN A PREPARATIONS IN
 VARIOUS SOLVENTS

Substance	Ref.	M.p., °C.	Solvent	Absorp- tion maxima (1%, 1 cm.)	<i>E</i>	ϵ	
Vitamin A ₁ (crystalline)	<i>a</i>	63-64	Ethanol	324	1,720	49,200	
		—	Methanol	324	1,760	50,800	
		—	2-Propanol	324	1,690	48,300	
		—	Ether	326	1,770	50,500	
		—	Isooctane	326	1,660	47,500	
		—	Hexane	326	1,680	47,900	
		—	Cyclohexane	326	1,600	45,700	
		<i>b</i>	—	—	325	1,750	—
		<i>c</i>	—	—	325	1,880 ± 40	—
		acetate	<i>d</i>	57-58	Ethanol	328	1,510
palmitate	<i>d</i>	27-28	Ethanol	328	940	—	
succinate	<i>d</i>	76-77	Ethanol	328	1,240	—	
2-naphthoate	<i>d</i>	74-75	Ethanol	328	1,090	—	
	<i>e</i>	—	—	328	1,185	33,900	
anthraquinone-2- carboxylate	<i>f</i>	121-122	Ethanol	330	1,065	—	
yellow form	<i>e</i>	126	—	328	938	26,800	
red form	<i>e</i>	118-120	—	328	1,090	31,100	
<i>p</i> -phenylazobenzoate	<i>f</i>	79-80	Petroleum ether- acetone	—	—	—	
Neovitamin A (crystalline)	<i>f</i>	59-60	—	328	1,675	—	
acetate	<i>f</i>	—	—	328	1,410	—	
anthraquinone-2- carboxylate	<i>f</i>	134-135	Benzene	333	1,020	—	
red form		134-136	—	—	—	—	
yellow form		121-122	—	—	—	—	
<i>p</i> -phenylazobenzoate		94-96	—	—	330	1,460	—
Vitamin A ₂	<i>a</i>	—	83% Methanol	345	—	—	
	<i>g</i>	—	—	351	1,460	—	
		—	—	287	820	—	
phenylazobenzoate	<i>g</i>	76-77	—	341	1,190	—	
Vitamin A ₃	<i>a</i>	—	83% Methanol	290	—	—	

^a J. R. Loofbourow, *Vitamins and Hormones*, 1, 109-155 (1943).

^b N. D. Embree, *Ann. Rev. Biochem.*, 16, 323-358 (1947).

^c R. A. Morton, *Ann. Rev. Biochem.*, 11, 365-390 (1942).

^d J. G. Baxter and C. D. Robeson, *J. Am. Chem. Soc.*, 64, 2407-2410 (1942).

^e T. H. Mead, S. W. F. Underhill, and K. H. Coward, *Biochem. J.*, 33, 589-600 (1939).

^f C. D. Robeson and J. G. Baxter, *Nature*, 155, 300 (1945); *J. Am. Chem. Soc.*, 69, 136-141 (1947).

^g E. M. Shantz, *Science*, 108, 417-419 (1948).

so far as the purity of the vitamin A was concerned; the potency of the oil in International units per gram was calculated by multiplying the extinction value at 328 m μ by a conversion factor of 2000.²²⁵

²²⁵ First specified in contract form No. PDP 76 for vitamin oils and concentrates issued by the War Food Administration in 1944.

Another method for establishing the amount of absorption at 328 $m\mu$ which is caused by vitamin A is by the use of acid clays which specifically adsorb vitamin A and thus separate it from the impurities which interfere with the spectrophotometric determination. Awapara, Mattson, Mehl, and Deuel²²⁶ have demonstrated that the vitamin A in margarines or butters can be quantitatively removed from a benzene solution by passing the non-saponifiable extract over a thin layer of floridin.²²⁷ When the absorption curve of the eluate is subtracted from that of the untreated extract, the difference shows a typical vitamin A curve. One complication which arises is the fact that the carotenoids are also adsorbed by floridin, even when an extremely thin layer is employed. In case such products are present in the extract, corrections must be made for their absorption. The vitamin A-floridin reaction involves the development of a deep blue color on the clay, which is extremely sensitive. Apparently, a chemical reaction occurs, since it has been impossible to remove the vitamin A from the floridin by means of other eluents.

Since vitamin A is particularly sensitive to irradiation with ultraviolet light,²² another method for establishing the proportion of the absorption at 328 $m\mu$ which is referable to vitamin A is from the difference in absorption before and after irradiation of the sample.^{228,229} Neal and Luckmann²²⁹ have successfully applied this method to the determination of vitamin A in margarine.

Wilkie and DeWitt²³⁰ employ chromatographic adsorption of vitamin A extracts obtained after saponification of margarine, which aids in removing the irrelevant absorption before the spectrophotometric determination is carried out. However, the eluates of the vitamin-A-containing layer of the chromatographic column still do not show characteristic curves for vitamin A.

Morton and Stubbs²³¹ have developed a formula to correct for irrelevant absorption. By determining the extinction values of a known mixture of anthracene and vitamin A, it is possible to obtain correction for the *E* (328 $m\mu$) value based upon observations at 313 and 338.5 $m\mu$ to afford a true vitamin A value. The Morton and Stubbs correction averages 6% for molecular distillates, while correction factors of 11–16% are usually necessary for oils.²³² The need for correction arises from the presence of anhydrovitamin A and sometimes oxidation products.

²²⁶ J. Awapara, F. H. Mattson, J. W. Mehl, and H. J. Deuel, Jr., *Science*, *104*, 602–604 (1946).

²²⁷ A. Emmerie and C. Engel, *Rec. trav. chim.*, *53*, 283–289 (1939).

²²⁸ R. W. Little, *Ind. Eng. Chem., Anal. Ed.*, *16*, 288–293 (1944).

²²⁹ R. H. Neal and F. H. Luckmann, *Ind. Eng. Chem., Anal. Ed.*, *16*, 358–362 (1944).

²³⁰ J. B. Wilkie and J. B. De Witt, *J. Assoc. Official Agr. Chem.*, *28*, 174–186 (1945).

²³¹ R. A. Morton and A. L. Stubbs, *Analyst*, *71*, 348–356 (1946).

²³² R. A. Morton and A. L. Stubbs, *Biochem. J.*, *40*, lviii (1946); *42*, 195–203 (1948).

In the case of foods enriched with vitamin A, satisfactory results may be obtained by correction of the E (1%, 1 cm., 328 m μ) value with a blank obtained from an extract of the unenriched food.²³³ A fairly general procedure for the determination of vitamin A in food products is given by Oser *et al.*²³⁴

A more complete discussion of the spectrometric properties of vitamin A and of the carotenoids is given in Morton's monograph,⁷⁷ as well as in the review of Loofbourow,²²⁴ Recent developments in this field are included in the reviews of Morton,²³⁵ Hickman,¹⁸⁸ and Embree.²³⁶

(4) Reaction with Antimony Trichloride (Carr-Price Test)

One of the earliest recognized tests for vitamin A was the reaction between vitamin A and inorganic chlorides in anhydrous solvents. Under such conditions a development of color results. Of the inorganic chlorides, antimony trichloride has been used most effectively in an anhydrous chloroform solution. This reaction forms the basis for the widely employed Carr-Price method.¹⁵ A similar reaction is given by zinc chloride,¹⁴ arsenic chloride,¹⁴ ferric chloride,¹⁵ and stannic chloride.¹⁵ However, the latter salts have proved less satisfactory than the antimony trichloride for quantitative work, partly because of the rapidity with which the color fades.

Vitamin A₁ gives rise to two distinct spectral bands in the reaction with antimony trichloride. The principal one has a maximum absorption at 620 m μ , while the second, which is partially masked, shows an inflection in the curve at 583 m μ . Morton²³⁵ reports the value of the extinction E (1%, 1 cm.) at 617 m μ as 6000 \pm 200, while Baxter and Robeson³³ cite a value of 4800 at 622 m μ . Similarly, vitamin A₂ reacts with antimony trichloride to give two bands; the more effective one has a maximum extinction at 693 m μ , while the weaker band has a maximum absorption at 645 m μ .

The relative proportion of vitamins A₂ and A₁ can therefore be determined by the relationship between absorption at 693 and at 620 m μ after the addition of the antimony trichloride reagent. However, the weaker vitamin A₂-antimony trichloride band at 645 m μ extends as far as the 620 m μ area. Morton⁷⁷ assumes that the absorption at 620 m μ in the absence of vitamin A₁ is about one-seventh of that at 693 m μ . The following formula is proposed to calculate the ratio of A₂ to A₁ from the spectrophotometric data after the addition of antimony trichloride.

²³³ P. B. Hawk, B. L. Oser, and W. H. Summerson, *Practical Physiological Chemistry*, 12th ed., Blakiston, Philadelphia, 1947, p. 1047.

²³⁴ B. L. Oser, D. Melnick, and M. Pader, *Ind. Eng. Chem., Anal. Ed.*, 15, 724-729 (1943).

²³⁵ R. A. Morton, *Ann. Rev. Biochem.*, 11, 365-390 (1942).

²³⁶ N. D. Embree, *Ann. Rev. Biochem.*, 16, 323-358 (1947).

$$\frac{\text{concentration } A_2}{\text{concentration } A_1} = \frac{E (693 \text{ m}\mu)}{E (620 \text{ m}\mu) - \frac{1}{7} E (693 \text{ m}\mu)}$$

This overcomes the criticism of Lederer and Rathmann¹⁴⁷ as to the inaccuracy of determining vitamin A_2 simply on the basis of the ratio of E (693 $\text{m}\mu$) to E (620 $\text{m}\mu$) without correction of the 620 $\text{m}\mu$ value to allow for the effect of vitamin A_2 . Lederer, Verrier, Glaser, and Hüttner²³⁷ have shown that the natural color test inhibitors found in fatty acids cause a decreased absorption at 693 $\text{m}\mu$ without affecting the band at 645 $\text{m}\mu$ appreciably. However, the latter band may be displaced to 635 or 655 $\text{m}\mu$. Oxidation of the oil also lowers the 693 $\text{m}\mu$ band without affecting the 645 $\text{m}\mu$ band; the intensity of the 693 $\text{m}\mu$ band reappears later in full intensity. Morton⁷⁷ therefore recommends the application of the Carr-Price procedure for the determination of vitamin A_2 only when the non-saponifiable extracts are used. Although the carotenoids also react with antimony trichloride, the maximum value is considerably below that of vitamin A_1 , being at 590 $\text{m}\mu$. In determining the presence of vitamin A in a mixture of vitamin A and carotenoids, a correction for the E (620 $\text{m}\mu$) value can be made for carotene, based upon the colorimetric determination of this chromogen.

(5) Related Compounds Formed by Action of Acids on Vitamin A

Although the vitamins A are relatively stable toward alkalies, they are quite susceptible to inorganic acids. Because such acids develop a color with vitamin A, it has been proposed to use this reaction as a method for determining vitamin A.²³⁸ Thus, sulfuric acid, phosphoric acid,²³⁹ chloric acid,²⁴⁰ molybdenum phosphotungstic acid,²⁴¹ and trichloroacetic acid²⁴² have all been employed. They are sometimes used alone or in combination with a phenol.

a. Anhydrovitamin A. When vitamin A is treated with mineral acids, a transformation in the molecule results which is associated with a change in absorption spectrum.^{27, 128, 189, 243} The resulting product has been referred to as "spurious vitamin A," "cyclized" vitamin A, and anhydrovitamin A.^{128, 244} Undoubtedly, the last designation is the most appropriate.

²³⁷ E. Lederer, M. L. Verrier, R. Glaser, and M. C. Hüttner, *Bull. soc. chim. biol.*, 21, 629-648 (1939).

²³⁸ H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1942.

²³⁹ E. Kobayashi and K. Yamamoto, *J. Soc. Chem. Ind. Japan*, 27, 1060-1067 (1924).

Cited by H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, p. 80.

²⁴⁰ A. E. Pacini and M. H. Taras, *J. Am. Pharm. Assoc.*, 26, 721-723 (1937).

²⁴¹ N. Bezssonoff, *Bull. soc. chim. biol.*, 11, 294-307 (1929).

²⁴² W. R. Fearon, *Biochem. J.*, 19, 888-895 (1925).

²⁴³ D. C. Castle, A. E. Gillam, I. M. Heilbron, and H. W. Thompson, *Biochem. J.*, 28, 1702-1711 (1934).

²⁴⁴ N. D. Embree, *J. Biol. Chem.*, 128, 187-198 (1939).

Further light on the structure of anhydrovitamin A is afforded by the synthesis of an unsaturated hydrocarbon related to vitamin A, by Karrer *et al.*^{248,249} The compound was prepared by synthesis or by partial hydrogenation of anhydrovitamin A.

(b) *Properties of Anhydrovitamin A.* The most characteristic property of anhydrovitamin A is its absorption spectrum. In contradistinction to the single maximum absorption peak of either vitamin A₁ or vitamin A₂, anhydrovitamin A shows 3 or 4 maxima which have a much sharper structure. Although a number of values for absorption maxima have been

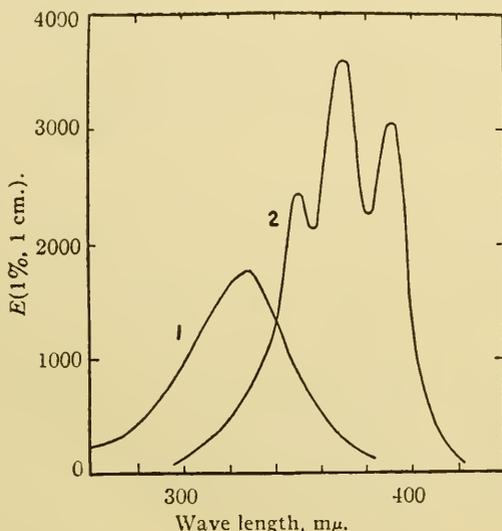


Fig. 8. Absorption spectra of: (1) vitamin A, and (2) anhydrovitamin A in absolute ethyl alcohol.²⁴⁵

recorded,^{77,140,159,243} the values obtained on the crystalline product by Shantz *et al.*²⁴⁵ are probably the most reliable. These absorption maxima are 351, 371, and 392 $m\mu$, and the corresponding E (1%, 1 cm.) values are 2500, 3650, and 3180, respectively. The absorption pattern for anhydrovitamin A is given in Figure 8.

The band at 392 $m\mu$ is always well defined, but the maximum at 351 $m\mu$ may be a sharp peak or only a marked inflection. Anhydrovitamin A reacts with antimony trichloride to give a peak having a maximum at the same wave length as that of vitamin A (620 $m\mu$). The extinction value, E (1%, 1 cm.), of the anhydrovitamin A-antimony trichloride product is

²⁴⁸ P. Karrer and R. Schwyzer, *Helv. Chim. Acta*, 31, 1055-1062 (1948).

²⁴⁹ P. Karrer and J. Benz, *Helv. Chim. Acta*, 31, 1048-1054 (1948).

5500,²⁴⁵ corresponding to a value of 4800³³ shown by vitamin A₁ and antimony trichloride. Crystalline anhydrovitamin A melts at 76–77°C.

Anhydrovitamin A₁ is quite stable in oil solutions, but purified concentrates and crystalline preparations deteriorate quite rapidly, even when kept at –35°C. When anhydrovitamin A is allowed to stand in solvents for any length of time, a pale yellow, rubbery precipitate is deposited which is probably a polymerization product. A yield of 50% of the theoretical amount of this material results, in 48 hours, from a petroleum ether solution of anhydrovitamin A which has been standing at room temperature. Anhydrovitamin A may be distilled in the molecular still, where it exhibits an elimination maximum 19° lower than that characteristic of vitamin A alcohol.

Anhydrovitamin A₂ shows the same absorption peaks as does anhydrovitamin A₁.^{59,140} However, these anhydrovitamins are probably not identical, since they are adsorbed with different affinities on a chromatographic column.

(c) *Distribution of Anhydrovitamin A.* Anhydrovitamin A is believed to occur naturally in some fish oils. Castle *et al.*²⁴³ obtained a yellow substance from halibut oil with an ultraviolet absorption spectrum similar to that given by cyclized vitamin A, while Heilbron *et al.*²⁷ found that the spectra of most volatile fractions of a prolonged vacuum distillation had a fine structure which followed a similar spectrometric pattern. Pritchard *et al.*¹⁸⁹ demonstrated that, on fractionation of a mammalian liver concentrate, the portion insoluble in 83% alcohol had slight inflections which suggested the presence of the anhydrovitamin A. Embree²⁴⁴ prepared a typical sample of this product from tuna liver oils in the low-boiling fractions distilled in the molecular still. Moreover, on molecular distillation of samples of halibut, pollack, and cod liver oils, anhydrovitamin A was found in the lowest boiling fractions to the extent of about 2%.

(d) *Vitamin A Activity of Anhydrovitamin A.* The early results of Embree,²⁴⁴ obtained on crude concentrates, indicated that anhydrovitamin A possesses no detectable vitamin A activity. However, the crystalline product investigated by Shantz *et al.*²⁴⁵ was shown to have a biological potency equivalent to 17,500 U.S.P. units per gram, which is 0.4% that of vitamin A. Samples of anhydrovitamin A, prepared in such a manner as to preclude the presence of vitamin A, assayed at 15,200 and 16,900 U.S.P. units per gram.²⁴⁵ Anhydrovitamin A is apparently not converted to vitamin A under such conditions but to a vitamin A isomer, *rehydrovitamin A*, which is stored in the liver as an ester.^{250,251}

b. Rehydrovitamin A. According to the recent work of Shantz,²⁵¹

²⁵⁰ E. M. Shantz, Report of the 114th Meeting of the American Chemical Society, Washington, D. C., 1948, Abstracts, pp. 16c–17c.

²⁵¹ E. M. Shantz, *J. Biol. Chem.*, 182, 515–524 (1950).

anhydrovitamin A, but is incapable of shifting the double bonds to their normal position. Thus, only a partial return to the normal vitamin A structure is possible, and merely a small degree of vitamin A activity results.

Rehydrovitamin A has two definite absorption maxima, at 351 and at 369 $m\mu$, and an inflection at 331 $m\mu$. A comparison of the absorption curves of anhydrovitamin A, rehydrovitamin A, and vitamin A is given in Figure 9.

c. Isoanhydrovitamin A. When anhydrovitamin A is allowed to stand for prolonged periods with alcoholic hydrochloric acid, the characteristic bands of anhydrovitamin A disappear and are replaced by new ones having maxima at 330, 350, and 370 $m\mu$.^{184,245} Although the absorption curve of isoanhydrovitamin A in the ultraviolet and that of rehydrovitamin A are quite similar, Shantz²⁵¹ believes that the differences in other physical properties preclude the possibility that the two compounds are identical.

d. Subvitamin A. Subvitamin A¹⁸⁴ is another vitamin derivative which is responsible for the distortion of the vitamin A absorption curve on the short wave length side of 328 $m\mu$. Embree and Shantz¹⁸⁴ suggested that subvitamin A is probably an oxygenated derivative of vitamin A, while Hawkins and Hunter¹⁸⁵ claim that it is vitamin A in which the double bond in the β -ionone ring has been oxidized. It possesses no biological activity.

CHAPTER VIII

DISTRIBUTION, PROPERTIES, AND CHEMISTRY OF THE PROVITAMINS D AND VITAMINS D

A. INTRODUCTION

There are many similarities between the vitamins D and the vitamins A. The most important one is the fact that both are found in the plant kingdom in the form of inactive precursors, while they are present as such only in animal products. A provitamin D compound, such as 7-dehydrocholesterol, may occur in the animal body, just as a provitamin A, *i.e.*, β -carotene, may be present in animal tissues. However, in the former case, the provitamin D may be synthesized by the animal, but it is not believed that any provitamin A can be produced *de novo*; such a provitamin A must have its origin in the diet.

Moreover, the animal possesses the capacity to transform the provitamins D to their active forms, just as it can change the provitamins A to vitamin A. However, the methods by which these reactions are brought about are quite distinct for the two provitamins. In the case of provitamin D, the activation can be effected by ultraviolet radiation of definite frequency, whereas a physical action of this nature causes destruction of the provitamins A, as well as of the active forms of vitamin A. It is not known at present what causes the provitamins A to be converted to vitamin A, although it is presumed that one or more enzyme systems may be responsible for the reaction.

Another difference between provitamins A and D is in their activation *in vitro*. While it is possible to convert a provitamin D very effectively to the corresponding vitamin D outside the body by irradiation and by other physical means, no physical or chemical methods are at present available for changing the provitamins A to their active forms.

Vitamin A and vitamin D are frequently, although not always, found associated in animal fats. For this reason, the recognition that vitamin A and vitamin D are two separate and distinct vitamins was long delayed. Cod liver oil, which cures xerophthalmia, is also known to be an especially effective medicinal agent for the treatment of rickets. When these fat-soluble vitamins are concentrated from cod liver oil by saponification, both

vitamins A and D are present in the non-saponifiable extract. However, because of the fact that the antixerophthalmic action of the non-saponifiable extract can be destroyed when the product is oxidized, while the antirachitic effect is preserved under such circumstances, it was first recognized that two distinct fat-soluble vitamins must occur.¹

As in the case of several other vitamins, the disease associated with a dietary vitamin D deficiency was known long before the vitamin itself was recognized. Trousseau,² in 1873, recommended cod liver oil for the treatment of rickets, while Hopkins^{3,4} and later Funk⁵ concluded that rickets was caused by the absence of an "accessory foodstuff" or a vitamin, as it was referred to later by Funk. Mellanby^{6,7} produced rickets in dogs experimentally and found that the condition could be cured by animal fats. He believed that the curative factor was "fat-soluble A," or that its distribution was similar to that of the latter vitamin. In 1922, Zucker and his co-workers⁸ demonstrated for the first time that the antirachitic factor in fish liver oils was concentrated in the non-glyceride portion of the fat, namely, in the unsaponifiable fraction. Recognition of the distribution of vitamin D was greatly aided by the fact that rickets could be produced experimentally in rats. The dietary procedures necessary to elicit rickets had been worked out just before this time by Sherman and Pappenheimer^{9,10} as well as by the brilliant work of McCollum and his associates.¹¹⁻¹⁵ The first phase in the discovery of the chemical nature of vitamin D was terminated by the demonstration that it is a vitamin entirely separate from vitamin A, which occurs as a component of the non-saponifiable residue.¹

¹ E. V. McCollum, N. Simmonds, J. E. Becker, and P. G. Shipley, *J. Biol. Chem.*, **53**, 253-312 (1922).

² A. Trousseau, *Lectures on Clinical Medicine*, Blakiston, 1873, Vol. II, p. 734 (translated by J. R. Cormack and P. V. Bazire from the 3rd ed., *Clinique médicale de l'Hotel Dieu de Paris*, Baillière, Paris, 1868).

³ F. G. Hopkins, *Analyst*, **31**, 385-404 (1906).

⁴ F. G. Hopkins, *J. Physiol.*, **44**, 425-460 (1912).

⁵ C. Funk, *Die Vitamine*, Wiesbaden, 1914.

⁶ E. Mellanby, *J. Physiol.*, **52**, liii-liv (1919).

⁷ E. Mellanby, *Lancet*, **196**, I, 407-412 (1919).

⁸ T. F. Zucker, A. M. Pappenheimer, and M. Barnett, *Proc. Soc. Exptl. Biol. Med.* **19**, 167-169 (1922).

⁹ H. C. Sherman and A. M. Pappenheimer, *Proc. Soc. Exptl. Biol. Med.*, **18**, 193-197 (1921).

¹⁰ H. C. Sherman and A. M. Pappenheimer, *J. Exptl. Med.*, **34**, 189-198 (1921).

¹¹ E. V. McCollum, N. Simmonds, H. T. Parsons, P. G. Shipley, and E. A. Park, *J. Biol. Chem.*, **45**, 333-341 (1920-1921).

¹² P. G. Shipley, E. A. Park, E. V. McCollum, N. Simmonds, and H. T. Parsons, *J. Biol. Chem.*, **45**, 343-348 (1920-1921).

¹³ E. V. McCollum, N. Simmonds, P. G. Shipley, and A. E. Park, *J. Biol. Chem.*, **47**, 507-527 (1921).

¹⁴ P. G. Shipley, E. V. McCollum, and N. Simmonds, *J. Biol. Chem.*, **49**, 399-410 (1921).

¹⁵ E. V. McCollum, N. Simmonds, P. G. Shipley, and E. A. Park, *J. Biol. Chem.*, **50**, 5-30, vi (1922).

Coincident with the development of our knowledge of the chemical nature of the antirachitic factor, reports were made as to the therapeutic action of sunlight as a curative agent for rickets. Sniadecki¹⁶ early inferred that sunlight constituted an effective treatment for rickets. In 1890, Palm,¹⁷ on the basis of geographical studies, correlated the incidence of rickets with the degree of sunlight. Cases of human rickets were cured by artificial light, according to Buchholz,¹⁸ while the influence of sunlight on the calcium assimilation of puppies was demonstrated in 1913 by Raczyński.¹⁹ The classical work in this field was carried out in 1919, when Huldshinsky^{20,21} proved, on the basis of x-ray studies, that severe rickets in children could be cured by irradiation with the quartz mercury vapor lamp. Shortly thereafter, Hess and Unger²² confirmed the report of Huldshinsky, and also demonstrated the effectiveness of sunlight in preventing rickets in rats on a rickets-producing diet.²³ In 1922, Hess and Gutman²⁴ came to the conclusion that the curative action brought about by cod liver oil and by irradiation is essentially the same.

The reason for the effectiveness of the two methods for the treatment of rickets became evident with the simultaneous discovery in 1924 by Steenbock and his associates²⁵⁻²⁷ and by Hess and co-workers²⁸⁻³¹ that antirachitic potency can be conferred upon foods by ultraviolet irradiation. It was realized almost immediately that the precursor of the antirachitic vitamin belongs in the category of provitamins. The active principle was found shortly thereafter to be a sterol-like compound³²⁻³⁴ which, before activation, had no rickets-preventing potency. During the next year

¹⁶ J. Sniadecki, *On the Physical Education of Children*, Wilno, 1822. Cited by W. Mozolowski, *Nature*, 143, 121 (1939).

¹⁷ T. A. Palm, *Practitioner*, 45, 271-279, 321-342 (1890).

¹⁸ E. Buchholz, *Verhandl. 21 ten Versamml. Gesell. Kinderhkl., Abt. Kinderhkl., 76ten Versamml. deut. Naturforsch., Aerzte, Breslau, 1904, 21*, 116-122 (1905), Wiesbaden.

¹⁹ J. Raczyński, *Compt. rend. assoc. intern. pediat.*, 1913, 308. Cited by H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, p. 342.

²⁰ K. Huldshinsky, *Deut. med. Wochschr.*, 45, 712-713 (1919).

²¹ K. Huldshinsky, *Z. orthop. Chim.*, 39, 426-451 (1919-1920).

²² A. F. Hess and L. J. Unger, *Proc. Soc. Exptl. Biol. Med.*, 18, 298 (1921).

²³ A. F. Hess, L. J. Unger, and A. W. Pappenheimer, *Proc Soc. Exptl. Biol. Med.*, 19, 8-12 (1921-1922).

²⁴ A. F. Hess and M. G. Gutman, *J. Am. Med. Assoc.*, 78, 29-31 (1922).

²⁵ H. Steenbock, *Science*, 60, 224-225 (1924).

²⁶ H. Steenbock and A. Black, *J. Biol. Chem.*, 61, 405-421 (1924).

²⁷ H. Steenbock and M. T. Nelson, *J. Biol. Chem.*, 62, 209-216 (1924).

²⁸ A. F. Hess, *Am. J. Diseases Children*, 28, 517-521 (1924).

²⁹ A. F. Hess and M. Weinstock, *Proc. Soc. Exptl. Biol. Med.*, 22, 5-6 (1924-1925).

³⁰ A. F. Hess and M. Weinstock, *Proc. Soc. Exptl. Biol. Med.*, 22, 6-7 (1924-1925).

³¹ A. F. Hess and M. Weinstock, *J. Biol. Chem.*, 62, 301-313 (1924).

³² A. F. Hess, M. Weinstock and F. D. Helman, *J. Biol. Chem.*, 63, 305-308 (1925).

³³ H. Steenbock and A. Black, *J. Biol. Chem.*, 64, 263-298 (1925).

³⁴ O. Rosenheim and T. A. Webster, *Lancet*, 1925, 1, 1025-1026.

(1925), the antirachitic vitamin,³⁵ which up to this time had no satisfactory name, was christened "vitamin D" by McCollum.³⁶

Cholesterol was ultimately shown to be devoid of provitamin D activity; the positive results obtained with impure cholesterol were ascribed to some impurity activated by ultraviolet irradiation.³⁷⁻⁴² Almost simultaneously several groups of workers came to the conclusion that the provitamin D is ergosterol or a sterol with a constitution similar to that of dehydrocholesterol.⁴³⁻⁴⁷

Crystalline vitamin D was isolated initially by Reerink and van Wijk^{48,49} in 1929; however, Linsert^{50,51} is generally given credit for being the first to obtain a pure preparation of vitamin D.

The multiple nature of vitamin D soon became evident with the demonstration⁵² of a new provitamin D, 22-dihydroergosterol, followed shortly thereafter by the synthesis of 7-dehydrocholesterol⁵³ and its isolation from hog skin.⁵⁴ It was found that the vitamin D obtained from tuna and hali-but oils as a crystalline ester consisted almost entirely of activated 7-dehydrocholesterol.⁵⁵⁻⁵⁹ In 1937 Schenck⁶⁰ succeeded in preparing the pure vitamin (D₃) from irradiated 7-dehydrocholesterol.

³⁵ E. V. McCollum, N. Simmonds, J. E. Becker, and P. G. Shipley, *Bull. Johns Hopkins Hosp.*, **33**, 229 (1922).

³⁶ E. V. McCollum, N. Simmonds, J. E. Becker, and P. G. Shipley, *J. Biol. Chem.*, **65**, 97-100 (1925).

³⁷ F. W. Schlutz and M. Morse, *Am. J. Diseases Children*, **30**, 199-209 (1925).

³⁸ O. Rosenheim and T. A. Webster, *J. Soc. Chem. Ind.*, **45**, 932 (1926).

³⁹ O. Rosenheim and T. A. Webster, *Biochem. J.*, **21**, 127-129 (1927).

⁴⁰ I. M. Heilbron, E. D. Kamm, and R. A. Morton, *J. Soc. Chem. Ind.*, **45**, 932 (1926).

⁴¹ I. M. Heilbron, E. D. Kamm, and R. A. Morton, *Biochem. J.*, **21**, 78-85 (1927).

⁴² R. Pohl, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 142-145 (1926).

⁴³ R. Pohl, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 185-191 (1926).

⁴⁴ A. Windaus and A. F. Hess, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 175-184 (1926).

⁴⁵ A. Windaus and F. Holtz, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 217-225 (1927).

⁴⁶ O. Rosenheim and T. A. Webster, *Lancet*, 1927, **I**, 306-307.

⁴⁷ O. Rosenheim and T. A. Webster, *Biochem. J.*, **21**, 389-397 (1927).

⁴⁸ E. H. Reerink and A. van Wijk, *Biochem. J.*, **23**, 1294-1307 (1929).

⁴⁹ E. H. Reerink and A. van Wijk, *Biochem. J.*, **25**, 1001-1010 (1931).

⁵⁰ O. Linsert, annotation in *Ann.*, **489**, 269 (1931).

⁵¹ A. Windaus, O. Linsert, A. Lüttringhaus, and G. Weidlich, *Ann.*, **492**, 226-241 (1931).

⁵² A. Windaus and R. Langer, *Ann.*, **508**, 105-114 (1933).

⁵³ A. G. Boer, E. H. Reerink, A. van Wijk, and J. van Niekerk, *Proc. Acad. Sci. Amsterdam*, **39**, 622-632 (1936); *Chem. Abst.*, **30**, 5636 (1936).

⁵⁴ A. Windaus and F. Bock, *Z. physiol. Chem.*, **245**, 168-170 (1937).

⁵⁵ H. Brockmann, *Z. physiol. Chem.*, **241**, 104-115 (1936).

⁵⁶ H. Brockmann, *Z. physiol. Chem.*, **245**, 96-102 (1937).

⁵⁷ H. Brockmann and A. Busse, *Z. physiol. Chem.*, **249**, 176-180 (1937).

⁵⁸ E. J. H. Simons and T. F. Zucker, *J. Am. Chem. Soc.*, **58**, 2655 (1936).

⁵⁹ G. A. D. Haslewood and J. C. Drummond, *Chemistry & Industry*, **14** (*J. Soc. Chem. Ind.*), **55**, 598-599 (1936).

⁶⁰ F. Schenck, *Naturwissenschaften*, **25**, 159 (1937).

The terminology introduced by Askew and co-workers⁶¹⁻⁶⁴ and by Windaus *et al.*^{51,65,66} has persisted in the literature until the present. Two active fractions were obtained by distillation of irradiated ergosterol. The first of these, which Windaus called vitamin D₁, was later shown by the same worker, in collaboration with Dithmar and Fernholz,⁶⁷ to be impure; it consisted of the active vitamin D with an inactive decomposition product, lumisterol. Askew also found that one of his fractions consisted of the active vitamin D₁, *calciferol*, combined with two inactive components, one of which was lumisterol. As a result of this confusion, Windaus suggested the retention of the designation, vitamin D₂, for the active vitamin D obtained by irradiation of ergosterol; it was recognized that this is identical with calciferol. No one has suggested a revision in the nomenclature since that time, and the D vitamins consist of a group of substances in which the first member, vitamin D₁, does not exist. The irradiation product of 7-dehydrocholesterol⁶⁸ has been termed vitamin D₃ by the Windaus group.⁶⁹ The activated product obtained by Windaus and Langer,⁵² from 22-dihydroergosterol, after synthesis of the latter, has been designated as vitamin D₄.

As early as 1935, Bills⁷⁰ recognized that vitamin D exists in multiple forms. The figure of six different active compounds which was first proposed⁷⁰ was later changed to eight.⁷¹ The fact that these are frequently associated was demonstrated by the results of Bills *et al.*⁷² obtained with a variety of fish liver oils, which were found to have widely varying potencies when tested on rats or on chickens.

It is now generally recognized that a number of additional products can be included in the category of provitamins D, although none of the activated forms have potencies approaching those exhibited by vitamins D₂ or D₃, or even by vitamin D₄. The added provitamins D include 7-dehydrostosterol,⁷³ 7-hydroxycholesterol, and 7-dehydrocampesterol, which has

⁶¹ F. A. Askew, R. B. Bourdillon, H. M. Bruce, R. G. C. Jenkins, and T. A. Webster, *Proc. Roy. Soc. London*, B107, 76-90 (1930).

⁶² F. A. Askew, R. B. Bourdillon, H. M. Bruce, R. G. C. Jenkins, and T. A. Webster, *Proc. Roy. Soc. London*, B107, 91-100 (1930).

⁶³ T. C. Angus, F. A. Askew, R. B. Bourdillon, H. M. Bruce, R. K. Callow, C. Fischermann, J. S. L. Philpot, and T. A. Webster, *Proc. Roy. Soc. London*, B108, 340-359 (1931).

⁶⁴ F. A. Askew, R. B. Bourdillon, H. M. Bruce, R. K. Callow, J. S. L. Philpot, and T. A. Webster, *Proc. Roy. Soc. London*, B109, 488-506 (1932).

⁶⁵ A. Windaus, A. Lüttringhaus, and M. Deppe, *Ann.*, 489, 252-269 (1931).

⁶⁶ A. Windaus, F. v. Werder, and A. Lüttringhaus, *Ann.*, 499, 188-200 (1932).

⁶⁷ A. Windaus, K. Dithmar, and E. Fernholz, *Ann.*, 493, 259-271 (1932).

⁶⁸ A. Windaus, H. Lettré, and F. Schenck, *Ann.*, 520, 98-106 (1935).

⁶⁹ A. Windaus, F. Schenck, and F. v. Werder, *Z. physiol. Chem.*, 241, 101-103 (1936).

⁷⁰ C. E. Bills, *Cold Spring Harbor Symposia Quant. Biol.*, 3, 328-340 (1935).

⁷¹ C. E. Bills, Paper read at convention of the American Medical Association, May 14, 1936. Cited by C. E. Bills *et al.*, *J. Nutrition*, 13, 435 (1937).

⁷² C. E. Bills, O. N. Massengale, M. Imboden, and H. Hall, *J. Nutrition*, 13, 435-452 (1937).

⁷³ W. Wunderlich, *Z. physiol. Chem.*, 241, 116-124 (1936).

recently been prepared by Ruigh.⁷⁴ Campesterol is a C₂₈ steroid which was originally isolated by Fernholz and MacPhillamy.⁷⁵ Activated 7-dehydrostigmasterol, which differs from ergosterol only by having an ethyl group on the C₂₄ atom in place of the methyl group, is practically devoid of vitamin D activity,⁷⁶ although it is frequently considered to be in this category.⁷⁷ Moreover, the provitamin D activity is not abolished by conversion of the Δ^{22} -ethenoid linkage of ergosterol into the oxide nor by epimerization of the hydroxyl group on the third carbon. Thus, both ergosterol-22-oxide⁷⁸ and epi-ergosterol have been shown to be active provitamins D. This is also true for irradiated epi-7-dehydrocholesterol, prepared from epi-cholesterol, which has been found to have one-tenth the activity of vitamin D₃.⁷⁹ Lumisterol, an irradiation product of ergosterol, has also been reported to have a positive provitamin D activity,⁸⁰ while another synthetic product, dihydrotachysterol or A.T.10 (antitetrany substance No. 10—see pages 767, 768), possesses some antirachitic action without further activation.⁸¹ It has been claimed that mussel provitamin D produces a vitamin D with a potency as great as that of vitamins D₂ or D₃;⁸² its structure is not as yet known. Slight provitamin D activity has also been ascribed to $\Delta^{5,7}$ -androstadiene-3,17-diol^{78,83} and to 3-hydroxy-5,7-choladienic acid.⁸⁴

Although it would appear that most of the natural forms of the vitamins D have been identified, it seems quite probable that many synthetic provitamins D and vitamins D will come to our attention in the future. The historical background of the rickets problem was reviewed by Park,⁸⁵ while Bills⁸⁶ has considered the subject of vitamin D from the historical standpoint. The latter review includes an excellent bibliography up to 1935. Reed *et al.*⁸⁷ also published a monograph on vitamin D.

B. PROVITAMINS D

The provitamins D are considered to be those compounds which, when subjected to a satisfactory procedure for their activation, are converted

⁷⁴ W. L. Ruigh, *J. Am. Chem. Soc.*, **64**, 1900–1902 (1942).

⁷⁵ E. Fernholz and H. B. MacPhillamy, *J. Am. Chem. Soc.*, **63**, 1155–1156 (1941).

⁷⁶ O. Linsert, *Z. physiol. Chem.*, **241**, 125–128 (1936).

⁷⁷ H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945.

⁷⁸ K. Dimroth and J. Paland, *Ber.*, **72**, 187–190 (1939).

⁷⁹ A. Windaus and J. Naggatz, *Ann.*, **542**, 204–218 (1939).

⁸⁰ I. M. Heilbron and E. R. H. Jones, *Ann. Rev. Biochem.*, **9**, 135–172 (1940).

⁸¹ F. v. Werder, *Z. physiol. Chem.*, **260**, 119–134 (1939).

⁸² A. G. Boer, J. van Nickerk, E. H. Reerink, and A. van Wijk, *U. S. Patent No.* 2,163,659 (June 27, 1939).

⁸³ A. Butenandt, E. Hausmann, J. Paland, D. v. Dresler, and U. Meinerts, *Ber.*, **71**, 1316–1322 (1938).

⁸⁴ G. A. D. Haslewood, *Biochem. J.*, **33**, 454–456 (1939).

⁸⁵ E. A. Park, *Physiol. Revs.*, **3**, 106–163 (1923).

⁸⁶ C. E. Bills, *Physiol. Revs.*, **15**, 1–97 (1935).

⁸⁷ C. I. Reed, H. C. Struck, and I. E. Stack, *Vitamin D*, Univ. Chicago Press, 1939.

into the corresponding form of vitamin D. All of the provitamins which are known at present are cyclopentanophenanthrene derivatives containing the steroid nucleus. All have an hydroxy group in position 3, which is usually esterified in the natural product; they have 2 double bonds in conjugate positions in ring B at C₅-C₆ and C₇-C₈. The hydrocarbon chain on C₁₇ is of great importance in establishing the nature of the vitamin D activity. Without such a side chain, the molecule cannot be changed to an active vitamin D. Moreover, slight changes in the nature of the side chain will greatly alter the physiological response of the resultant vitamin D formed from the provitamin D. Vitamin D₂ made from ergosterol, which has the side chain $-\text{CH}(\text{CH}_3)\text{CH}:\text{CHCH}(\text{CH}_3)\text{CH}(\text{CH}_2)_2$, on C₁₇, has only a low percentage of the vitamin D activity in chickens that is possessed by vitamin D₃, in which the side chain is $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$. The saturation of the double bond on carbons 22 and 23 in ergosterol results in a reduction of the potency of the resultant vitamin to 10% of its original level.

When the provitamins D are activated by any one of the various procedures into the active forms of vitamin D, the invariable change involves the rupture of the B ring of the steroid nucleus between carbons 9 and 10. The methyl group attached to C₁₀ is changed to a methylene group, while the extra hydrogen thus rendered available is used to saturate C₉.

1. Occurrence of the Provitamins D

(1) Ergosterol

Ergosterol, or provitamin D₂, was discovered by Tanret⁸⁸ in the sclerotium of the parasitic fungus ergot (*Claviceps purpurea*), in 1879. This investigator named the compound *ergotinine*, and later *ergostérine*.⁸⁹ He reported that it differs in composition from cholesterol and the phytosterols.⁹⁰ Gérard,⁹¹⁻⁹⁴ after examination of the sterols from a number of plants, was able to demonstrate that a taxonomic relationship exists in the distribution of ergosterol in nature. Ergosterol was shown to be characteristic of the cryptogams, just as the phytosterols are the prevailing sterols in the phanerogams (see Chapter IV).

Ergosterol is present in lower plants such as brown algae, slime fungi, bacteria (*Staphylococcus* spp.), *Mucor* spp., yeast, *Penicillium* spp., and

⁸⁸ C. Tanret, *Ann. chim. phys.* [5], 17, 493-512 (1879).

⁸⁹ C. Tanret, *Compt. rend.*, 108, 98-100 (1889).

⁹⁰ C. Tanret, *Ann. chim. phys.* [6], 20, 289-297 (1890).

⁹¹ E. Gérard, *Compt. rend.*, 114, 1544-1546 (1892).

⁹² E. Gérard, *Compt. rend.*, 121, 723-726 (1895).

⁹³ E. Gérard, *J. pharm. chim.* [6], 1, 601-608 (1895).

⁹⁴ E. Gérard, *Compt. rend.*, 126, 909-911 (1898).

lichens.^{86,95} It is almost certain that the mushroom substance investigated many years ago by Braconnot,⁹⁶ Vauquelin,⁹⁷ and Gobley⁹⁸ was ergosterol. Zellner⁹⁹ reported a number of additional varieties of mushrooms which contain ergosterol. The most practical source of this sterol is dried yeast,¹⁰⁰ in which ergosterol makes up 90 to 100% of the total sterols.⁷⁷ Ergosterol may also be found in some of the higher plant forms. Thus, it has been reported to comprise 5% of the non-saponifiable fraction of cottonseed oil, while its proportion of the total sterols in *Scopolia* root amounts to 1.4%.⁷⁷

Although the main sources of ergosterol are undoubtedly the cryptogams, there are well-authenticated instances in which it is found in the animal. It has been proved that ergosterol comprises 19 to 25% of the sterols in the red snail (*Arion empiricorium*),^{101,102} while 22% of the sterol content of the earthworm (*Lumbricus terrestris*) has been found to consist of this sterol. Windaus and Stange¹⁰³ also noted that ergosterol is to be found in the hen egg, although it is believed that its presence in this source is a reflection of the ergosterol which has been ingested. A similar explanation has been advanced to explain the presence of vitamin D₂ in eggs, inasmuch as this is not the natural form of vitamin D in this species. Bethke and co-workers^{104,105} noted that vitamin D may be found in the tissues and egg-yolks of hens fed irradiated ergosterol or cod liver oil, the type of vitamin D found depending upon that present in the feed. The same variations were also reported in milk, coincident with variations in the type of vitamin D in the diet.¹⁰⁶ Small amounts of vitamin D₂ have been isolated from fish liver oils by Brockmann and Busse,¹⁰⁷ although the chief vitamin here is vitamin D₃.

(2) 7-Dehydrocholesterol

7-Dehydrocholesterol is the most prevalent provitamin D in the higher animals and man. Boer and co-workers⁵³ were the first to demonstrate the presence of this sterol as a contaminant in cholesterol of unknown origin. That it is a naturally occurring product is indicated by the results of

⁹⁵ C. Tanret, *Ann. chim. phys.* [8], 15, 313-330 (1908).

⁹⁶ H. Braconnot, *Ann. chim. phys.* [1], 79, 265-304 (1811).

⁹⁷ M. Vauquelin, *Ann. chim. phys.* [1], 85, 5-25 (1813).

⁹⁸ M. Gobley, *J. pharm. chim.* [3], 29, 81-91 (1856).

⁹⁹ J. Zellner, *Chemie der höheren Pilze*, Engelmann, Leipzig, 1907.

¹⁰⁰ C. Tanret, *Compt. rend.*, 147, 75-77 (1908).

¹⁰¹ A. Windaus, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 185-192 (1936).

¹⁰² F. Bock and F. Wetter, *Z. physiol. Chem.*, 256, 33-41 (1938).

¹⁰³ A. Windaus and O. Stange, *Z. physiol. Chem.*, 244, 218-220 (1936).

¹⁰⁴ R. M. Bethke, P. R. Record, C. H. Kirk, and D. C. Kennard, *Poultry Sci.*, 15, 326-335 (1936).

¹⁰⁵ R. M. Bethke, P. R. Record, O. H. M. Wilder, and C. H. Kirk, *Poultry Sci.*, 15, 336-344 (1936).

¹⁰⁶ R. M. Bethke, W. E. Krauss, P. R. Record, and O. H. M. Wilder, *J. Nutrition*, 11, 21-30 (1936).

¹⁰⁷ H. Brockmann and A. Busse, *Z. physiol. Chem.*, 256, 252-270 (1938).

Windaus and Bock,⁵⁴ who were able to separate it from pigskin, where it makes up 3 to 6% of the total sterols. Just how far down the scale of the

TABLE 1
PROVITAMIN D CONTENT OF VARIOUS ANIMAL AND PLANT MATERIALS^a

Animal	Tissue	Provitamin D in sterols, %
Vertebrata		
Man	Skin	0.15-0.43
Cattle	Skin	0.18
	Pancreas	0.18
Cow	Blood serum	0.15
	Brain	0.01
	Placenta	0.18
Calf	Spleen	0.045
	Skin	0.68
	Heart	0.032
Mice	Lung	0.025
	Skin	0.87
Chicken	Feet	1.0-4.0
	Trunk	0.001-0.01
Animal		Provitamin D in sterols, %
Invertebrata		
Lugworm (<i>Arenicola marina</i>)		4-12
Mussels (<i>Mytilus edulis</i>)		9-10
Oysters (<i>Ostrea spp.</i>)		5-6
Leeches (<i>Hirudinea spp.</i>)		4
Crabs (<i>Cancer pagurus</i>)		0.32
Plant sources		
Plant	Provitamin D in sterols, %	
Sea anemones	2-10	
Orchard grass	0.80	
Rye grass	1.5	
Wheat germ oil	0.8	
Seaweed	0.008	
Cabbage	0.05	
Spinach	1.0	
String beans	0.1	

^a Adapted from H. R. Rosenberg, *The Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, p. 347.

animal kingdom it is to be found is uncertain; however, its appearance may coincide with that of ergosterol, since one species of whelk, *Buccinum undatum*, contains this provitamin.^{101,102}

Ergosterol and 7-dehydrocholesterol are the only provitamins D which occur as such in nature. Considerable amounts of the provitamins have been demonstrated in the sterols of various vertebrates, invertebrates, and plants, but the nature of the compound is uncertain. These sterols are listed in Table 1, which has been assembled by Rosenberg.⁷⁷

2. Properties of the Provitamins D

All of the provitamins are characterized by solubility in fat solvents and insolubility in water. They can be readily crystallized from the lower

TABLE 2
ABSORPTION CHARACTERISTICS OF PROVITAMINS D₂ AND D₃ IN ETHANOL^{a, b}

Absorption maxima and minima		
λ , A. ^a	ϵ^a	E (1%, 1 cm.) ^b
Ergosterol (provitamin D ₂)		
Min. 2300	1,430	36.1
Max. 2620	6,940	175
Min. 2630	6,850	173
Max. 2710	10,000	253
Min. 2755	8,580	217
Max. 2820	10,600	268
Min. 2890	5,450	138
Max. 2930	6,060	153
Min. 3175	35	0.8
7-Dehydrocholesterol (provitamin D ₃)		
Min. 2300	1,500	39.1
Max. 2625	7,400	193
Max. 2710	10,400	271
Min. 2760	8,830	230
Max. 2815	10,750	280
Min. 2880	5,530	144
Max. 2930	6,150	160
Max. 3210	36	0.9

^a T. R. Hogness, A. E. Sidwell, Jr., and F. P. Zscheile, Jr., *J. Biol. Chem.*, 120, 239-256 (1937), p. 242.

^b J. R. Loofbourow, *Vitamins and Hormones*, 1, 109-155 (1943), p. 118.

alcohols, from which they separate on the addition of a molecule of water or solvent of crystallization.

The provitamins ergosterol, 7-dehydrocholesterol, 7-dehydrositosterol, and 7-dehydrostigmasterol produce almost identical absorption spectra¹⁰⁸ with 4 well-defined maxima. These occur at 2625, 2710, 2815, and 2930

¹⁰⁸ H. Brockmann, *Ergeb. Vitamin-Hormonforsch.*, 2, 55-103 (1939).

A.¹⁰⁹ The peak at 2815 Å. has the greatest absorption. The molecular absorption coefficient K at 2815 Å. is reported⁷⁷ to be at about 30×10^3 . Earlier reviews of the role of absorption spectra in the investigation of the vitamin D precursors is included in the papers of Loofbourow¹¹⁰⁻¹¹² and Brockmann.¹⁰⁸ The absorption maxima and minima of ergosterol and 7-dehydrocholesterol are shown in Table 2, while Figure 1 gives a graphic representation of their absorption spectra.

The melting points of the several provitamins vary sufficiently to be of use in characterization. All exhibit optical rotation. These values are summarized in Table 3.

TABLE 3
MELTING POINTS AND SPECIFIC ROTATIONS OF PROVITAMINS D

Name	M.p., °C.	$[\alpha]_D^{20}$
Ergosterol (nearly anhyd.) ^a	168	-132° (CHCl ₃)
7-Dehydrocholesterol ^b	149-150	-122.5° (benzene)
Epi-7-dehydrocholesterol ^c	124-126	-70.5° (CHCl ₃)
22-Dihydroergosterol ^d	152-153	-109° (CHCl ₃), 19°C.
7-Dehydrositosterol ^e	144-145	-116° (CHCl ₃)
7-Dehydrostigmasterol ^e	154	-113.15° (benzene)
"Mussel" provitamin ^f	149-150	-116° (benzene)
"Periwinkle" provitamin ^g	137-137.5	-124° (benzene)

^a C. E. Bills and E. M. Honeywell, *J. Biol. Chem.*, **80**, 15-23 (1928).

^b A. G. Boer, E. H. Reerink, A. van Wijk, and J. van Niekerk, *Proc. Acad. Sci. Amsterdam*, **39**, 622-632 (1936).

^c A. Windaus and J. Nagatz, *Ann.*, **542**, 204-218 (1939).

^d A. Windaus and R. Langer, *Ann.*, **508**, 105-114 (1933).

^e H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945.

^f A. G. Boer, J. van Niekerk, E. H. Reerink, and A. van Wijk, *U. S. Patent No. 2,163,659* (June 27, 1939).

^g A. G. Boer, J. van Niekerk, E. H. Reerink, and A. van Wijk, *U. S. Patent No. 2,216,719* (Oct. 18, 1940).

3. Structure of the Provitamins D

The natural provitamins are all sterols, although some of the synthetic compounds prepared by irradiation can no longer be classed in this category. The primary irradiation products—the lumisterols—still retain the steroid nucleus, although they have assumed a stereoisomeric configuration. On the other hand, the tachysterols, which are the irradiation products next in sequence, have lost their typical steroid ring structures; however, they

¹⁰⁹ T. R. Hogness, A. E. Sidwell, Jr., and F. P. Zscheile, Jr., *J. Biol. Chem.*, **120**, 239-256 (1937).

¹¹⁰ J. R. Loofbourow, *Bull. Basic Sci. Research*, **3**, 101-156, 201-236 (1931).

¹¹¹ J. R. Loofbourow, *Bull. Basic Sci. Research*, **4**, 59-112 (1932).

¹¹² J. R. Loofbourow, *Bull. Basic Sci. Research*, **3**, 257-262 (1931).

are to be classed as potent provitamins D. Although the justification for the general steroid structure has been discussed earlier (Chapter IV), proof of the structure of the important provitamins D will be briefly outlined below.

(1) *Ergosterol (Provitamin D₂)*

Ergosterol has an empirical formula of $C_{28}H_{44}O$, as has been determined by analysis of the crystalline 3,5-dinitrobenzoate and also of the halogenonitrobenzoate esters.^{114,115} On catalytic hydrogenation, ergosterol absorbs 6 hydrogen atoms, with the formation of ergostanol, $C_{28}H_{50}O$ ¹¹⁶; this indicates the presence of 3 double bonds. On the basis of the empirical formula, ergosterol must contain 4 rings. This is readily proved by the fact that it is converted to the Diels hydrocarbon, $C_{18}H_{16}$, on dehydrogenation with selenium.¹¹⁷ In 1933, Rosenheim and King¹¹⁸ identified this

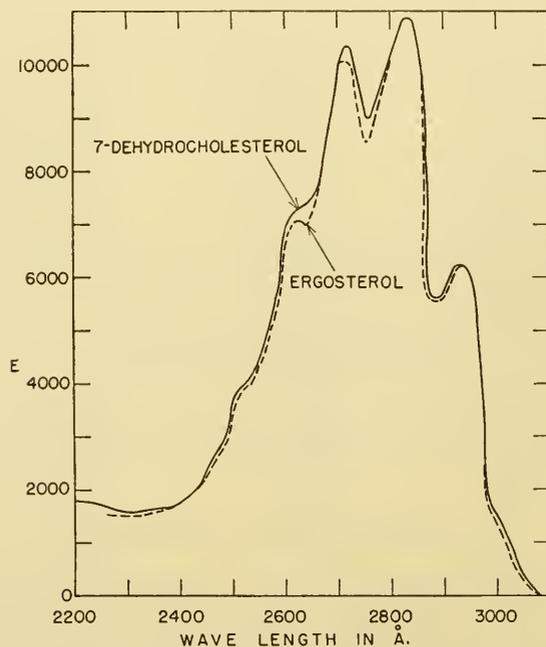


Fig. 1. Absorption spectra of 7-dehydrocholesterol and ergosterol in ethanol according to Hogness *et al.*¹⁰⁹ and Loofbourow.¹¹³

¹¹³ J. R. Loofbourow, *Vitamins and Hormones*, 1, 109-155 (1943).

¹¹⁴ A. Windaus, F. v. Werder, and B. Gschaider, *Ber.*, 65, 1006-1009 (1932).

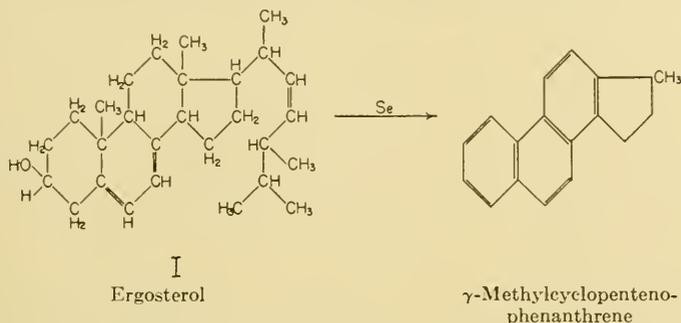
¹¹⁵ A. Windaus and A. Lüttringhaus, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 4-7 (1932).

¹¹⁶ A. Windaus and O. Linsert, *Ann.*, 465, 148-166 (1928).

¹¹⁷ O. Diels and A. Karstens, *Ann.*, 478, 129-137 (1930).

¹¹⁸ O. Rosenheim and H. King, *Chemistry & Industry*, 11 (*J. Soc. Chem. Ind.*, 52) 299-301 (1933).

hydrocarbon with γ -methylpentenophenanthrene. This is a typical reduction product characteristic only of the sterols. On the basis of these reactions and of others, ergosterol has been assigned the structure (I) shown here. The above reaction involves the following changes.



The close relationship of ergosterol (I) to cholesterol (VI) can readily be demonstrated by its conversion to an acid common to both of the sterols when the saturated hydrocarbons produced from the two sterols are oxidized. After ergostanol (II) is converted to ergostanyl chloride (III), it is readily reduced to the saturated hydrocarbon ergostane, $C_{28}H_{50}$ (IV), by reduction with sodium and amyl alcohol.^{119,120} The latter hydrocarbon can then be oxidized to a monocarboxylic acid, $C_{23}H_{38}O_2$, which is identified as nor-allocholic acid¹²¹ (V). This is the same acid as that obtained from cholesterol (VI) after conversion to cholestane (VII).

The formation of nor-allocholic acid from ergostane and cholestane indicates that the parent sterols have the same steric configuration. This can also be assumed from the fact that both ergosterol and cholesterol are precipitable with digitonin, indicating a β -type of configuration for the hydroxyl group.

The data likewise indicate that the basic steroid nuclei of cholesterol and of ergosterol are similar, and also that the first 5 carbon atoms on the side chain are the same. Since ergosterol contains one more carbon atom than does cholesterol, it is presumed that this must be in the side chain. The fact that this is the case can be readily demonstrated by strenuous oxidation of ergostanol (VIII) and cholestanol (IX) with chromic acid. After such treatment, a ketone containing 9 carbon atoms ($C_9H_{18}O$) (X) is obtained from ergostanol, while a corresponding compound with 8 carbon atoms ($C_8H_{16}O$) (XI) is formed from cholestanol.¹²² Further information about the side chain of ergosterol is afforded by the reaction with ozone. Under such conditions, methylisopropylacetaldehyde is formed.^{122,123}

¹¹⁹ F. Reindel and E. Walter, *Ann.*, **460**, 212-224 (1928).

¹²⁰ A. Windaus and W. Grosskopf, *Z. physiol. Chem.*, **124**, 8-14 (1923).

¹²¹ C. K. Chuang, *Ann.*, **500**, 270-281 (1933).

¹²² A. Guiteras, Z. Nakamiya, and H. H. Inhoffen, *Ann.*, **494**, 116-126 (1932).

¹²³ F. Reindel and H. Kipphan, *Ann.*, **493**, 181-190 (1932).

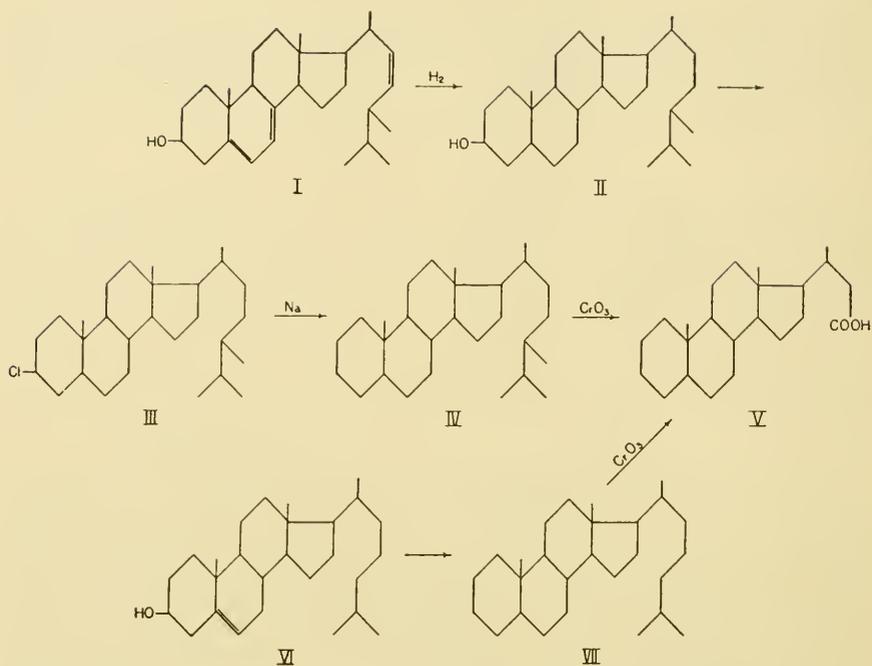


Fig. 2. The conversion of ergosterol (I) and cholesterol (VI) to the same nor-allo-cholanic acid (V).

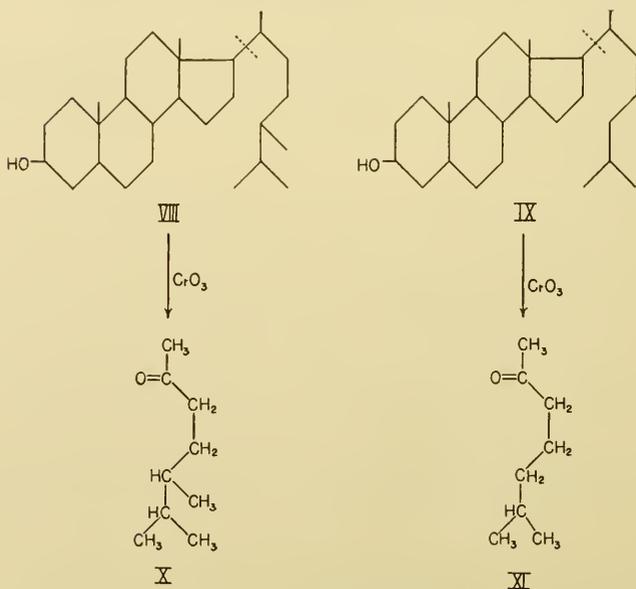
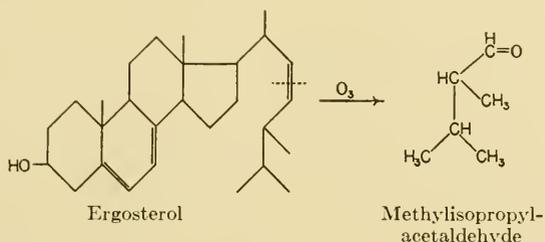


Fig. 3. A comparison of the oxidation products of ergostanol (VIII) and cholestanol (IX) with chromic acid.

This is readily identified by its semicarbazone or dinitrophenylhydrazone derivative, as well as by its conversion to methylisopropylacetic acid on oxidation. These reactions indicate that the side chain of ergosterol contains an unsaturated linkage between carbons 22 and 23.



The presence of an hydroxyl group on ergosterol is indicated by its ability to form esters. The fact that the alcohol group is attached to position 3 in common with other sterols is indicated by the demonstration by Fernholz and Chakravorty¹²⁴ that the same 3-acetoxy-nor-allocholanic acid is obtained on oxidation of acetylated ergostanol as is derived from dihydrocholesterol acetate.

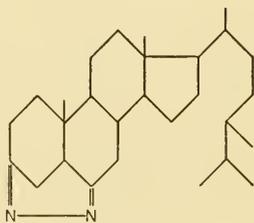
The final proof of the structure of ergosterol necessitates the establishment of the position of the double bonds. One of these has already been shown to occur in the side chain between carbons 22 and 23. The second one is in the 5,6 position, which is the location of the single unsaturated linkage of cholesterol.¹²⁵ This has been proved by a complicated set of reactions involving oxidation with perbenzoic acid to form ergostadiene-triol monobenzoate.¹²⁶ This compound contains one secondary and one tertiary alcohol group. After saponification and acetylation, an ergostadiene-triol diacetate is formed which is readily reduced to ergostene-triol diacetate by catalytic hydrogenation. On treatment with hydrochloric acid, the single double bond in the latter compound rearranges, and the resulting triol is readily saturated.⁷⁷ This becomes ergostane triol after saponification. The latter contains two of the hydroxyl groups in α,β position as determined from the Criegee reaction with lead tetraacetate. On chromic acid oxidation, an hydroxy-diketone is formed which, on treatment with hydrochloric acid, splits out water to produce a diketone. The unsaturated diketone ergostenedione is readily transformed into the corresponding saturated diketone, ergostadione. The pyridazine derivative can be formed from this by treatment with hydrazine. Since one ketone group is known to be in position 3 in place of the original hydroxyl group, the second one must be in position 6, since the pyridazine will be formed

¹²⁴ E. Fernholz and P. N. Chakravorty, *Ber.*, 67, 2021-2026 (1934).

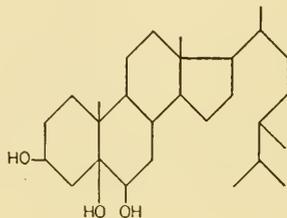
¹²⁵ A. Windaus, H. H. Inhoffen, and S. v. Reichel, *Ann.*, 510, 248-259 (1934).

¹²⁶ A. Windaus and A. Lüttringhaus, *Ann.*, 481, 119-131 (1930).

only by reaction with a ketone group 3 carbons removed from the first one. The diketone is, therefore, ergosta-3,6-dione and the ergostane-triol has the constitution ergosta-3,5,6-triol. The second double bond can, therefore, be only in the 5,6 position.



Pyridazine derivative
of ergosta-3,6-dione



Ergostane-
3,5,6-triol

The location of the third double bond can likewise be determined. The fact that it forms a conjugate bond is indicated by the refractive index,¹²⁷ by its x-ray pattern,¹²⁸ and also by its ultraviolet absorption spectrum. This third double bond cannot be present in the side chain, so that it must be conjugated to the $\Delta^{5,6}$ -linkage. This would place it at the 7,8 position. The fact that ergosterol is readily reduced with sodium and amyl alcohol,¹²⁹ as well as that it forms an addition product with maleic anhydride,¹³⁰ is further evidence of conjugation of 2 unsaturated linkages. The 7,8 position is also indicated by the maleic anhydride condensation, since this is only effected when the 2 double bonds are in the same ring. The formation of toluene-2,3,4,5-tetracarboxylic acid on nitric acid oxidation offers further proof of the location of the third double bond. The double bonds in ergosterol are therefore located in the 5,6-, 7,8-, and 22,23-positions.

(2) 7-Dehydrocholesterol (Provitamin D₃)

The structure of 7-dehydrocholesterol has been established by virtue of its synthesis from cholesterol by Windaus, Lettré, and Schenck.⁶⁸ The basic structure of cholesterol has been established in an earlier section (see Chapter IV). The location of the double bonds is also evident because the conjugate system is the same as in ergosterol.

¹²⁷ K. v. Auwers and E. Wolter, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse*, III, 101-119 (1931).

¹²⁸ G. E. R. Schulze, *Z. physik. Chem.*, A171, 436-444 (1934).

¹²⁹ A. Windaus and J. Brunken, *Ann.*, 460, 225-235 (1928).

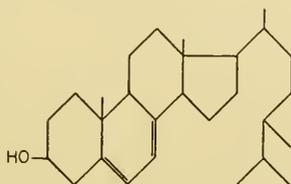
¹³⁰ A. Windaus and A. Lüttringhaus, *Ber.*, 64, 850-854 (1931).



7-Dehydrocholesterol

(3) *22-Dihydroergosterol (Provitamin D₄)*

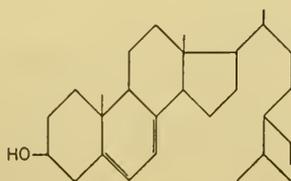
The structure of 22-dihydroergosterol is readily established by its synthesis from ergosterol on the absorption of one molecule of hydrogen. It is evident that the reduction has occurred in the side chain, inasmuch as it is no longer possible to obtain methylisopropylacetaldehyde on ozonolysis. The double bonds in ring B are obviously in the same position as in ergosterol.



22-Dihydroergosterol

(4) *Other Provitamins D*

Provitamin D₅ is 7-dehydrositosterol. Its structure can readily be determined by analogy to the other provitamins. It differs from provitamins D₂, D₃, and D₄ only in the side chain. The formulas of epi-ergosterol and epi-7-dehydrocholesterol are readily established, since they are entirely similar to their corresponding *trans* derivatives except for the *cis* position of the hydroxyl group in position 3. The compositions of "mussel" and of "periwinkle" provitamins D are not known.



7-Dehydrositosterol

4. Synthesis of the Provitamins D

None of the provitamins D has been completely synthesized, although several of them have been prepared from closely related sterol compounds. The preparation of the 7-dehydro compounds has been the most involved, but a similar procedure may be followed in preparing these from the appropriate 7-dihydrosterol.

(1) 7-Dehydrocholesterol

Windaus, Lettré, and Schenck⁶⁸ have accomplished an entirely satisfactory synthesis of 7-dehydrocholesterol (XVII) from cholesterol (VI). In this method, cholesteryl acetate (XII) is oxidized by chromic acid to 7-oxo-cholesteryl acetate (XIII). When this is treated with aluminum isopropoxide in isopropanol, 7-hydroxycholesterol (XIV) is formed. As a result of a simultaneous ester interchange, the free alcohol is obtained. After benzylation of the dihydroxy compound, the dibenzoate (XV) is thermally decomposed into the monobenzoate of 7-dehydrocholesterol (XVI), a molecule of benzoic acid being simultaneously freed. On saponification, provitamin D₃ is set free (XVII).

Another synthesis involves the use of cholesterol benzoate as the starting material.¹³¹ The 7-hydroxycholesterol can be converted into the 7-dehydro compound by the utilization of amines,^{132,133} whereby isodehydrocholesterol is obtained as a by-product.¹³⁴ Less satisfactory methods involve direct oxidation of cholesterol by means of mild oxidants¹³⁵ such as peroxides, the use of quinones as dehydrating agents,^{136,137} and also the application of succinodehydrogenase in the presence of light.¹³⁶

(2) 22-Dihydroergosterol

22-Dihydroergosterol, or provitamin D₄, has been prepared by Windaus and Langer⁵² by hydrogenation of the side chain of ergosterol. This can readily be accomplished by first acetylating the hydroxyl group, and then protecting the double bonds at the 5,6 and 7,8 positions by formation of the addition product with maleic anhydride. After catalytic hydrogenation, the maleic anhydride is removed by heat, and the free provitamin may be obtained after saponification of its ester.

¹³¹ H. R. Rosenberg and J. M. Tinker (to E. I. du Pont de Nemours & Co.), *U. S. Patent No. 2,215,727* (Sept. 20, 1940).

¹³² G. A. D. Haslewood, *J. Chem. Soc.*, 1938, 224-228.

¹³³ H. R. Rosenberg (to E. I. du Pont de Nemours & Co.), *U. S. Patent No. 2,209,934* (July 30, 1940).

¹³⁴ A. Windaus, O. Linsert, and H. J. Eckhardt, *Ann.*, 534, 22-41 (1938).

¹³⁵ J. Waddell, *U. S. Patent Nos. 2,028,364* (May 16, 1933), and 2,056,992 (Oct. 13, 1936).

¹³⁶ N. A. Milas and R. Heggie, *J. Am. Chem. Soc.*, 60, 984-985 (1938).

¹³⁷ P. P. T. Sah, *Rec. trav. chim.*, 59, 454-460 (1940).

(3) 7-Dehydrostigosterol

The synthesis of 7-dehydrostigosterol or provitamin D₅ from stigosterol is readily accomplished⁷³ by the Windaus method for 7-dehydrocholesterol. Soybean oil is used as a source of stigosterol.

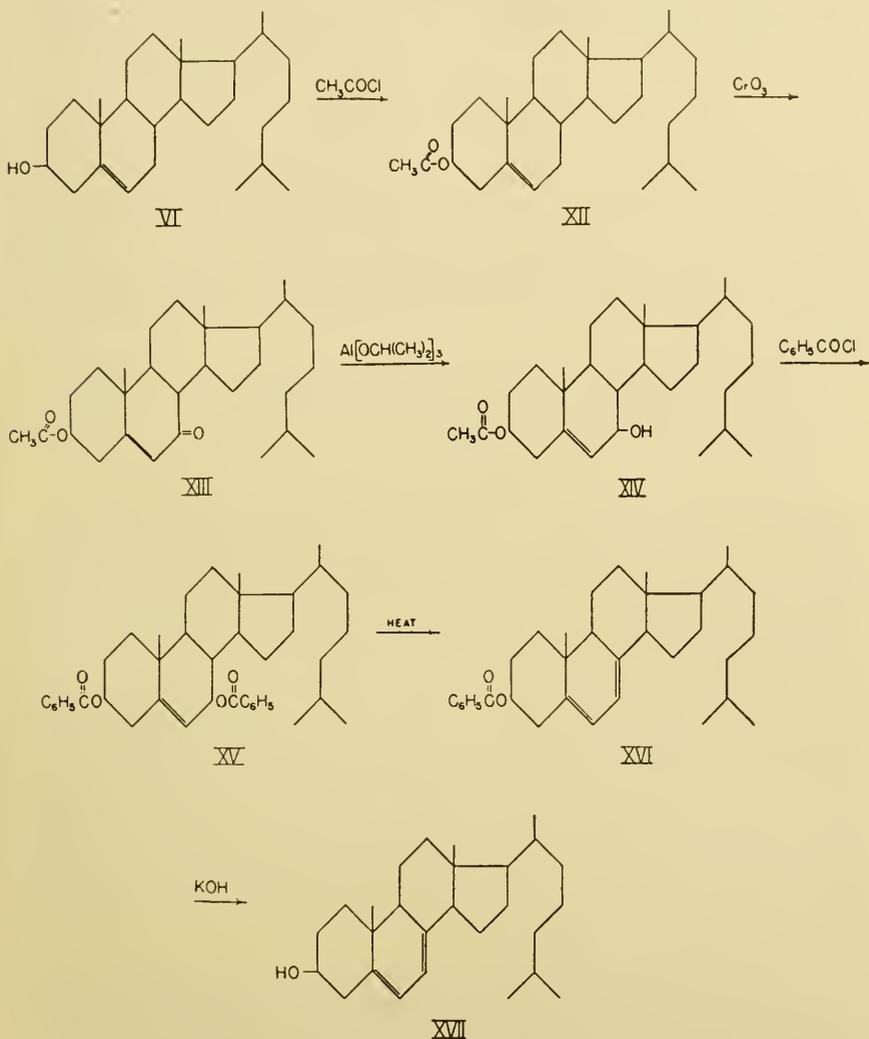


Fig. 4. The synthesis of 7-dehydrocholesterol (XVII) from cholesterol (VI).

(4) Other Provitamins D

Epi-7-dehydrocholesterol can be produced by the Windaus method for the preparation of 7-dehydrocholesterol, but using the epi-cholesterol

in place of cholesterol as the starting material.⁷⁹ It may also be prepared by epimerization of the hydroxyl group of 7-dehydrocholesterol.¹³⁸ This is accomplished by oxidation with aluminum *tert*-butoxide to 7-dehydrocholestenone which, on subsequent reduction with aluminum isopropoxide, produces the *epi*-isomer¹³⁸ in a yield of 1.25%.

Epi-ergosterol has been obtained in a 1.3% yield by reduction of ergostenone with aluminum isopropoxide.¹³⁹ It has not been obtained in pure form.

7-Dehydrostigmasterol can be synthesized from stigmasterol⁷⁶ by the Windaus method used for 7-dehydrocholesterol. However, it is not certain whether or not this sterol should be classed as a provitamin D, because of the slight antirachitic activity of its irradiation product. No synthesis is reported in the literature for 22,23-oxidoergosterol. Rosenberg⁷⁷ suggests that it might be synthesized by oxidation of the maleic anhydride addition product of ergosterol ester followed by thermal decomposition of the addition product to the ester, and saponification of the latter compound to the provitamin.

C. CONVERSION OF THE PROVITAMINS D TO THE VITAMINS D

1. Introduction

In order to effect the change of the physiologically inert provitamin D to the active form, it is necessary to supply a definite quantity of energy. This can be accomplished by the use of ultraviolet light present in sunlight or obtained from artificial sources of radiant energy, or by cathode rays and other similar types of radiation.

Although the healing action of sunlight on rickets had been known for many years, it was not realized until the work of Huldshinsky,^{20,21} and especially that of Hess, Pappenheimer, and Weinstock,¹⁴⁰ that the shorter ultraviolet waves of sunlight, or the still shorter waves of artificial light, account for this therapeutic action. The association of this curative effect with a chemical reaction caused by the ultraviolet light became evident as soon as it was demonstrated almost simultaneously by the Steenbock^{27,33} and by the Hess³¹ groups that inert rations and oils can be activated as antirachitic agents by irradiation.

As soon as the observation was reported that antirachitic potency could be conferred on foods by the process of irradiation, the nature of the chemical reaction involved became the concern of many chemists. It was evident that the product which was acted upon was of a sterol-like nature, in

¹³⁸ A. Windaus and O. Kaufmann, *Ann.*, 542, 218-224 (1939).

¹³⁹ A. Windaus and K. Buchholz, *Ber.*, 71, 576-578 (1938); 72, 597-599 (1939).

¹⁴⁰ A. F. Hess, A. M. Pappenheimer, and M. Weinstock, *Proc. Soc. Exptl. Biol. Med.*, 20, 14-16 (1922).

view of the demonstration by Hess, Weinstock, and Helman³² that phyto-sterol, cholesterol, and—to a lesser extent—lanolin could be activated. As has been discussed elsewhere, several provitamins were shortly recognized. These included ergosterol¹³⁻¹⁷ and later 7-dehydrocholesterol,⁶⁰ as well as 22-dihydroergosterol.⁵² A number of additional provitamin D compounds have since been discovered, but none possesses the potential activity of the groups which were first recognized.

2. Processes of Activation

Ultraviolet irradiation has been most widely employed for the activation of the provitamins D. It was first assumed that the reaction could be carried out with equal efficiency by light of wave lengths within the principal absorption range of ergosterol, which lies between 305 and 230 μ .¹⁴¹⁻¹⁴³ However, Rosenberg⁷⁷ states that the greatest efficiency obtains with ergosterol when light of a wave length of 281 μ is employed. This value coincides with the principal absorption peak of this sterol. In the case of 7-dehydrocholesterol, Bunker, Harris, and Mosher¹⁴⁴ obtained the best results when monochromatic light of 296.7 μ was employed for activation. These workers state that light of the same wave length is most effective in curing rickets in rats when a direct irradiation of the animals is employed; however, Knudson and Benford¹⁴⁵ found that light with a wave length of 280.4 μ is best for this purpose. Since it is known that several intermediate products are produced before vitamin D is ultimately formed, it is surprising that light with such a narrow range should act most effectively in the total conversion. However, two of these intermediate compounds—lumisterol and tachysterol—do exhibit absorption maxima at 280 μ .

Several types of lamps have been successfully employed for providing the ultraviolet light for the irradiation process. These include the magnesium and carbon arcs, the mercury vapor lamp, and cored carbon electrodes impregnated with different metals.⁷⁷ The bismuth vapor lamp has also been shown to give satisfactory results.¹⁴⁶ The best products are obtained for commercial use when the range of light is limited to the area between 275 and 300 μ .^{147,148} This is done by the use of certain filters. To

¹⁴¹ S. K. Kon, F. Daniels, and H. Steenbock, *J. Am. Chem. Soc.*, **50**, 2573-2581 (1928).

¹⁴² T. A. Webster and R. B. Bourdillon, *Biochem. J.*, **22**, 1223-1230 (1928).

¹⁴³ A. L. Marshall and A. Knudson, *J. Am. Chem. Soc.*, **52**, 2304-2314 (1930).

¹⁴⁴ J. W. M. Bunker, R. S. Harris, and L. M. Mosher, *J. Am. Chem. Soc.*, **62**, 508-511 (1940).

¹⁴⁵ A. Knudson and F. Benford, *J. Biol. Chem.*, **124**, 287-299 (1938).

¹⁴⁶ E. H. Reerink and A. van Wijk (to N. V. Philips' Gloeilampenfabrieken, Holland), *U. S. Patent* No. 1,904,751 (Apr. 18, 1933).

¹⁴⁷ E. H. Reerink and A. van Wijk, *Strahlentherapie*, **40**, 728-732 (1931).

¹⁴⁸ T. H. Rider, G. Sperti, G. P. Goode, and H. G. Cassidy, *J. Am. Med. Assoc.*, **106** 452-456 (1936).

filter out light with wave lengths shorter than 270–275 $m\mu$,¹⁴⁹ benzene,^{146,150} xylene,¹⁵⁰ diphenyl in benzene solution,¹⁵¹ or 5% lead acetate¹⁵² has been used. Carbon disulfide¹⁵³ filters out light in the area of 312–313 $m\mu$. Special types of glass have been produced which allow a selective transmission of light of wave lengths of 275 to 300 $m\mu$ only.⁷⁷

Other methods of activation have also proved effective in transforming the provitamins D to the corresponding vitamins D. These include cathode rays^{154,155} alone or, in some cases, in the presence of catalysts such as iron or uranium salts.¹⁵⁶ Canal rays have been used, as well as the α -, β -, and γ -rays of radioactive elements. A German patent¹⁵⁷ describes the use of corpuscular rays as a method of activation. Moore and De Vries¹⁵⁸ have reported activation by the use of radium emanation. Although the reports on the effectiveness of x-rays are conflicting, it is probable that the negative results of Goldblatt¹⁵⁹ are correct. Finally, electrons of high frequency have been widely used,¹⁶⁰ while alternating current of high frequency has also been employed.^{161,162} Bills⁸⁶ has reported that radio waves of high intensity and short wave length are completely ineffective. Another contribution to the method of *in vivo* activation is the suggestion that it is brought about by the Gurwitsch rays or the so-called mitogenetic radiations.¹⁶³ Ultraviolet rays with wave lengths of 190 to 250 $m\mu$ are supposedly produced in the living cell, although the existence of such radiations has been denied by some workers.^{164–166}

The energy of the reaction involved in the activation of the sterols has been investigated by a number of workers.^{141,143,145,167} Pohl⁴³ concluded

¹⁴⁹ N. V. Philips' Gloeilampenfabrieken (Holland), *German Patent* No. 634,146 (Aug. 18, 1936).

¹⁵⁰ A. G. Boer, J. van Niekerk, E. H. Reerink, and A. van Wijk, *U. S. Patent* No. 2,216,719 (Oct. 18, 1940).

¹⁵¹ O. Linsert (to Winthrop Chem. Co., Inc.), *U. S. Patent* No. 1,902,785 (March 21, 1933).

¹⁵² G. Sperti, R. J. Norris, R. B. Withrow, and H. Schneider (to General Development Lab.), *U. S. Patent* No. 1,982,029 (Nov. 27, 1934).

¹⁵³ N. V. Philips' Naamlooze Vennootsch. Gloeilampenfabrieken (Holland), *British Patent* No. 385,626 (Dec. 20, 1932).

¹⁵⁴ A. Knudson and C. N. Moore, *J. Biol. Chem.*, **81**, 49–64 (1929).

¹⁵⁵ R. M. Hoffman and F. Daniels, *J. Biol. Chem.*, **115**, 119–130 (1936).

¹⁵⁶ A. J. Pacini (to Research Products, Inc.), *U. S. Patent* No. 1,983,944 (Dec. 11, 1934).

¹⁵⁷ K. Hembd and Vitam Fabrik, *German Patent* No. 577,170 (Aug. 10, 1933).

¹⁵⁸ R. B. Moore and T. De Vries, *J. Am. Chem. Soc.*, **53**, 2676–2681 (1931).

¹⁵⁹ H. Goldblatt, *Ergeb. allgem. Pathol., Path. Anat., Abt. 2*, **25**, 58–491 (1931).

¹⁶⁰ Brit. Thompson-Houston Co., *British Patent* No. 292,926 (Aug. 22, 1928). A. Knudson (to Sun-A-Sured, Inc.), *U. S. Patent* No. 2,007,765 (July 9, 1935).

¹⁶¹ I. G. Farbenindustrie, *Austrian Patent* No. 119,210 (Apr. 15, 1930).

¹⁶² C. C. Whittier, *U. S. Patent* Nos. 2,106,779; 2,106,780; (to Nutrition Research Labs., Inc., Chicago) 2,106,781; 2,106,782 (Feb. 1, 1938).

¹⁶³ H. Mai, *Abhandl. Kinderhkl. Grenzgeb.*, **45**, 1–81 (1937).

¹⁶⁴ K. H. Kreuchen, *Angew. Chem.*, **47**, 185–186 (1934).

¹⁶⁵ E. N. Harvey, *Naturwissenschaften*, **12**, 165–169 (1924).

¹⁶⁶ J. Levine and A. H. Steinhaus, *Am. J. Physiol.*, **133**, 361–362P (1941).

¹⁶⁷ R. W. Haman and H. Steenbock, *Ind. Eng. Chem., Anal. Ed.*, **8**, 291–293 (1936).

that the activation probably involves the addition of energy by an electron displacement. The energy required to produce one U.S.P. unit of vitamin D has been calculated by Harris *et al.*,¹⁶⁸ as 7.5×10^{13} quanta. For the production of a healing effect equivalent to one unit of vitamin D on direct irradiation of rats, Knudson and Benford¹⁴⁵ calculated the following energy values: 2653 Å., 287,000 ergs; 2804 Å., 226,000 ergs; 2894 Å., 395,000; 2967 Å., 280,000 ergs; 3024 Å., 553,000 ergs; 3128 Å., 27,545,000 ergs. There is no evidence, however, that the energy used for activation of the provitamin remains stored in the molecule of the vitamin D so produced. Bills and co-workers¹⁶⁹ were unable to demonstrate any difference in the heats of combustion of the provitamins D and of the inactivated products.

A number of factors alter the ease with which the provitamins D are activated. The effect of the wave length of the light has already been discussed. Variations are dependent upon the physical state of the provitamin. Thus, although dry material can be activated,¹⁷⁰ the yield is poor because activation of the provitamin occurs at first only on the surface. Although this less exposed provitamin D can be activated by further irradiation, this treatment will cause a destruction of the vitamin D already produced on the surface. Askew and collaborators¹⁷¹ describe a method of activation of the provitamin D while in the vapor form. However, the most effective synthesis of vitamin D occurs when the provitamin D is in solution. The yield is increased when the solution of provitamins is agitated.¹⁷² This can be accomplished very efficiently when a special quartz irradiation chamber is employed which is built concentrically around a mercury vapor lamp¹⁷³; the solution of provitamin D is passed continuously through this apparatus, which is so constructed as to cause a considerable agitation of the solution.

The solvent employed, also, has some influence upon the efficiency of conversion of the provitamins to the vitamins. Activation is said to occur more effectively in ether than in alcohol.¹⁷⁴ Cyclohexane and dioxane have been employed as well, either alone or in a mixture with benzene, ethyl acetate, or triethanolamine. Oil is sometimes used as a solvent for the provitamins. An improvement in yield is claimed when compounds are

¹⁶⁸ R. S. Harris, J. W. M. Bunker, and L. M. Mosher, *J. Am. Chem. Soc.*, **60**, 2579-2580 (1938).

¹⁶⁹ C. E. Bills, F. G. McDonald, La M. N. Be-Miller, G. E. Steel, and M. Nussmeier, *J. Biol. Chem.*, **93**, 775-785 (1931).

¹⁷⁰ H. H. Beard, R. E. Burk, H. E. Thompson, and H. Goldblatt, *J. Biol. Chem.*, **96**, 307-312 (1932).

¹⁷¹ F. A. Askew, R. B. Bourdillon, and T. A. Webster, *Biochem. J.*, **26**, 814 (1932).

¹⁷² A. Windaus, K. Westphal, F. v. Werder, and O. Rygh, *Nachr. Ges. Wiss. Göttingen Math. physik. Klasse*, **III**, 45-59 (1929).

¹⁷³ F. Seitz, *Vitamine und Hormone und Ihre technische Darstellung. Darstellung von Vitamin-Präparaten*, Leipzig, 1939, p. 50; Advance Scientific Pub., New York, 1944.

¹⁷⁴ C. E. Bills, E. M. Honeywell, and W. M. Cox, *J. Biol. Chem.*, **92**, 601-604 (1932).

added which protect the vitamin D after it has been formed. Ethylene and alkali hydroxide have been used for this purpose.¹⁷⁵ A British patent¹⁷⁶ has been issued for the use of photosensitizers such as eosin, erythrosin, dibromodinitrofluorescein, di-iodofluorescein, and isoquinolin Red, which presumably aid in the activation reaction.

The activation of the provitamin involves only a rearrangement, and no oxidation occurs. In fact, the presence of oxygen may have an extremely deleterious effect upon this reaction.^{48,172,177-179} The intermediates formed during the irradiation are quite susceptible to oxidation with molecular oxygen, although both the provitamins D and the vitamins D, either crystalline or in the form of resins^{63,169} are relatively stable toward oxidation. Angus *et al.*⁶³ have found that the presence of oxygenated compounds renders it difficult to isolate the crystalline vitamin D, although the yield is not greatly affected.

The temperature coefficient of the activation reaction is an extremely small value. Bills and Brickwedde¹⁸⁰ found that an impure cholesterol was readily activated at -183°C ., while Webster and Bourdillon¹⁴² noted little difference in the effectiveness of irradiation between -18°C . and -78°C .. The latter authors did find, however, that the products prepared by irradiation in liquid oxygen (-183°C .) or liquid nitrogen (-195°C .), possessed definitely lower biological activity. It is claimed that the effect is somewhat enhanced when irradiation is regulated by spectral analysis in a device connected with the irradiation apparatus and traversed by the reaction liquid,¹⁸¹ or when it occurs at the boiling point of the solvent.¹⁸² This has been ascribed to a more uniform activation of all the molecules present.⁷⁷ Since most bimolecular reactions have a high temperature coefficient and are, in general, inhibited by very low temperatures, it was early suggested that the activation involves a monomolecular reaction. Such is now known to be the case.

3. Chemistry of the Activation Products

The activation of provitamins D is a complicated series of reactions involving the formation of several intermediate products,^{48,142,177,178,180,183}

¹⁷⁵ I. G. Farbenindustrie, *British Patent* No. 321,992 (Aug. 25, 1928).

¹⁷⁶ E. Merck Co., *British Patent* No. 286,665 (Sept. 6, 1928).

¹⁷⁷ A. Smakula, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 49-64 (1928).

¹⁷⁸ C. E. Bills, E. M. Honeywell, and W. M. Cox, *J. Biol. Chem.*, *80*, 557-563 (1928).

¹⁷⁹ A. Windaus, *Nachr. Ges. Wiss. Göttingen, Math. Physik. Klasse, III*, 36-57 (1930).

¹⁸⁰ C. E. Bills and F. G. Brickwedde, *Nature*, *121*, 452 (1928).

¹⁸¹ W. Zimmermann and W. Frankenburger (to Winthrop Chem. Co., New York), *U. S. Patent* No. 1,896,191 (Feb. 7, 1933).

¹⁸² G. B. Ellis (to Société Usines Chimiques de Rhône-Poulenc), *British Patent* No. 335,277 (Nov. 12, 1930).

(1) *Lumisterols*

Lumisterol is the first product formed on irradiation of the provitamin. It was originally referred to as "sterol X." Lumisterol₂, C₂₈H₄₈OH, is the predominant product formed when ergosterol is irradiated⁶⁷ with light of wave lengths 285 to 313 mμ. After about 40% of the ergosterol has been changed, lumisterol₂ can readily be prepared in the following manner:

The unchanged ergosterol is first removed by crystallization from methanol. The methyl alcohol is removed by evaporation from the mother liquor. The residue is then dissolved in acetone and the addition product of lumisterol₂ and vitamin D₂ (formerly called vitamin D₁) is separated by crystallization. This addition product is readily broken down by acetylation. When this mixture is subjected to fractional crystallization from acetic acid, lumisterol₂ acetate crystallizes out first. Pure lumisterol may be prepared by saponification of the ester.

Apparently, lumisterol is invariably formed on irradiation, and this step cannot be by-passed. Lettré¹⁸⁸ and Dimroth¹⁸⁹ have shown that, even on irradiation of ergosterol for a short period, lumisterol₂ is produced before the next product, tachysterol₂, is formed. Apparently, lumisterol₂ is a necessary precursor of the latter compound, although Lettré states that it is not yet certain whether tachysterol can be derived from ergosterol without the intermediation of lumisterol.

There is every evidence that lumisterol₂ is a compound closely related to ergosterol. Molecular weight determinations and elementary analyses are identical for these two compounds; the hydroxyl group is also present in the irradiated product, as well as in the ergosterol, since lumisterol₂ forms esters. Three double bonds are present in lumisterol₂, as determined from the reaction with perbenzoic acid and from catalytic hydrogenation.^{67,190} One of these is on the side chain between carbons 22 and 23, since on ozonolysis methylisopropylacetaldehyde is formed.¹²² The other two double bonds are conjugated, as can be judged from the absorption spectrum of lumisterol₂. These must be on the same ring, which can be only ring B or C. The ring system of lumisterol₂ and of ergosterol must therefore be identical. This conclusion is likewise based upon the demonstration that the same hydrocarbon, C₁₈H₁₆ (γ-methylcyclopentenophenanthrene), results on selenium dehydrogenation of both sterols,^{122,190} and upon the fact that the same toluene-tetracarboxylic acid¹⁹¹ is obtained on nitric acid oxidation of lumisterol₂ and ergosterol.^{122,188} When lumisterol₂ is treated with perbenzoic acid, a triol is formed which can be converted to a diacetate. When the latter compound is acted upon by mercuric acetate,

¹⁸⁸ H. Lettré, *Ann.*, 511, 280-291 (1934).

¹⁸⁹ K. Dimroth, *Ber.*, 70, 1631-1636 (1937).

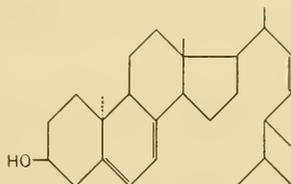
¹⁹⁰ K. Dimroth, *Ber.*, 68, 539-542 (1935).

¹⁹¹ F. Reindel and K. Niederländer, *Ann.*, 482, 264-279 (1930).

dehydrolumisterol results.¹⁹² All of these reactions are analogous to those given by ergosterol. The conclusion seems justified that the double bonds must occur between carbons 5,6 and 7,8.¹⁹³

Several reactions of lumisterol₂ are markedly different from those of ergosterol. In the first place, lumisterol₂ does not form an addition product with digitonin; moreover, no bimolecular compound results when lumisterol is irradiated in the presence of eosin. Finally, upon complete hydrogenation of lumisterol₂, the hexahydro compound formed differs from hexahydroergosterol.

The difference between lumisterol₂ and ergosterol is now known to be one of stereoisomerism. The point of difference cannot be on C₃, since pyrocalciferol, in which an epi arrangement on C₃ occurs, yields a bimolecu-



Lumisterol₂

lar compound with eosin. It is now certain that the structural difference between ergosterol and lumisterol₂ is due to a steric difference in the position of the methyl group on carbon 10.

Lumisterol₃¹⁸⁷ and lumisterol₄¹⁸⁶ have been prepared from 7-dehydrocholesterol and 22-dihydroergosterol, respectively. They closely resemble lumisterol₂, except that they fail to form addition products with their corresponding vitamins as is the case with lumisterol₂. This addition compound of lumisterol₂ and vitamin D₂ has the molecular ratio 1:1. Lumisterol₂ can be converted into vitamin D₂ by irradiation, although it is devoid of any biological activity. It is presumed that lumisterol₃ and lumisterol₄ would yield vitamins D₃ and D₄ on additional irradiation.

(2) Tachysterols

Tachysterol, C₂₈H₄₃OH, is the second known product which is formed in the progressive activation of provitamin D₂. It was named *tachysterin* by Windaus, Lüttringhaus, and Busse.¹⁹⁴ The name denotes its outstanding property, *i.e.*, the speed with which it reacts. Lumisterol presumably yields first a protachysterol, which is very unstable and which has not been

¹⁹² I. M. Heilbron, F. S. Spring, and P. A. Stewart, *J. Chem. Soc.*, 1935, 1221-1223.

¹⁹³ I. M. Heilbron and F. S. Spring, *Chemistry & Industry*, 13, 795-797 (1935).

¹⁹⁴ A. Windaus, A. Lüttringhaus, and P. Busse, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 150-159 (1932).

prepared in pure form. It rapidly undergoes a transformation to tachysterol.¹⁹⁵

The methods employed for the preparation of tachysterol and for its purification differ considerably from those used in the synthesis of lumisterol. The destruction of ergosterol is allowed to proceed to 60% instead of to the 40% limit as in the preparation of lumisterol; shorter wave lengths are used in the irradiation than in the synthesis of lumisterol. The first step in the preparation of tachysterol from the irradiation mixture involves the separation of unchanged ergosterol by crystallization from a methanol solution. Tachysterol₂ can then be isolated from the irradiation mixture as an addition product of citraconic anhydride. On thermal decomposition, the adduct yields tachysterol₂. It can be further purified by the preparation of tachysterol-3,5-dinitro-4-methylbenzoate, which forms well-shaped crystals. This method was first used by Windaus, von Werder, and Lüttringhaus⁶⁶ in 1932.

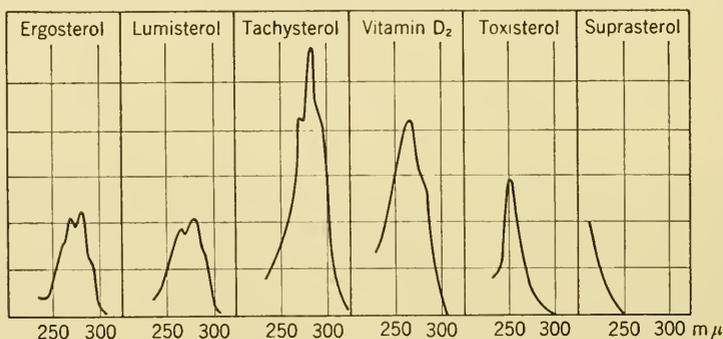


Fig. 6. Absorption curves of ergosterol and its irradiation products in 0.2% ethereal solution.⁷⁷

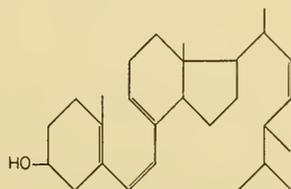
Tachysterol₂ is an isomer of both ergosterol and lumisterol, since they all have an identical empirical formula. However, it differs markedly in reaction from each of its precursors. For example, it contains 4 double bonds instead of the 3 found in ergosterol or lumisterol₂. When tachysterol₂ acetate is treated with citraconic anhydride, the addition product formed has one less double bond; 2 additional double bonds can be demonstrated in this adduct by catalytic hydrogenation. A fourth double bond which can be hydrogenated was demonstrated by treatment with perbenzoic acid.¹⁸⁸

Since tachysterol₂ has 4 double bonds, only 3 rings instead of 4 are required to satisfy the valence requirements. The fourth additional double bond which results from the ring opening must be in conjugation with the other 2 double bonds in ring B, as indicated by the absorption spectrum.

¹⁹⁵ A. Windaus and E. Auhagen, *Z. physiol. Chem.*, 196, 108-120 (1931).

It is believed that ring B is ruptured between positions 9 and 10. The proof in this case is largely based upon the fact that this point of cleavage has been proved for vitamin D₂. These 2 products are closely related, as evidenced by the fact that both yield an identical dihydro derivative when reduced with sodium in alcohol.¹⁹⁶

The position of the double bonds is apparently shifted with the increase in their number. They are believed to occur at the 10,5-, 6,7-, and the 8,9-positions. This supposition is based largely upon the failure of tachysterol₂ to form a ketone on oxidation.¹⁹⁷ Vitamin D₂, when oxidized, has been shown to form a ketone, C₁₉H₃₂O, which has been formed by the cleavage of the double bond between carbons 7 and 8. Tachysterol₂ therefore does not have a double bond in this position. The structure which is in harmony with these observations is given below:



Tachysterol₂

On irradiation tachysterol₂ is converted into vitamin D₂. It has no antirachitic activity itself, and is about one-half as toxic as vitamin D₂. As in the case of the lumisterols, there are several tachysterols which are formed from the respective provitamins. Thus, tachysterol₃ has been prepared from 7-dehydrocholesterol¹⁸⁷ in the same manner as that by which tachysterol₂ is produced from ergosterol. Windaus and Güntzel¹⁸⁶ obtained tachysterol₄ from 22-dihydroergosterol by an analogous procedure. Tachysterols₃ and ₄ can also be isolated by forming the addition products with citraconic aldehyde. None has been prepared in crystalline form, although the esters are crystallizable.

The tachysterols are readily autoxidized. Their susceptibility to oxidation is far greater than that of ergosterol or of any other irradiation products. The absorption spectrum is given in Figure 6.

a. Dihydrotachysterol₂. Dihydrotachysterol₂, also referred to as A.T.10 (antitetany compound No. 10), is of considerable interest because of its therapeutic value in deranged calcium metabolism.

Dihydrotachysterol can readily be prepared by reduction of the 3,5-dinitro-4-methyl benzoic acid ester of tachysterol with sodium ethoxide¹⁹⁸

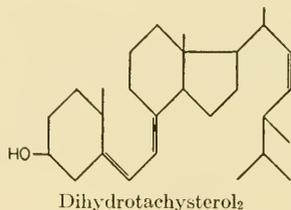
¹⁹⁶ M. Müller, *Z. physiol. Chem.*, **233**, 223-234 (1935).

¹⁹⁷ W. Grundmann, *Z. physiol. Chem.*, **252**, 151-154 (1936).

¹⁹⁸ O. Dolmer and F. v. Werder (to Winthrop Chem. Co.), *U. S. Patent* No. 2,070,117 (Feb. 9, 1937; in Germany, July 27, 1934).

and subsequent saponification of the reduced product. Dihydrotachysterol₂ can be crystallized readily, although this is not possible with unreduced tachysterol.

On the basis of the absorption spectrum and of catalytic hydrogenation experiments and the oxidative degradation reaction, von Werder⁸¹ assigned the accompanying formula to dihydrotachysterol₂. The pure prod-



uct melts at 125–127°C. and has absorption maxima at 242, 251, and 261 m μ .

Dihydrotachysterol₂ is slightly effective as an antirachitic agent.⁸¹ This administration of this product results in an increase in blood calcium, for which reason it has been used in the treatment of idiopathic hypoparathyroidism, as well as of the postoperative tetany following the injury or removal of too great a portion of this gland.^{199,200} Although tachysterol has a slight tendency to increase the level of blood calcium, its effectiveness is only about one-tenth that of the reduced product.⁷⁷

(3) *Vitamins D*

The vitamins D are the products formed by the further irradiation of the various tachysterols. The distribution, properties, and structure of these substances will be discussed in Section D of this chapter.

The vitamins D are not necessarily the terminal products produced by irradiation of the provitamins. When they are subjected to further action by ultraviolet light, they rapidly lose their biological potency and are transformed into products which have a toxic action. These products are toxisterol, suprasterol I, and suprasterol II. It is not known whether these products are formed in sequence or whether each results as a primary decomposition product of vitamin D when slightly different methods of treatment are employed.

(4) *Toxisterols*

Toxisterol, also frequently called Substance 248 because of the prominence of the absorption band at 248 m μ , was so named by Laquer and Lin-

¹⁹⁹ F. Holtz, *Merck's Jahresber.*, 47, 20–23 (1934).

²⁰⁰ F. Holtz, *Klin. Wochschr.*, 13, 104 (1934).

sert²⁰¹ (*toxisterin*). Morton, Heilbron, and Kamm²⁰² originally demonstrated that, on irradiation of ergosterol, the three absorption maxima at 293.5, 282, and 270 $m\mu$ were gradually replaced by a band of great intensity at 247–248 $m\mu$ (see Fig. 5). These latter investigators incorrectly ascribed the single absorption band to vitamin D. However, Smakula¹⁷⁷ concluded that the substance responsible for the absorption band at 248 $m\mu$ is not vitamin D. This assumption gained firm support from the experiments of Bills, Honeywell, and Cox,¹⁷⁸ who showed that the appearance of this band coincided not with the development of vitamin D, but rather with the disappearance of its antirachitic potency.

Toxisterol has not been isolated in pure form. Bills *et al.*¹⁷⁸ and van Wijk and Reerink²⁰³ regarded it as an isoergosterol. However, it was later shown to differ from this class, since it is not precipitable with digitonin.²⁰⁴ Apparently, toxisterol is formed more readily when the irradiation is carried out in alcohol than when ether or oil is employed.^{178,203,205–209}

When vitamin D is moderately over-irradiated, a toxic-calcifying property all out of proportion to the antirachitic property appears.^{194,207} The earlier preparation of vitamin D, Vigantol, first made by I. G. Farbenindustrie,^{181,210,211} apparently contained toxisterol along with vitamin D; this was responsible for the toxic reactions following its administration. The deleterious effects of toxisterol have also been pointed out by Laquer and Linsert²⁰¹ and by Windaus *et al.*¹⁹⁴ It has no antirachitic activity.¹⁷⁸

(5) *Suprasterols I and II*

Two additional products of over-irradiation were first recognized by Windaus, Gaede, Köser, and Stein²¹² in 1930. These workers coined the name *suprasterine* for these two substances. Setz¹⁸⁴ assumes that the suprasterols I and II are formed simultaneously. Bills⁸⁶ considers that the formation of toxisterol precedes that of suprasterols I and II; the latter products, however, may be formed simultaneously. The assumption is based upon the fact that the absorption spectrum of Substance 248 is essentially that of the suprasterols rather than of a mixture of toxisterol and

²⁰¹ F. Laquer and O. Linsert, *Klin. Wochschr.*, 12, 753–754 (1933).

²⁰² R. A. Morton, I. M. Heilbron, and E. D. Kamm, *J. Chem. Soc.*, 1927, 2000–2005T.

²⁰³ A. van Wijk and E. H. Reerink, *Nature*, 122, 648 (1928).

²⁰⁴ W. M. Cox and C. E. Bills, *J. Biol. Chem.*, 88, 709–713 (1930).

²⁰⁵ W. E. Dixon and J. C. Hoyle, *Brit. Med. J.*, 2, 832–835 (1928).

²⁰⁶ L. J. Harris and T. Moore, *Biochem. J.*, 23, 261–273 (1929).

²⁰⁷ J. C. Hoyle, *J. Pharm.*, 40, 351–372 (1930).

²⁰⁸ J. C. Hoyle and H. Buckland, *Biochem. J.*, 23, 558–565 (1929).

²⁰⁹ R. Kern, M. F. Montgomery, and E. U. Still, *J. Biol. Chem.*, 93, 365–380 (1931).

²¹⁰ I. G. Farbenindustrie, *British Patent* No. 296,093 (Nov. 15, 1928).

²¹¹ J. Y. Johnson (to I. G. Farbenindustrie), *British Patent* No. 316,803 (Sept. 25, 1929).

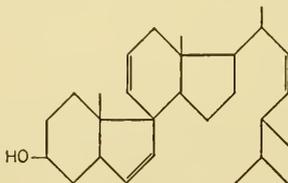
²¹² I. G. Farbenindustrie, *German Patent* No. 499,524 (Aug. 28, 1930).

²¹³ A. Windaus, J. Gaede, J. Köser, and G. Stein, *Ann.*, 483, 17–30 (1930).

the suprasterols. The latter substances would then be considered to be the terminal compounds in the sequence of irradiation products formed from the provitamins.

Suprasterol₂ I, C₂₈H₄₃OH, melts at 104°C., has a specific rotation in chloroform at 18° (D line) of -76°, and shows absorption only below 250 mμ. Suprasterol₂ II, also C₂₈H₄₃OH, melts at 110°C., is dextro-rotatory ($[\alpha]_D^{19} = +62.9^\circ$ in chloroform) and has spectral absorption only below 250 mμ.⁸⁶ Both products have an hydroxyl group and form esters. The separation of the two isomers has been rendered possible by the difference in solubility of their allophanic acid esters.

There appear to be 3 double bonds in both suprasterols₂ I and II, as indicated by the results of catalytic hydrogenation and by titration with perbenzoic acid.¹⁹⁶ Since only 3 double bonds exist, there must be a closure of ring B, which is open in tachysterol and vitamin D. Because no absorption occurs in the spectral region above 220 mμ, it must be concluded that the double bonds are not conjugated. However, since the typical hydrocarbon formed on dehydrogenation of sterol compounds with selenium, namely γ -methylcyclopentenophenanthrene, is not formed from the suprasterol, it appears certain that the suprasterols do not have the steroid nucleus. Müller¹⁹⁶ has suggested the following structure for the suprasterols:



Suprasterols₃ and ₄ have been obtained by the over-irradiation of 7-dehydrocholesterol⁷⁷ and 22-dihydroergosterol,¹⁸⁶ respectively. The suprasterols are only slightly toxic and are entirely ineffective as antirachitic agents.⁸⁶

D. VITAMINS D

A number of different compounds have been shown to possess antirachitic potency. Only two of these have been prepared in a pure state from natural sources. Several of the others have been synthesized by irradiation of provitamins, and it has been possible to separate them in pure form from such relatively highly concentrated preparations. It is not known how many of the latter group of compounds so prepared are present in nature. This inability to obtain other members of the vitamin D group from natural sources is due to the difficulties encountered in isolation and purification. This is especially the case since some of the more uncommon mem-

bers of the vitamin D group may only occur as minor constituents in a mixture containing the other vitamins D.

Ten different provitamins D are known, as determined by the fact that, on irradiation, antirachitic substances originate. There must therefore be ten different vitamins D which correspond to these provitamins D. Of these, only four vitamins have been prepared in pure form. The composition of the others can be surmised by analogy in those cases in which the structure of the provitamin is known.

As indicated earlier, vitamin D₁ is not a true vitamin, since it is a molecular compound of vitamin D₂ and lumisterol₂. Vitamin D₂, also referred to as calciferol or viosterol, is derived from the irradiation of ergosterol. On activation of 7-dehydrocholesterol, vitamin D₃ originates. Vitamin D₄ consists of activated 22-dihydroergosterol, while vitamin D₅ is generally considered to be the vitamin originating from the irradiation of 7-dehydrostosterol. Of these, only vitamins D₂ and D₃ have been isolated¹⁰⁷ in pure form from fish liver oils. On the basis of molecular distillation of cod liver oil, it has been suggested²¹³ that six different vitamins D are present. These consist of two which occur in major amounts, two others in lesser amounts, and the remaining two in traces. Rosenberg⁷⁷ refers to one of the latter as vitamin D₆, but it has not been further identified. Other fish liver oils, such as those from spearfish (*Tetrapturus* spp.) and from white sea bass (*Cynoscion nobilis*), show elimination curves differing from each other and also varying from that of cod liver oil. It is therefore presumed that these oils contain distinct types of vitamin D.

One factor which has helped qualitatively in the study of the vitamins D is the difference in their physiological behavior when administered to various species of animals. Although diverse cod liver oils are not entirely uniform in composition, an especially prepared sample has been used for many years as the reference standard in the United States. This is assumed to possess maximum activity in the case of the test animals commonly used, namely the rat and the chicken. On the other hand, although vitamin D₂ is very effective in curing rickets in the rat, it possesses very little potency in this respect for the chicken. Pure vitamin D₃, prepared from irradiated 7-dehydrocholesterol, is equally active in the case of the rat and of the chicken, and compares favorably with the vitamin D in cod liver oil. However, Bills and co-workers⁷² reported that the vitamin D isolated from the white sea bass (*Cynoscion nobilis*) is 300% more active in the chick than is cod liver oil, while that from the Pacific spiny dogfish (*Squalus suckleyi*) is about 230% more active. It has been suggested⁷⁷ that such unusually high potencies may be due to the presence of an unknown vitamin D rather than to some hypothetical synergistic factor.

Turkeys and chickens do not necessarily respond in the same manner to

²¹³ K. C. D. Hickman and E. Le B. Gray, *Ind. Eng. Chem.*, **30**, 796-802 (1938).

TABLE 4
DISTRIBUTION OF VITAMINS D IN VARIOUS FISH OILS^a

Source of oil	Zoological name	Potency, I.U. per g.
Blue-fin tuna, liver	<i>Thunnus thynnus</i>	40,000
Broadbill swordfish, liver	<i>Xiphias gladius</i>	10,000
Yellow-fin tuna, liver	<i>Neothunnus macropterus</i>	10,000
Black sea bass, liver	<i>Stercolepis gigas</i>	5,000
Boceaccio (rockfish), liver	<i>Sebastes paucispinis</i>	2,100
Red rockfish, liver	<i>Sebastes ruberrimus</i>	1,500
Black rockfish (priest fish), liver	<i>Sebastes mystinus</i>	1,500
China rockfish, liver	<i>Sebastes nebulosus</i>	1,400
"Ling cod," liver	<i>Ophiodon elongatus</i>	1,300
Chinook (king) salmon, liver	<i>Oncorhynchus tshawytscha</i>	1,300
Halibut, liver	<i>Hippoglossus hippoglossus</i>	1,200
Rabbitfish (burrfish), liver	<i>Chilomycterus schoepfi</i>	1,100
Striped rockfish (reina), liver	<i>Sebastes elongatus</i>	1,000
Starry flounder, liver	<i>Platichthys stellatus</i>	1,000
Boston (Atlantic) mackerel, liver	<i>Scomber scombrus</i>	750
Sablefish, liver	<i>Anoplopoma fimbria</i>	600
Atlantic pufferfish, liver	<i>Sphocroides maculatus</i>	570
Chum (dog) salmon, liver	<i>Oncorhynchus keta</i>	400
Black horse (blue sucker), mesentery	<i>Cycleptus elongatus</i>	400
Greenland halibut, liver	<i>Reinhardtius hippoglossoides</i>	260
Rex sole, liver	<i>Glyptocephalus zachirus</i>	150
Pacific sand-dab, liver	<i>Citharichthys (Orthopsetta) sordidus</i>	120
Cod, liver	<i>Gadus morrhua</i>	100
Herring, entire body	<i>Clupea harengus</i>	100
Lemon sole (Georges Bank flounder), liver	<i>Pseudopleuronectes americanus dig- nabilis</i>	90
Sardine (pilchard), entire body	<i>Sardinops caerulea</i>	80
Goosefish (sea devil), liver	<i>Lophius piscatorius</i>	70
Pollack liver	<i>Pollachius virens</i>	50
Menhaden, entire body	<i>Brevoortia tyrannus</i>	50
Shark, liver	<i>Selachioidei (spp.)</i>	50
Salmon, trimmings	<i>Oncorhynchus (spp.)</i>	40
Greenland halibut, body, minus liver	<i>Reinhardtius hippoglossoides</i>	30
Big skate, liver	<i>Raia binoculata</i>	25
Dogfish (Pacific), liver	<i>Squalus suckleyi</i>	20
Flathead (muddy) catfish, body	<i>Pilodictis (Leptops) olivaris</i>	20
Golden perch (fresh-water drum), mes- entery	<i>Aplodinotus grunniens</i>	11
Buffalo, mesentery	<i>Ictiobus (Megastomatobus) cyprinella</i>	10
Haddock, liver	<i>Melanogrammus aeglefinus</i>	10
Channel catfish (spotted), mesentery	<i>Ictalurus punctatus</i>	5
Spiny dogfish (Atlantic), liver	<i>Squalus acanthias</i>	3
Capelin, entire body	<i>Mallotus villosus</i>	3
Ratfish, liver	<i>Hydrolagus (Chimaera) colliciei</i>	2
Gray sole (witch flounder), liver	<i>Glyptocephalus cynoglossus</i>	<1
Sturgeon (Great Lakes), liver	<i>Acipenser rubicundus</i>	0

^a C. E. Bills, *Physiol. Revs.*, 15, 1-97 (1935), p. 13.

the different types of vitamins D.²¹⁴ Moreover, Russian workers²¹⁵ have reported that seal oil is a more effective antirachitic agent for man than it is for rats. Vitamin D₃ is reputedly somewhat more effective in man than is vitamin D₂.⁷⁷

1. Occurrence of the Vitamins D

Vitamin D is distributed in nature only to a limited extent. It is practically absent from the plant kingdom, although significant amounts of provitamins D occur in vegetables. Vitamin D has been observed^{216,217} to occur in vegetables. The presence of vitamin D has been noted^{216,217} in mushrooms growing in open woods, for instance, in the morel or May mushroom (*Morchella esculenta*), the edible boletus (*Boletus edulis*, *B. badius*), chanterelle (*Cantharellus cibarius*), and the edible turban-top (*Helvella mitra (esculenta)*). It occurs in insignificant amounts in the edible meadow (button) mushroom (*Agaricus (Psalliota) campestris*) grown commercially in dark cellars,²¹⁶ but was also found in slightly larger amounts in those growing in open meadows.²¹⁷ These provitamins may, in exceptional cases, be transformed into vitamin D in the plant, as has been reported for cacao shells (*Theobroma cacao*)²¹⁸ and for hay.²¹⁹

On the other hand, vitamin D is widely distributed in the animal kingdom, but the species which contain significant amounts of this substance are relatively few.²²⁰ The largest concentrations of the vitamins D are to be found in the fishes, where they are chiefly present in the liver and in some cases in the viscera. The amount of vitamin D present varies markedly in different species. Thus, the liver oil of the blue-fin tuna (*Thunnus thynnus*) contains as much as 40,000 I.U. per gram, while the liver oil of the European sturgeon (*Acipenser sturio*) is devoid of this vitamin. Morton²²¹ has indicated that the vitamin D content of fish liver oils may reach 200,000 I.U. per gram in exceptional cases. The comparative values in various fish liver oils are summarized in Table 4.

The vitamin D content of fish oils varies with the season and with such biological factors as age, food supply, climate, sex, and nutritional condition.²²² In the case of the halibut, there is a large amount of oil in the liver

²¹⁴ T. H. Jukes and T. D. Sanford, *J. Nutrition*, **18**, 71-85 (1939).

²¹⁵ E. J. Michlina, M. J. Leiserovskaia, and N. N. Milanova, *Kazan. Med. Chim. Zhur.*, **33**, 64-67 (1937); *Chem. Zentr.*, **169**, II, 3108 (1938); *Chem. Abstr.*, **34**, 5896 (1940).

²¹⁶ A. Scheunert and J. Reschke, *Deut. med. Wochschr.*, **57**, 349-351 (1931).

²¹⁷ A. Scheunert, M. Schieblich, and J. Reschke, *Z. physiol. Chem.*, **235**, 91-96 (1935).

²¹⁸ W. A. Knapp and K. H. Coward, *Analyst*, **59**, 474-478 (1934).

²¹⁹ H. Steenbock, E. B. Hart, C. A. Elvehjem, and S. W. F. Kletzien, *J. Biol. Chem.*, **66**, 425-440 (1925).

²²⁰ P. A. Coppens and G. A. Metz, *Arch. neerland. Physiol.*, **18**, 407-415 (1933).

²²¹ R. A. Morton, *Ann. Rev. Biochem.*, **11**, 365-390 (1942).

²²² E. Poulsson and F. Ender, *Skand. Arch. Physiol.*, **66**, 92-96 (1933).

during the summer with a low vitamin D content, while in the winter the livers contain less oil, but the latter has a higher vitamin D potency. The vitamin D content of the Norwegian cod, which is caught at the time when the nutritional condition is poor, following spawning, is higher than that of the Newfoundland cod, which is in a much better nutritional state when obtained.²²² Those fishes which have a low content of liver fat generally belong to the species in which the highest content of vitamin D is found.²²³ Fish roe²²⁴ and turtle eggs contain moderate amounts of the vitamin. In the case of the latter, it has been demonstrated that the season of the year is an exceedingly important factor, as is also the nature of the food.

In addition to the fishes, such lower forms as the copepods of the zooplankton contain some vitamin D.^{225,226} This is a probable source of the vitamin D in the cod, which consumes large quantities of zooplankton.

There is practically no vitamin D in the fat of other animals.²²⁰ The marine birds, which live largely on fish, are an exception to this rule, but here the vitamin D deposited is obviously the result of the large amount constantly available in the diet. In the case of higher animals, a concentration of vitamin D is found in the milk and eggs in sufficient amounts to afford the essential quantity necessary to assure the growth of the newborn.

Cow milk is, in general, a relatively poor source of vitamin D.²²⁷ Ordinarily it contains about 5 to 8 I.U. per quart, in the case of cows kept indoors, while values of 17 to 26 I.U. per quart are found in the milk of cows kept outdoors in June.²²⁸ Bechtel and Hoppert²²⁹ reported figures of 5 I.U. for winter milk and 44 I.U. for summer milk. The value of 35 I.U. per quart is based upon the vitamin D content of butter, or 8-60 I.U. per 100 grams.²³⁰ Human milk has a somewhat higher content of vitamin D than cow milk; Drummond and co-workers²³¹ obtained a value of 60 I.U. per quart for this product. Colostrum, the milk secreted during the first few days after the birth of the young, contains from 6 to 10 times the amount of vitamin D that is present in mature milk in the case of the cow,²³² and about 3 times the amount in the case of the human.

²²² A. F. Hess, C. E. Bills, and E. M. Honeywell, *J. Am. Med. Assoc.*, *92*, 226-229 (1929).

²²⁴ E. P. Daniel and H. E. Munsell, *U. S. Dept. Agr. Misc. Pub.*, No. 275, 1-176 (1937).

²²⁵ A. M. Copping, *Biochem. J.*, *28*, 1516-1520 (1934).

²²⁶ J. Drummond and E. R. Gunther, *J. Exptl. Biol.*, *11*, 203-209 (1934).

²²⁷ H. E. Honeywell, R. A. Dutcher, and C. D. Dahle, *J. Nutrition*, *2*, 251-256 (1930).

²²⁸ J. E. Campion, K. M. Henry, S. K. Kon, and J. Mackintosh, *Biochem. J.*, *31*, 81-88 (1937).

²²⁹ A. E. Bechtel and C. A. Hoppert, *J. Nutrition*, *11*, 537-549 (1936).

²³⁰ H. J. Heinz Co., *Nutritional Data*, Pittsburgh (1950).

²³¹ J. C. Drummond, C. H. May, and N. E. G. Richardson, *Brit. Med. J.*, *II*, 757-760 (1939).

²³² J. van Niekerk and M. S. C. Blicik, *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, *9*, 25-26 (1939).

The concentration of vitamin D in milk is increased by several methods in commercial practice. One of these involves a direct addition of vitamin D concentrate to milk, to yield a potency of 150 or 300 I.U. per quart.^{233,234} Propylene glycol is frequently employed as a solvent. A second procedure of little importance commercially because of the slight effectiveness is the direct irradiation of the cow with sunlight or with artificial ultraviolet light.^{223,229} However, the potency of cow milk may be increased from 5 I.U. per quart in the unirradiated animals to 17–26 I.U. per quart,²²³ or even as high as 44²²⁹ I.U. after the animals have been exposed to sunlight.

One of the most widely employed methods for the augmentation of the vitamin D content of milk entails the feeding of large doses of irradiated yeast to the cows. When the quantities of such vitamin D administered are controlled, a so-called "metabolized" vitamin D milk is produced which contains a minimum of 400 I.U. per quart. Some years ago Krauss and Bethke^{235,236} demonstrated that the vitamin D content of milk is increased by feeding irradiated ergosterol to the cows. Irradiated yeast feeding was later shown to be even more effective.^{237,233} A cod liver oil concentrate, Vitex, was also fed, with good results.²³⁹ The ewe, likewise, has been shown to respond to feedings of irradiated yeasts and molds by producing milk with an increased vitamin D content, while irradiated ergosterol was found to be less effective in producing this increase,²⁴⁰ when fed in equivalent amounts.

Still another procedure for the production of high vitamin D milk employs the direct irradiation of the milk. While this is the cheapest method, it is also the most difficult one with which to produce satisfactory results, as over-irradiation produces milk with an unpleasant taste and odor.²⁴¹ Specific methods which have been proposed to overcome this difficulty have been reviewed by Diemair.²⁴² There is a sufficient amount of pro-vitamin D in milk to allow for a maximum production of 200 I.U. per quart; however, the vitamin D level in commercial irradiated milks has been standardized to a vitamin D content of 135 I.U. per quart.

The eggs of most birds are also good sources of vitamin D. It occurs ex-

²³³ T. F. Zucker, *Am. J. Pub. Health*, 23, 10–18 (1923).

²³⁴ D. J. Barnes, *J. Mich. State Med. Soc.*, 22, 242–246 (1933).

²³⁵ W. E. Krauss and R. M. Bethke, *J. Biol. Chem.*, 92, x–xi (1931).

²³⁶ W. E. Krauss, R. M. Bethke, and C. F. Monroe, *J. Nutrition*, 5, 467–477 (1932); *Ohio Agr. Exptl. Sta., Bimonthly Bull.*, No. 156, 117–121 (1932).

²³⁷ B. H. Thomas and F. L. MacLeod, *Science*, 73, 618–620 (1931).

²³⁸ W. E. Krauss and R. M. Bethke, *Ohio Agr. Exptl. Sta., Bimonthly Bull.*, 18, No. 162, 77–80 (1933).

²³⁹ W. E. Krauss, R. M. Bethke, and M. Wilder, *Ohio Agr. Exptl. Sta., Bimonthly Bull.*, 18, No. 1, 15–19 (1933).

²⁴⁰ W. O. Kirk, *Iowa State Coll. J. Sci.*, 13, 235–238 (1939).

²⁴¹ K. G. Weckel and H. C. Jackson, *Food Research*, 1, 419–426 (1936).

²⁴² W. Diemair, *Chem. Fabrik*, 14, 51–54 (1941).

TABLE 5
RELATIVE EFFECTIVENESS OF VITAMIN D FROM DIFFERENT SOURCES FOR RATS AND CHICKENS^a

Zoological name	Name of fish oil or sterol	Vitamin D per 100 g. dist., ^b I.U.	Response obtained		Efficacy ratio, %
			Femur ash, %	Vitamin D per 100 g., I.U.	
<i>Hippoglossus hippoglossus</i>	Halibut	17.5	45.42	15.1	86
<i>Eopsetta jordani</i>	Round-nosed sole (flounder)	18.9	45.57	15.4	81
<i>Thunnus thynnus</i>	Tuna, blue-fin (Cal.)	7.2	35.98	<i>c</i>	<i>c</i>
		57.6	42.55	9.3	16
		42.4	41.03	7.4	17
<i>Thunnus secundadorsalis</i>		84.9	45.92	16.5	19
	Tuna, New England	7.9	39.99	6.4	81
<i>Gerno alalunga</i>		9.2	41.45	8.0	87
	Albacore	20.5	44.42	12.6	61
<i>Neothunnus macropterus</i>	Tuna, yellow-fin	61.4	46.80	19.7	32
	Tuna (oceanic bonito) striped	10.7	45.23	14.6	136
<i>Katsuwonus vagans</i>		9.6	35.62	<i>c</i>	<i>c</i>
		56.5	45.12	14.3	25
<i>Sarda sarda</i>		8.4	34.31	<i>c</i>	<i>c</i>
	Atlantic bonito	58.0	45.93	16.5	28
<i>Scomber (Pneumatophorus) diego</i>	Pacific mackerel	9.8	44.01	11.8	120
<i>Xiphias gladius</i>	Swordfish	10.1	45.53	15.3	151
<i>Stereolepis gigas</i>	Black sea bass	7.8	41.98	8.5	109
<i>Epinephelus analogus</i>	Cabrilla	11.0	40.83	7.2	65
<i>Cynoscion nobilis</i>	White sea bass	2.8	42.19	8.8	314
<i>Erisicion macdonaldi</i>		6.0	45.74	15.9	265
	Totuava	10.0	34.90	<i>c</i>	<i>c</i>
		59.8	44.47	12.7	21

Zoological name	Name of fish oil or sterol	Vitamin D per 100 g. dist., ^b I.U.	Response obtained		Efficacy ratio, %
			Femur ash, %	Vitamin D per 100 g., I.U.	
<i>Anoplopoma fimbria</i>	Sablefish	6.8	43.59	11.0	162
<i>Ophiodon elongatus</i>	Ling cod	9.8	45.64	15.6	159
<i>Sebastes paucispinis</i>	Bocaccio (rockfish)	15.8	45.66	15.7	99
<i>Anarhichas lupus</i>	Atlantic wolf-fish	8.7	44.46	12.7	146
<i>Cetorhinus maximus</i>	Basking shark	9.9	43.08	10.1	102
<i>Squalus suckleyi</i>	Pacific spiny dogfish	5.2	41.94	8.4	162
<i>Pollachius virens</i>	Green pollack (coalfish)	4.0	42.50	9.2	230
<i>Urophycis chuss</i> and <i>U. tenuis</i>	Squirrel hake, mud hake	8.5	38.12	4.3	51
<i>Sardinops caerulea</i>	Sardine (pilchard)	8.7	43.91	11.6	133
<i>Gadus morhua</i>	Cod (control)	10.7	43.96	11.7	109
		10.0	42.97	9.9	99
		8.0	42.08	8.6	108
		8.0	40.89	7.3	91
		8.0	41.53	8.0	100
		4.0	38.81	5.1	128
	Maize oil (control)	0.0	35.12	^c	
	Irradiated ergosterol	200	40.00	6.4	3.2
		403	43.30	10.4	2.6
		1800	46.50	18.5	1.0
	Irradiated cholesterol	8.0	41.72	8.2	103
	Irradiated 7-dehydrocholesterol	13.0	43.93	11.7	90

^a Adapted from C. E. Bills, O. N. Massengale, M. Imboden, and H. Hall, *J. Nutrition*, 13, 435-452 (1937), p. 442.

^b Based on assays on rats.

^c Response too low for significant interpretation.

clusively in the yolk, dissolved in the fatty substances in that portion of the egg. The average content of the yolks of hen eggs has been reported as 300 I.U. per 100 grams.²³⁰ Although the values for vitamin D have been shown to remain fairly constant with ordinary diets, increased amounts of vitamin D can be introduced into the egg when the hen is irradiated,²⁴³ or when the diet of the hens contains larger amounts of vitamin D.^{244,245}

The type of vitamin D which is excreted in the milk¹⁰⁶ and in the egg¹⁰⁵ is the same as that ingested. This was beautifully demonstrated by Bethke and his co-workers,¹⁰⁶ who showed that about ten times as much vitamin D given as "metabolized" vitamin D milk was required for the chick as was the case when irradiated milk was used. In the first case the vitamin arising from the irradiated yeast was D₂; this is very ineffectively utilized by chickens.²⁴⁶ In the irradiated milk, the product formed was vitamin D₃, which resulted from the activation of the 7-dehydrocholesterol already present in milk. This form of vitamin D is well utilized by chickens.

Unfortunately, the records for the distribution of vitamins D do not indicate which type of vitamin D is present. Brockmann and Busse were the first to prepare vitamins D₃ and D₂ from halibut liver and tuna liver oils, respectively.¹⁰⁷ Although both vitamins D₂ and D₃ have been isolated from fish liver oils, it is known that vitamin D₃ is usually present in a larger proportion. Moreover, Bills and co-workers^{70,72} have suggested that at least six different vitamins D occur in cod liver oil. The efficacy of different fish liver oils in preventing or curing rickets in the rat (vitamin D₂ or D₃) and the chicken (vitamin D₃) offers a clue to the varying proportions of vitamins D in the different oils. These data are summarized in Table 5.

2. Properties of the Vitamins D

The known vitamins D have properties which are almost identical. They are white crystalline solids which are soluble in organic (fat) solvents and insoluble in aqueous media. They all have an absorption maximum at 265 m μ in hexane or in diethyl ether.

(1) Vitamin D₂

Vitamin D₂, also referred to as calciferol or activated ergosterol, has a molecular weight of 396. It melts at 115–117°C. and has a specific rotation ($[\alpha]_D^{25}$) of +103° in absolute alcohol, +82.6° in acetone, +33.3° in petroleum ether, and +91.2° in diethyl ether.¹⁰³

²⁴³ G. H. Maughan and E. Maughan, *Brit. J. Phys. Med.*, **7**, 137–138 (1932).

²⁴⁴ G. H. Maughan and E. Maughan, *Science*, **77**, 198 (1932).

²⁴⁵ G. M. De Vaney, H. E. Munsell, and H. W. Titus, *Poultry Sci.*, **12**, 215–222 (1933).

²⁴⁶ O. N. Massengale and M. Nussmeier, *J. Biol. Chem.*, **87**, 423–426 (1930).

In hexane or diethyl ether, vitamin D₂ has an absorption maximum at 265 m μ ¹⁰⁸ and an absorption minimum at 310 m μ . The molecular extinction coefficient (ϵ) is 18,200¹⁰⁸, while the value for E (1%, 1 cm.) equals 460^{159,247} at 265 m μ ; the figures are both 0 at the minimum point on the curve (310 m μ). The complete absorption curve is given in Figure 7.

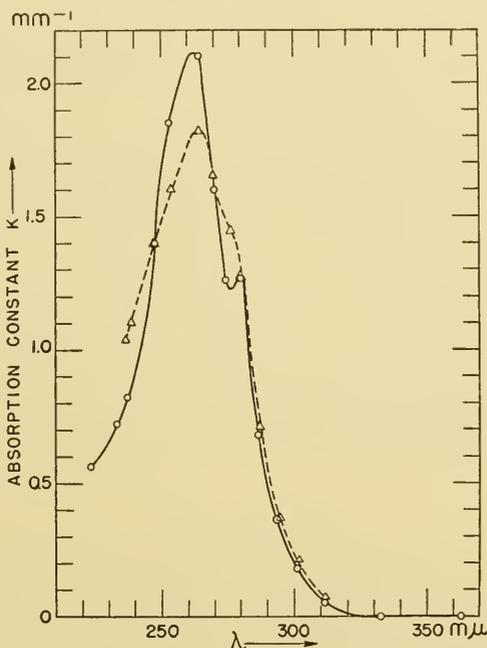


Fig. 7. The absorption spectra of vitamin D₂ (O—O) and of vitamin D₃ (Δ - Δ) in 0.02% solution in hexane.^{55,57} $K = 45$ to 46×10^3 .

Two crystalline esters of vitamin D₂ can be readily prepared. These include the 3,5-dinitrobenzoate, which melts at 148–149°C. and has an optical rotation ($[\alpha]_D^{20}$) in benzene of +55°. The second common ester is the *p*-nitrobenzoate, which melts at 93°C., and has a specific rotation, ($[\alpha]_D^{20}$) of +104° in chloroform.

Crystalline vitamin D₂ is stable over long periods when stored in the dark in sealed tubes from which oxygen has been excluded. It is also quite stable when dissolved in olive oil and stored under similar conditions. After 5 years, more than 50% was found to be still present.

Vitamin D₂ is destroyed by heat. In spite of the fact that it can be sublimed at 125°C. if a high vacuum is employed, it is partially decomposed into pyrocalciferol and isopyrocalciferol, although these decomposition

²⁴⁷ S. K. Crews and E. L. Smith, *Analyst*, 64, 568–570 (1939).

products are more readily formed at 160–190°C. Vitamin D₂ is generally considered to have a biopotency of 40,000,000 I.U. per gram. Although it possesses maximum activity as an antirachitic agent for the rat, it is almost completely ineffective for the chicken.

(2) Vitamin D₃

Vitamin D₃, or activated 7-dehydrocholesterol, has a molecular weight of 384. It melts more than 30° lower than vitamin D₂, at 82–83°C. The specific rotation ($[\alpha]_D^{20}$) in acetone is +83.3°. ¹⁰⁸

The absorption maximum in hexane is identical with that of vitamin D₂, namely, at 265 m μ . However, it has slightly higher extinction coefficients. The molecular extinction (ϵ) is reported as 19,200, ¹⁰⁸ while the *E* (1%, 1 cm.) value is 500. The complete absorption curve is given in Figure 7.

Vitamin D₃, likewise, forms crystalline esters with the nitrobenzoic acids. The 3,5-dinitrobenzoate develops two polymorphic forms which melt at 129° and 140°C. and have $[\alpha]_D^{20} = +98^\circ$ in chloroform. The *p*-nitrobenzoate ester melts at 127°C. and shows an optical rotation ($[\alpha]_D^{20}$) of +114° in chloroform. Vitamin D₃ has a biopotency of 40,000,000 I.U. per gram. It is equally effective for rats and for chickens. ⁵³

(3) Vitamin D₄

Vitamin D₄, or activated 22-dihydroergosterol, melts at 107–108°C. ²⁴⁸ The specific rotation in acetone was found to be +89.3°. Vitamin D₄ also forms a 3,5-dinitrobenzoate ester which melts at 135–136°C. and has a $[\alpha]_D^{18}$ value of +94.5° in acetone. It has been shown to have a biopotency in rats of from 20,000,000 to 30,000,000 I.U. per gram; in the case of the chicken, it was found to have an activity of approximately 20% of that in the rat. ⁷⁷ McDonald ²⁴⁹ indicates that it has a greater effectiveness than has irradiated ergosterol in the chicken, but less than that of cod liver oil. However, it compares favorably with some samples of tuna oil in this respect.

(4) Other Vitamins D

Little can be said about the properties of the other members of the vitamin D group. Some data on biopotencies have, however, been reported. Vitamin D₅ (activated 7-dehydrositosterol) has an activity of 500,000 I.U. per gram in rats, while its effectiveness in the chick is 1 : > 13. ²⁵⁰

²⁴⁸ A. Windaus and G. Trautmann, *Z. physiol. Chem.*, **247**, 185–188 (1937).

²⁴⁹ F. G. McDonald, *J. Biol. Chem.*, **114**, lxx (1936).

²⁵⁰ W. Grab, *Z. physiol. Chem.*, **243**, 63–89 (1936).

Activated 7-dehydrostigmasterol is potent only to the extent of 100,000 I.U. per gram in the rat, while its activity in the chicken is uncertain.⁷⁷ The biological response is of such a low order that one may question its inclusion as one of the D vitamins.

The effectiveness of mussel vitamin D is equal, in the rat,⁸² to that of vitamins D₂ or D₃; in the chicken it has 100%⁸² of the efficacy which it displays in the case of the rat. This represents an activity similar to that of vitamin D₂. Activated epi-7-dehydrocholesterol has been found to be 10% as active as vitamin D₃ in the rat, while its potency in the chicken is as yet undetermined.⁷⁷ The activated form of 22,23-oxidoergosterol is only "feebly" active.⁷⁷ The potencies of other substances possessing vitamin-D-like activity in the rat are the following (per gram): activated 5,7-androstadiene-3,17-diol, 100,000 I.U.^{78,83}; activated 3-hydroxy-5,7-choladienic acid, 100,000 I.U.⁸⁴; and dihydrotachysterol, 200,000 I.U.⁸¹

3. Standards for the Vitamins D

Because of the varying potency of different vitamin D preparations, a standard by which to gauge unknown samples is necessary. This is particularly advisable inasmuch as no satisfactory chemical methods are available, especially when it becomes essential to differentiate among the different types of the vitamin D.

The International Unit (I.U.) of vitamin D is considered to be the primary universal standard. It was defined by an International Vitamin Conference held by the League of Nations in 1934²⁵¹ as the effect caused by 0.025 μ g of pure crystalline vitamin D₂ dissolved in one milligram of olive oil. The following properties are ascribed to the preparation used as the standard:⁷⁷

Calciferol or vitamin D₂, C₂₈H₄₈OH

(a) Colorless, acicular crystals, odorless. M.p. 114.5–117 °C. in open capillary.

(b) Specific rotation:

in alcohol, $[\alpha]_D^{20} = +101^\circ$ to $+102.5^\circ$

$[\alpha]_{5461}^{20} = +119^\circ$ to $+122^\circ$

in chloroform, $[\alpha]_D^{20} = +52^\circ$

$[\alpha]_{5461}^{20} = +62^\circ$

(c) Absorption spectrum. In alcohol or other suitable non-absorbing solvent, it shows a smooth curve with maximum at 265 m μ . E (1%, 1 cm.) = 470–485.

This replaces the earlier International Unit of 1931, which is now reported to be unsatisfactory.²⁵²

²⁵¹ *League of Nations Quarterly Bull. Health Organization*, 4, No. 3 (Sept., 1935), 540–542. Memorandum on the International Standard for Vitamin D and Its Application.

²⁵² N. T. Gridgeman, H. Lees, and H. Wilkinson, *Analyst*, 65, 493–496 (1940).

However, secondary standards are employed in the United States and in Great Britain. In the United States we have the U.S. Pharmacopoeia Reference cod liver oil, while in England a similar standard is used for the M.R.C. Unit (Medical Research Unit). The reference cod liver oil used here has been accurately standardized against the International Standard by bioassays on rats, and its potency has been determined on the basis of such tests. The U.S.P. units refer to those established by the use of the U.S.P. Reference cod liver oil. Since birds, and especially chickens, are unable to utilize vitamin D₂ efficiently, the International Standard is not of importance in establishing the potency of vitamin preparations used in chick feeding. However, the standard employed in this case is the "A.O.-A.C. Chick Unit," introduced by the Association of Official Agricultural Chemists, which is defined²⁵³ as the potency of one unit of vitamin D of the U.S.P. Reference cod liver oil when determined under standard conditions. Since the vitamin D₃ content of various cod liver oils varies in relation to its vitamin D₂ content, it would be advisable to replace the chick standard by one composed of crystalline vitamin D₃. Still better, if the International Standard were changed to crystalline vitamin D₃ instead of vitamin D₂, the primary standard would serve equally well for the rat and for the chicken.

One International Unit is therefore equivalent to one U.S.P. Unit, one M.R.C. Unit, one Coward Unit, 5-6 Poulsson Units, 6-8 Laquer Units, 2.6 Prophylactic Units, 3.25 ADMA Units, 1.66 Oslo Units, or 0.025 mg. of crystalline vitamin D₂.

4. Structures of the Vitamins D

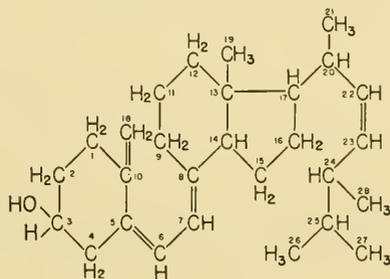
The provitamins and the corresponding vitamins have closely related structures; each pair has the same empirical formula, and thus it is obvious that they must represent isomers. Moreover, the several D vitamins differ from each other only in the side chain; the sterol structure of the nucleus is the same in all cases for the provitamins, and the modified structure derived from the steroid nucleus after activation of the provitamins is the same for all of the vitamins.

Although the structural formulas have not been worked out for all the known D vitamins, very complete proof is available in the case of vitamin D₂. It has been possible to demonstrate by certain key reactions that the general structure of vitamin D₃ (exclusive of the side chain) is identical with that of vitamin D₂. In the case of the other D vitamins, the formulas have been deduced by analogy.

²⁵³ Association of Official Agricultural Chemists, *Methods of Analysis*, 5th ed., 1940, pp. 371-373.

(1) Vitamin D₂

As early as 1932, Windaus and co-workers¹¹⁵ demonstrated that vitamin D₂ has an empirical formula of C₂₈H₄₄O, which is identical with that of ergosterol, from which it is derived. As a result of the investigations of a large number of workers, the structure of vitamin D₂ has been thoroughly elucidated. The formula which is universally accepted is given here.

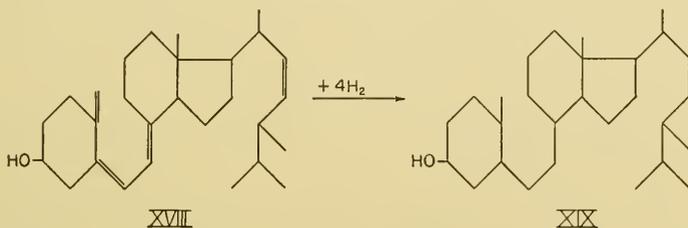


XVIII

Vitamin D₂

The oxygen present in the molecule must be in an hydroxyl group, since vitamin D₂ is usually present in nature in the form of an ester. The position of this secondary alcohol group must logically be on position 3; this is the location where it is invariably found in a large variety of sterols, some of which are provitamins D.

Important information on the structure of vitamin D₂ (XVIII) can be gleaned from the number and position of the double bonds. When this vitamin is catalytically hydrogenated, 4 molecules of hydrogen are absorbed (XIX—octahydrovitamin D₂), which can be interpreted only as indicative of the presence of 4 unsaturated linkages.²⁵⁴



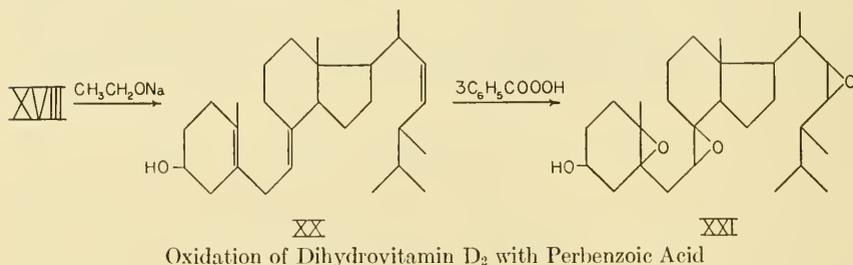
XVIII

XIX

However, only 3 double bonds can be demonstrated by titration with perbenzoic acid. This discrepancy between the two different methods for the demonstration of the double bonds can be brought into harmony by employing a different preliminary procedure for the perbenzoic acid tests. If vitamin D₂ (XVIII) is first reduced with sodium ethylate, one molecule of hydrogen is absorbed and a dihydrovitamin D₂ (XX) is produced. Two

²⁵⁴ R. Kuhn and E. F. Möller, *Angew. Chem.*, 47, 145-149 (1934).

different isomers of dihydrovitamin D₂ are simultaneously formed. In the case of dihydrovitamin D₂ I, 3 additional double bonds are demonstrable by oxidation,²⁵⁵ and a crystalline trioxide (XXI) is formed.²⁵⁶



Since vitamin D₂ has thus been shown to have 4 double bonds instead of the 3 present in ergosterol, while at the same time both the provitamin D₂ and the vitamin D₂ have an identical number of carbon and hydrogen atoms, the conclusion is inescapable that vitamin D₂ can have only 3 rings in place of the 4-ring system present in the sterols. This supposition is further borne out by the results obtained when vitamin D₂ is subjected to dehydrogenation with selenium. Whereas all sterols yield the same characteristic hydrocarbon, namely, γ -methylcyclopentenophenanthrene, on such treatment, vitamin D₂ and tachysterol₂ fail to produce the above typical hydrocarbon.¹⁸⁸

The position of the double bonds is the next consideration of importance in establishing the structure of vitamin D₂. One of these occurs in the side chain between carbons 22 and 23, just as it does in ergosterol, since on ozonolysis of vitamin D₂ methylisopropylacetaldehyde, (CH₃)₂·CH·CH·(CH₃)·CHO, is formed.¹²²

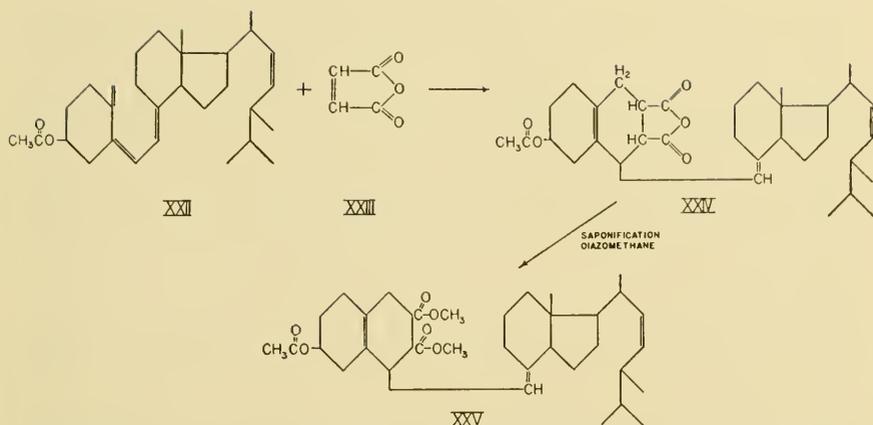
The fact that the remaining 3 double bonds are probably in conjugation with each other can be deduced from the absorption spectrum and the magnitude of the extinction coefficient. Absolute proof of the conjugation of two of its double bonds is afforded by the fact that vitamin D₂ acetate (XXII) forms an addition product with maleic anhydride (XXIII).²⁵⁷

The maleic anhydride addition product of vitamin D₂ acetate (XXIV) has been the starting material for carrying out the several reactions necessary for the complete elucidation of the remaining facts concerning the structure of vitamin D₂. When the latter compound is subjected to saponification, a dicarboxylic acid is formed; on treatment with diazomethane this product forms the corresponding dimethyl ester (XXV), which exists in two isomeric forms.

²⁵⁵ A. Windaus and C. Roosen-Runge, *Z. physiol. Chem.*, **260**, 181-184 (1939).

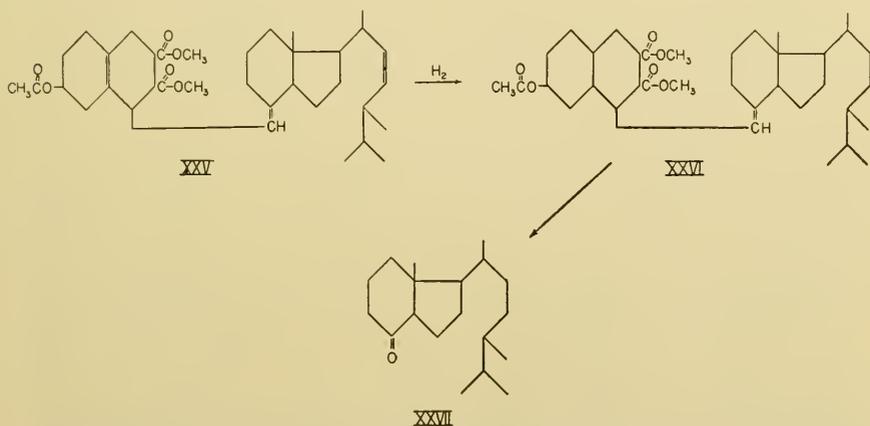
²⁵⁶ S. v. Reichel and M. Deppe, *Z. physiol. Chem.*, **239**, 143-146 (1936).

²⁵⁷ A. Windaus and W. Thiele, *Ann.*, **521**, 160-175 (1935).



Preparation of the Dimethyl Ester of the Maleic Anhydride Addition Product of Vitamin D₂ Acetate (XXV)

The first reaction of the dimethyl ester (XXV) which is useful in establishing the structure of vitamin D₂ involves catalytic hydrogenation followed by ozonolysis of the dihydro compound (XXVI) so obtained. The chief product of the reaction with ozone is a saturated ketone, C₁₉H₃₄O (XXVII), as determined by analysis of its semicarbazone and of its oxime. The number of carbons would seem to indicate that this compound consists of rings C and D of the ergosterol molecule, the side chain, and the angular methyl group located on C₁₃ between rings C and D. Since no methylisopropylacetaldehyde results on treatment with ozone, one must conclude that the double bond in the side chain has previously been saturated.

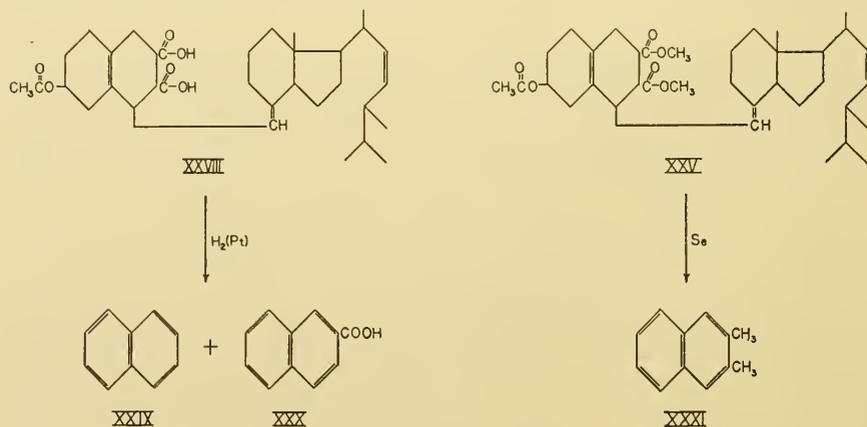


Formation of a Ketone (XXVII) Following Ozonolysis of the Reduction Product (XXVI) of the Dimethyl Ester of the Maleic Acid Addition Product of Vitamin D₂ Acetate (XXV)

The ketone group must be located on what was the C ring of the ergosterol molecule. Presumably it would occur at the position where the C ring was attached to the rest of the molecule. In ergosterol, this connection is through carbons 8 and 9. However, since only one carbon in the saturated ketone has been oxidized, it would appear that, in vitamin D₂, ring B no longer exists and only one point of combination with the rest of the molecule occurs. This hypothesis is supported by earlier evidence that only 3 rings are possible in vitamin D₂, as contrasted with the 4 cyclic groups in ergosterol.

The fact that the point of union of ring C with the rest of the molecule is changed to a ketone group, indicates that the ketone has replaced a double bond. Such a double bond could not occur between carbons 9 and 10, since C₁₀ is quaternary. It then follows that the ketone can only be at C₈, and the double bond in vitamin D₂ must have a linkage between carbons 7 and 8. The cleavage of ring B of ergosterol must have occurred between C₉ and C₁₀ before vitamin D₂ synthesis can take place.

The final proof of the structure of ring A, and the nature of the connection between ring A and the portion of the molecule isolated as the saturated ketone, can be deduced by reduction of the original dimethyl ester and its acid. When this is carried out on the free dicarboxylic acid (XXVIII) by platinum dehydrogenation, naphthalene (XXIX) and naphthoic acid (XXX) are the products. On the other hand, when a selenium dehydrogenation is performed on the diester (XXV), 2,3-dimethylnaphthalene (XXXI) is obtained. Earlier work in which a dehydrogenation of dicarboxylic acid esters was carried out proved that the methyl groups originate from the carboxyl groups.²⁵⁸



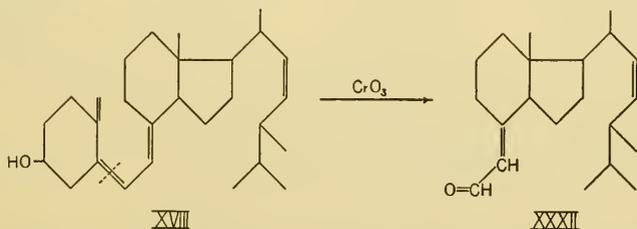
Reduction Reactions of the Dicarboxylic Acid (XXVIII) and of the Dimethyl Ester (XXV) of the Maleic Acid Anhydride Addition Product of Vitamin D₂ Acetate

²⁵⁸ W. Thiele and G. Trautmann, *Ber.*, 68, 2245-2247 (1935).

An interpretation of the above results allows one to deduce the positions of the third and fourth double bonds in vitamin D₂. In the first place, it is indicated that when maleic anhydride combines with vitamin D₂, a hydronaphthalene derivative is formed. A reaction of this nature is possible only when the two new bonds are in conjugation with the third double bond known to be at the 7,8 position. This would place the two new bonds in vitamin D₂ at the 5,6 and 10,18 positions. The addition of maleic anhydride has apparently been on C₆ and C₁₈.

Further proof that the side-chain methyl group, designated as number 18 in ergosterol, has become a methylene group in vitamin D₂ has been furnished by oxidation experiments. Thus, an acid permanganate converts this methylene group into formic acid; in the presence of ozone it yields formaldehyde.²⁵⁷

These results are sufficient to establish the structure of vitamin D as given earlier. Moreover, other types of approach have yielded confirmatory evidence that the postulated structure for vitamin D₂ is correct. Thus, permanganate oxidation yields, in addition to formic acid, an unsaturated ketone which can be changed to the same saturated ketone (XXVII) obtained by direct oxidation of the 22-dihydro derivative, when selective hydrogenation is employed. If vitamin D₂ (XVIII) is carefully oxidized with chromic acid, a rupture of the 5,6 bond obtains, and one is able to isolate the doubly unsaturated aldehyde (XXXII).²⁵⁹ This compound corresponds to the products which one would expect to obtain from the postulated vitamin D₂.⁷⁷



a. Pyrocalciferol and Isopyrocalciferol. When vitamin D₂ is heated at 160–190°C. in the absence of air, it is converted to two new products—pyrocalciferol and isopyrocalciferol. These are of aid in establishing the structure, not only of vitamin D₂ but also of some of the intermediate irradiation products. These pyro-compounds separate as crystalline addition products; after acetylation they can be isolated by fractional crystallization.

On the basis of molecular weight determinations and of elementary analyses, one must conclude that they, also, are isomers of vitamin D₂ and

²⁵⁹ I. M. Heilbron, R. N. Jones, K. M. Samant, and F. S. Spring, *J. Chem. Soc.*, 1936, 905–907.

of ergosterol. It is believed, on the basis of catalytic hydrogenation¹⁹⁶ as well as of perbenzoic acid titration,²⁶⁰ that the pyro-compounds contain only three double bonds. This would indicate that a ring closure occurs when the pyrocompounds are formed. Furthermore, when dehydrogenated with selenium, pyro- and isopyrocalciferols yield γ -methylcyclopentophenanthrene, which is the characteristic product yielded by compounds having the intact steroid nucleus.¹⁹⁶ This latter evidence seems to prove that the ring closure involves the same carbons which were concerned in the case of the ring cleavage when ergosterol was changed to vitamin D₂.

Of the three double bonds in the pyrocalciferols, one obviously is in the side chain between carbons 22 and 23. The remaining two double bonds are in conjugation, since the pyro-compounds form addition products with maleic anhydride. Further proof that they exist on the same ring is afforded by their behavior when subjected to nitric acid oxidation; in this case, toluene 2,3,4,5-tetracarboxylic acid is obtained.¹²²

The difference between pyro- and isopyrocalciferol is probably one of steric configuration. These relationships are indicated in Table 6.

TABLE 6
COMPARISON OF PROPERTIES OF PYRO- AND ISOPYROCALCIFEROL WITH THOSE OF ERGOSTEROL

Treatment employed	Results of reaction on		
	Pyrocalciferol	Isopyrocalciferol	Ergosterol
Digitonin ^a	No precipitation	Precipitation	Precipitation
Dehydrogenation with eosin in visible light ^b	Bimolecular compound	No reaction	Bimolecular compound
Dehydrogenation with mercuric acetate	$\Delta^9,11$ -Dehydro-lumisterol ₂ ^a	$\Delta^9,11$ -Dehydro-ergosterol ^c	$\Delta^9,11$ -Dehydro-ergosterol ^{c,d,e}

^a H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945.

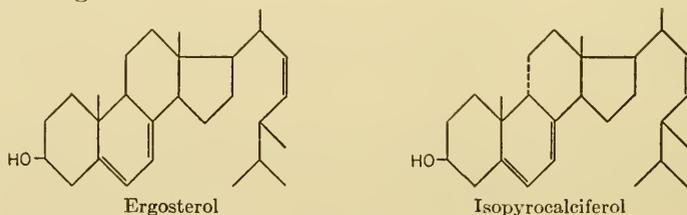
^b T. Kennedy and F. S. Spring, *J. Chem. Soc.*, 1939, 250-253.

^c A. Windaus and K. Dimroth, *Ber.*, 70, 376-379 (1937).

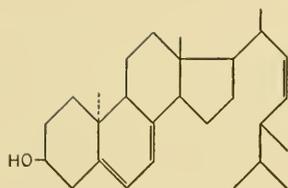
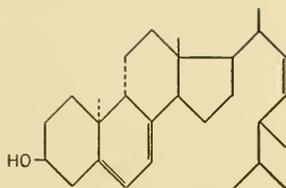
^d I. M. Heilbron, F. S. Spring, and P. A. Stewart, *J. Chem. Soc.*, 1935, 1221-1223.

^e M. Müller, *Z. physiol. Chem.*, 231, 75-84 (1935).

From the data presented in Table 6, it is evident that isopyrocalciferol and ergosterol differ only in spatial arrangement in position 9, while lumisterol₂ and pyrocalciferol differ from ergosterol and isopyrocalciferol solely by the position of the substituents on carbon 10. Comparative formulas are given here.



²⁶⁰ P. Busse, *Z. physiol. Chem.*, 214, 211-222 (1933).

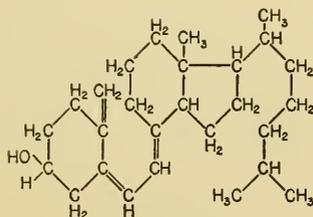
Lumisterol₂

Pyrocalciferol

The pyro-compounds are broken down on irradiation with ultraviolet light, but apparently an entirely different set of reactions obtains from that which occurs when ergosterol is subjected to similar treatment.¹⁸⁹ Furthermore, the pyro- and isopyrocalciferol have no antirachitic efficacy, and no biologically active products can be demonstrated after their irradiation. The ineffectiveness of the irradiation products is further corroborated by the observation that no absorption bands occur in the critical spectral region between 248 and 320 m μ . Finally, the reactions caused by irradiation of the pyro- and isopyro-compounds are reversible, and presumably involve only a rearrangement of the double bonds on ring B.

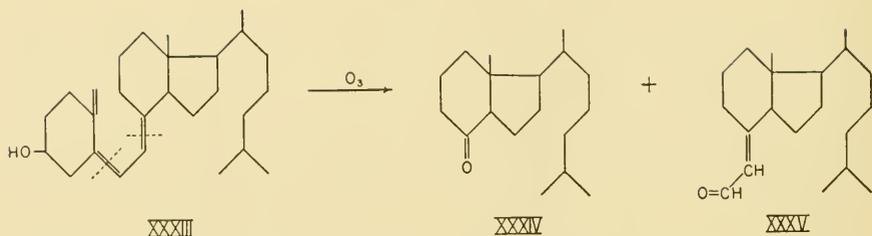
(2) Vitamin D₃

Since vitamin D₃ is derived from 7-dehydrocholesterol, by a series of reactions similar to those which occur when vitamin D₂ is synthesized from ergosterol, one can tentatively assign the accompanying formula to vitamin D₃ (XXXIII).



XXXIII

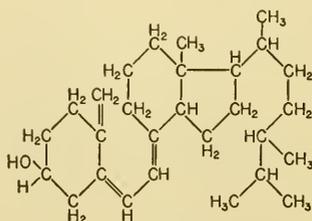
When vitamin D₂ is oxidized with ozone, formaldehyde, a saturated ketone (XXXIV), and an unsaturated aldehyde (XXXV) are the products formed.^{107, 108} These compounds are characterized by the semicarbazones and by the 2,4-dinitrophenylhydrazone derivatives. Thus, it would appear that such compounds could originate only by cleavage of the 10,18, 5,6, or 7,8 double bonds. Since these are the same positions at which the unsaturated linkages occur in the case of vitamin D₂, it would appear to be definite that the structure is identical with the latter except for the side chain on C₁₇.



a. **Pyrovitamin D₃ and Isopyrovitamin D₃.** When vitamin D₃ is subjected to a temperature of 200°C., it is converted to pyro- and isopyrovitamin D₃²⁶¹ by a reaction analogous to that which occurs with vitamin D₂. The analogy can be carried further since, on dehydrogenation with mercuric acetate, pyrovitamin D₂ forms 9,11-dehydrolumisterol₃, while isopyrovitamin D₃ forms 7,8,9,11-dehydrocholesterol. Ergosterol bears the same relationship to isopyrocalciferol as 7-dehydrocholesterol does to isopyrovitamin D₃; moreover, pyrocalciferol is closely related to lumisterol₂, just as pyrovitamin D₃ is quite similar to lumisterol₃.

(3) Vitamin D₄

Vitamin D₄ is the vitamin formed when the provitamin, 22-dihydroergosterol, is activated. On the basis of the structures of vitamins D₂ and D₃, Windaus and Trautmann²⁴⁸ have assigned the structure shown here to vitamin D₄ (XXXVI).

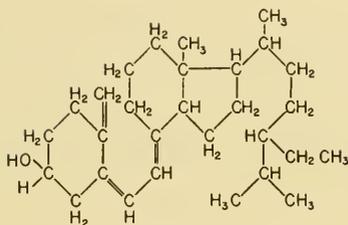


XXXVI
Vitamin D₄

(4) Vitamin D₅

Vitamin D₅ (XXXVII), which is the activated 7-dehydrositosterol, would be expected to have a structure analogous to that of vitamins D₂, D₃, and D₄, except for the variation in the side chain. Although proof has not been deduced that such is the case, one cannot fail to arrive at that conclusion in view of the evidence available. The formula for vitamin D₅ would, therefore, be that given here.

²⁶¹ A. Windaus, M. Deppe, and C. Roosen-Runge, *Ann.*, 537, 1-10 (1938).



XXXVII

Vitamin D₃

(5) General Structural Requirements for the Vitamins D

On the basis of the known constitution of vitamins D₂ and D₃, and of the known structure of other provitamins, it is generally agreed that certain structural requirements must obtain for vitamin D activity. A compound of this nature must be a derivative of a steroid in which the B ring is ruptured between carbons 9 and 10. There is also a requirement that three conjugated double bonds exist at the 10,18-, 5,6-, and 7,8-positions. The hydroxyl group on carbon 3 is another prerequisite. Vitamin D activity occurs only where the alcohol group is free, or in the esters which can be hydrolyzed in the organism. Non-hydrolyzable esters and ethers of vitamin D are inactive.²⁶² Epimerization of the hydroxyl group also greatly alters the biological activity, although the effectiveness is not entirely lost in the case of activated epi-7-dehydrocholesterol.

Another requirement for biological activity is the presence of a chain attached at carbon 17. The absence of the side chain (as in 5,7-androstadiene-3,17-diol),^{73,83} or even the presence of a four-membered chain such as occurs in activated 3-hydroxy-5,7-choleadienic acid,⁸⁴ practically inactivates the compound. Even slight changes in the normal side chains in the naturally occurring sterols are accompanied by marked alterations in biological potency.

5. Synthesis of the Vitamins D

Although considerable information is available concerning methods for the synthesis of the vitamins D by activation of their provitamins (see Section C), no complete synthesis of any members of the vitamin D group has been accomplished. Milas and Alderson (XXXVIII),²⁶³ Dimroth (XXXIX),^{264,265} and Aldersley and Burkhardt (XL)²⁶⁶ have synthesized

²⁶² A. Windaus and O. Rygh, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse, III*, 202-216 (1928).

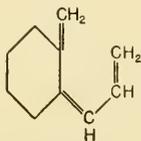
²⁶³ N. A. Milas and W. L. Alderson, *J. Am. Chem. Soc.*, 61, 2534-2537 (1939).

²⁶⁴ K. Dimroth, *Ber.*, 71, 1333-1345, 1346-1350 (1938).

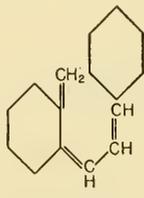
²⁶⁵ K. Dimroth and H. Jonsson, *Ber.*, 71, 2658-2662 (1938).

²⁶⁶ J. B. Aldersley and G. N. Burkhardt, *J. Chem. Soc.*, 1938, 545.

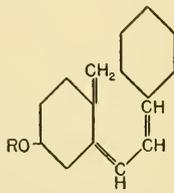
products which contain the three conjugated double bonds attached to a cyclohexane ring. The biological efficacy of these products has not been investigated. Milas²⁶⁷ has also reported the preparation of biologically active alkyl ethers of vitamin D from metal vitaminates. The methyl ether is reported as having a potency of 7,500,000 U.S.P. units per gram.



XXXVIII



XXXIX



XL

Possible Simplified Synthetic Products Related to the Vitamins D

²⁶⁷ N. A. Milas, *U. S. Patent* No. 2,410,893 (Nov. 12, 1946).

CHAPTER IX

DISTRIBUTION, PROPERTIES, AND CHEMISTRY OF THE VITAMIN E GROUP

1. Introduction

The fact that additional members of the group of fat-soluble vitamins exist was not recognized until considerable information had already been collected about vitamins A and D. The first suggestion that another fat-soluble vitamin may occur came from the work of Evans and Bishop¹⁻⁷ in 1922 and 1923. These investigators found that rats failed to reproduce when fed a purified diet containing adequate amounts of both vitamins A and D and of the other foodstuffs then recognized as required. This deficiency could be overcome if an extract containing the missing essential factor was added. It was believed to be a vitamin, and was named "vitamine E."

Confirmation of the existence of this new fat-soluble vitamin came from many quarters. The biological activity was also found to be much more far-reaching than was originally realized. Mattill and his associates,⁸ as well as Mattill alone,⁹ reported the development of a type of sterility in male rats deprived of a dietary "X substance"; the deficiency was slower in appearing in male rats than it was in female animals, but the testicular damage was found by Mason to be permanent.^{10,11} It is also not entirely certain whether or not Mattill and Conklin¹² should be given the credit for the discovery of vitamin E, since as early as 1920 these workers reported that disturbances in reproduction occurred in rats fed milk diets.

Evans and Burr¹³ found that weanling rats whose mothers were on restricted diets, presumably lacking in vitamin E, developed paralysis; this

¹ H. M. Evans and K. S. Bishop, *Science*, *56*, 650-651 (1922).

² H. M. Evans and K. S. Bishop, *Am. J. Physiol.*, *63*, 396-397 (1922).

³ H. M. Evans and K. S. Bishop, *J. Am. Med. Assoc.*, *81*, 889-892 (1923).

⁴ H. M. Evans and K. S. Bishop, *J. Metabolic Research*, *1*, 319-333 (1922).

⁵ H. M. Evans and K. S. Bishop, *J. Metabolic Research*, *1*, 335-356 (1922).

⁶ H. M. Evans and K. S. Bishop, *J. Metabolic Research*, *3*, 201-231 (1923).

⁷ H. M. Evans and K. S. Bishop, *J. Metabolic Research*, *3*, 233-316 (1923).

⁸ H. A. Mattill, J. S. Carman, and M. M. Clayton, *J. Biol. Chem.*, *61*, 729-740 (1924).

⁹ H. A. Mattill, *Am. J. Physiol.*, *79*, 305-315 (1927).

¹⁰ K. E. Mason, *Proc. Natl. Acad. Sci. U. S.*, *11*, 377-382 (1925).

¹¹ K. E. Mason, *J. Exptl. Zool.*, *45*, 159-229 (1926).

¹² H. A. Mattill and R. E. Conklin, *J. Biol. Chem.*, *44*, 137-158 (1920).

¹³ H. M. Evans and G. O. Burr, *J. Biol. Chem.*, *76*, 273-297 (1928).

could be cured by changing the diet to a natural food mixture, or it could be prevented when the mother was given a diet containing adequate wheat germ oil or other vitamin-E-rich products. Vitamin E was found by Adamstone^{14,15} to be concerned not only with the growth of young chicks but also with the development of the embryo. Eggs from hens on a vitamin-E-low diet were found to have a lowered hatchability. Moreover, degeneration of the testis occurred in adult male birds on a diet lacking vitamin E.¹⁶

Following the work of Evans and Burr,¹³ a number of workers found that vitamin E is apparently of considerable importance in relation to normal muscle metabolism. Goettsch¹⁷ and Pappenheimer,¹⁸ working singly and together,¹⁹ reported the occurrence of a specific muscular dystrophy in rats, rabbits, and guinea pigs which were receiving a vitamin-E-free diet.

The addition of vitamin E to the rations has also been found to produce a growth-promoting activity in 5- to 7-month-old rats raised on a vitamin-E-low diet.^{20,21} This effect was shown by Olecott and Mattill²² to occur in late adolescence; it appears at a later age in the female than in the male. Martin²³ is of the opinion that the antisterility and growth-promoting effects are due to different factors; he was able to effect a partial separation of the specific active components by fractional crystallization of vitamin E concentrates. Such an effect is not surprising, in view of the demonstration by Adamstone²⁴ of the possible relation of this vitamin to cell division.

One of the most important functions of vitamin E which can readily be determined by studies *in vitro* is its antioxidant action. Thus, the destructive effect of ferrous sulfate on vitamin A²⁵ can be prevented if the food is mixed with wheat germ oil,²⁶ which is a rich source of vitamin E. Simultaneously, Mattill²⁷ showed that diets which produced sterility were usually subject to rapid development of rancidity. The addition of a wheat germ oil concentrate, which was rich in vitamin E, prevented the oxidative rancidity.

The question of greatest importance at this stage of the development was whether the antioxidant and antisterility effects of wheat germ oil are to be

¹⁴ F. B. Adamstone, *J. Morphol.*, *52*, 47-90 (1931).

¹⁵ F. B. Adamstone, *Anat. Record*, *60*, No. 4, Suppl., 36-37 (1934).

¹⁶ F. B. Adamstone and L. E. Card, *J. Morphol.*, *56*, 339-359 (1934).

¹⁷ M. Goettsch, *Proc. Soc. Exptl. Biol. Med.*, *27*, 564-567 (1930).

¹⁸ A. M. Pappenheimer, *Proc. Soc. Exptl. Biol. Med.*, *27*, 567-568 (1930).

¹⁹ M. Goettsch and A. M. Pappenheimer, *J. Exptl. Med.*, *54*, 145-169 (1931).

²⁰ H. M. Evans, *J. Nutrition*, *1*, 23-28 (1928).

²¹ G. A. Emerson and H. M. Evans, *J. Nutrition*, *14*, 169-178 (1937).

²² H. S. Olecott and H. A. Mattill, *J. Nutrition*, *14*, 305-315 (1937).

²³ G. J. Martin, *J. Nutrition*, *13*, 679-685 (1937).

²⁴ F. B. Adamstone, *Science*, *80*, 450 (1934).

²⁵ J. H. Jones, *J. Biol. Chem.*, *75*, 139-146 (1927).

²⁶ N. Simmonds, J. E. Becker, and E. V. McCollum, *J. Am. Med. Assoc.*, *88*, 1047-1050 (1927).

²⁷ H. A. Mattill, *J. Am. Med. Assoc.*, *89*, 1505-1508 (1927).

attributed to an identical compound. In 1931, Olcott and Mattill²⁸ apparently answered this question in the negative; they were able to separate the antisterility and antioxidant fractions of wheat germ oil by distribution between petroleum ether and 92% methyl alcohol. Moreover, these workers later showed²⁹ that acetylation destroyed the antioxygenic property, while leaving the antisterility effect unimpaired. Finally, it was found that, although the vitamin E activity and the antioxidant action occurred in a number of vegetable oils, such as lettuce, tomato, corn, cottonseed, soybean, carrot, alfalfa, palm, and peanut, variations in the two effects were to be noted in the case of yeast fat, lard, cod liver oil, and castor oil. However, these earlier experiments were later found to be incorrect when more concentrated sources of the vitamin E were available for study. The relationship between the antisterility and antioxidant effects could not be finally clarified until the chemical nature of the several compounds having vitamin E activity was worked out.

The chemical nature of vitamin E has been elucidated by a brilliant series of investigations which have culminated in the synthesis of several of these compounds. As early as 1927, Evans and Burr³⁰ proved that vitamin E is a constituent of the non-saponifiable fraction of fats. Olcott and Mattill^{28,29} prepared concentrates of high potency by vacuum distillation, while Drummond *et al.*³¹ employed chromatographic adsorption on Brockmann alumina. The first clear-cut separation of pure crystallized esters resulted from the work of Evans, Emerson, and Emerson,³² who were able to prepare two different allophanates which showed biological activity. The ester having the higher activity was designated as α -tocopherol ($\tau\omicron\kappa\omicron\sigma$, childbirth; $\varphi\epsilon\rho\epsilon\upsilon\upsilon$ to bear; *ol*, indicating an alcohol). The presence of an alcoholic group in vitamin E was first demonstrated by Olcott³³ and by Drummond and associates³¹; this was definitely proved when crystalline esters were prepared.

It soon became evident that there are several tocopherols which possess varying degrees of biopotency. The allophanate prepared by Evans *et al.*,³² melting at 136–138°C., was subsequently further purified by recrystallization from acetone (m.p., 144–146°C.)³⁴ and was shown to have a lower activity than α -tocopherol; Emerson *et al.*³⁴ designated it as β -tocopherol. A third allophanate was prepared from cottonseed oil and was designated as γ -tocopherol.³⁴ This also was found to have a biological activity only one-

²⁸ H. S. Olcott and H. A. Mattill, *J. Biol. Chem.*, *93*, 59–64, 65–70 (1931).

²⁹ H. S. Olcott and H. A. Mattill, *J. Biol. Chem.*, *104*, 423–435 (1934).

³⁰ H. M. Evans and G. O. Burr, *Mem. Univ. Calif.*, *8*, 1–158 (1927).

³¹ J. C. Drummond, E. Singer, and R. J. Mac Walter, *Biochem. J.*, *29*, 456–471 (1935).

³² H. M. Evans, O. H. Emerson, and G. A. Emerson, *J. Biol. Chem.*, *113*, 319–332 (1936).

³³ H. S. Olcott, *J. Biol. Chem.*, *110*, 695–701 (1935).

³⁴ O. H. Emerson, G. A. Emerson, A. Mohammad, and H. M. Evans, *J. Biol. Chem.*, *122*, 99–107 (1938).

half to one-third of that of α -tocopherol. δ -Tocopherol represents the last member of the series to be added to the group of vitamins E³⁵; it has been found to possess the least antisterility effect and the greatest antioxidant activity of the group.

The work of Evans *et al.*³² was confirmed in 1937 by Todd, Bergel, Waldmann, and Work.^{36,37} According to later reports,³⁸ these workers prepared a new allophanate which they designated as β -tocopheryl allophanate. It was found to melt at 143.5–144.5°C. Apparently it is the same allophanate which was separated in impure form by Evans *et al.*,³² melting at 136–138°C., which was subsequently further purified by acetone recrystallization to yield a product with a melting point in agreement (144–146°C.)³⁴ with the preparation of the latter workers.

The structural relationships of the tocopherols were worked out by Fernholz.^{39,40} The tocopherols were shown to have the chroman ring. α -Tocopherol contains three methyl groups, and the structure was found to agree with the empirical formula $C_{29}H_{50}O_2$, suggested by Evans and co-workers.³² The β - and γ -forms have one less methyl group, which is in line with the formula of $C_{28}H_{48}O_2$, while δ -tocopherol has only one methyl group.

The chemical synthesis of *dl*- α -tocopherol was first accomplished by Karrer, Fritzsche, Ringier, and Salomon in 1938,^{41–43} and was later confirmed by Smith, Ungnade, and Prichard.⁴⁴ Two of the naturally occurring tocopherols, α and γ , have recently been prepared in crystalline form.^{45,46} Natural β -tocopherol could not be crystallized under similar treatment; β -tocopherol azobenzene carboxylate was the only ester of β -tocopherol—with the exception of the allophanate—which could be crystallized.

Several extensive reviews on the physiology of vitamin E include those of Evans,⁴⁷ Mattill,⁴⁸ and Mason,⁴⁹ while Smith⁵⁰ covered the chemical aspects of the field.

³⁵ M. Stern, C. D. Robeson, L. Weisler, and J. G. Baxter, *J. Am. Chem. Soc.*, **69**, 869–874 (1947).

³⁶ A. R. Todd, F. Bergel, H. Waldmann, and T. S. Work, *Nature*, **140**, 361–362 (1937).

³⁷ A. R. Todd, F. Bergel, H. Waldmann, and T. S. Work, *Biochem. J.*, **31**, 2247–2256 (1937).

³⁸ A. R. Todd, F. Bergel, and T. S. Work, *Biochem. J.*, **31**, 2257–2263 (1937).

³⁹ E. Fernholz, *J. Am. Chem. Soc.*, **59**, 1154–1155 (1937).

⁴⁰ E. Fernholz, *J. Am. Chem. Soc.*, **60**, 700–705 (1938).

⁴¹ P. Karrer, H. Fritzsche, B. H. Ringier, and H. Salomon, *Nature*, **141**, 1057 (1938).

⁴² P. Karrer, H. Fritzsche, B. H. Ringier, and H. Salomon, *Helv. Chim. Acta*, **21**, 520–525 (1938).

⁴³ P. Karrer, H. Fritzsche, B. H. Ringier, and H. Salomon, *Helv. Chim. Acta*, **21**, 820–825 (1938).

⁴⁴ L. I. Smith, H. E. Ungnade, and W. W. Prichard, *Science*, **88**, 37–38 (1938).

⁴⁵ J. G. Baxter, C. D. Robeson, J. D. Taylor, and R. W. Lehman, *J. Am. Chem. Soc.*, **65**, 918–924 (1943).

⁴⁶ C. D. Robeson, *J. Am. Chem. Soc.*, **65**, 1660 (1943).

⁴⁷ H. M. Evans, *J. Am. Med. Assoc.*, **99**, 469–476 (1932).

⁴⁸ H. A. Mattill, *J. Am. Med. Assoc.*, **110**, 1831–1837 (1938).

⁴⁹ K. E. Mason, *Vitamins and Hormones*, **2**, 107–153 (1944).

⁵⁰ L. I. Smith, *Chem. Revs.*, **27**, 287–329 (1940).

2. Occurrence of the Tocopherols (Vitamins E)

(1) *Distribution in Plant Tissues*

The various types of vitamin E are found almost exclusively in plants, and only to a minimal degree in the animal organism. All green plants which have been examined have been shown to contain demonstrable amounts of the tocopherols. Although wheat germ oil⁵¹ and other seed germ oils^{29,52} are generally considered to have the highest concentration of vitamins E, Dam and his colleagues⁵³ have brought forward some evidence to indicate that the green leafy vegetables and rose hips may contain more extractable tocopherol on a dry-weight basis than does wheat germ.⁴⁹ Mason⁴⁹ considers that the storage of these various vitamins E in the embryo of the seed may be an indication that they act as stabilizers of fats during the period of dormancy, or that they are present as a reserve to supply some special function in early development and growth.

The vegetable fats are relatively the highest sources of the tocopherols among the natural food products. The tocopherol content of some vegetable fats is recorded in Table 1. β -Tocopherol, which is not listed in the table, is found chiefly in wheat-germ oil.

The low content of vitamins E in olive oil and especially in coconut oil is of considerable interest. In fact, Bacharach and others,^{54,55} on the basis of earlier work, reported the total absence of vitamin E in olive oil and the small content in peanut oil. Lettuce and alfalfa contain considerable amounts of the vitamins, while oranges and bananas have a low content.⁵⁵ The lower plants, such as algae, fungi, liverworts (*Hepaticae*), mosses, ferns and primitive seed plants, are not believed to contain vitamin E.⁴⁹ Schopfer and Blumer⁵⁶ have definitely proven the absence of these compounds in one fungus, the saprophytic mold, *Phycomyces*.

The tocopherols occur in free (unesterified) forms in the seed oils.^{57,58} Rye germ oil is reported⁵⁷ to contain as much as 1050 milligram per cent. The proportion among the different tocopherols varies with different plants, and it is not always constant in the same plant. Thus, while European wheat germ oil contains principally β -tocopherol, with smaller amounts of the α -form, or about equal amounts⁵⁸ of the two types, the α -variety predominates in the American wheat berry, with a smaller quantity of γ -tocopherol. California wheat germ oil contains about twice as much of the

⁵¹ H. S. Olcott, *J. Biol. Chem.*, **107**, 471-474 (1934).

⁵² H. M. Evans and G. O. Burr, *Proc. Natl. Acad. Sci. U. S.*, **11**, 334-341 (1925).

⁵³ H. Dam, J. Glavind, I. Prange, and J. Ottesen, *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **16**, No. 7, 1-39 (1941).

⁵⁴ A. L. Bacharach, E. Alchorne, and H. E. Glynn, *Biochem. J.*, **31**, 2287-2292 (1937).

⁵⁵ H. L. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945.

⁵⁶ W. H. Schopfer and S. Blumer, *Z. Vitaminforsch.*, **9**, 344-349 (1939).

⁵⁷ W. Halden, *Monatsh.*, **77**, 197-205 (1947).

⁵⁸ A. R. Moss and J. C. Drummond, *Biochem. J.*, **32**, 1953-1956 (1938).

TABLE 1
 TOCOPHEROL (VITAMIN E) CONTENT OF SOME VEGETABLE OILS
 AND HYDROGENATED FATS^a

Oil	Tocopherol content, mg. per 100 g.			
	Total ^b	α	γ	δ
Babassu, crude.....	3	—	—	—
Carrot.....	162;500	—	0	0
Castor.....	50	—	—	—
Cacao butter.....	3-13	—	—	—
Coconut.....	3;8.3	3.6	—	4.7 ^c
Corn.....	87-250;102	12.6	89.4	—
Cottonseed.....	83-110;86	41	36	9
Linseed.....	113	—	—	—
Okraseed, crude.....	74	31	43	—
Olive.....	3-30	—	—	—
Palm.....	3-50;56	30	—	26 ^c
Peanut.....	22-59;34	13	14	7
Pecan, refined.....	45;42	20	22	—
Poppyseed, crude.....	44	—	—	—
Rapeseed.....	55	—	—	—
Rice bran.....	55-100;91	58	33	—
Safflower, crude.....	80	—	—	—
Soybean.....	92-280;168	20	98	50
Soybean phosphatide.....	200 ^d	—	—	—
Sunflowerseed, crude.....	70	—	—	—
Wheat germ.....	140-550;274	192	—	—

HYDROGENATED VEGETABLE SHORTENINGS				
Crisco.....	104.2	—	—	—
Margarine base stock.....	100.5	—	—	—
Primex.....	98.4	—	—	—
Sweetex.....	101.0	—	—	—

^a Data adapted from W. Lange, *J. Am. Oil Chemists' Soc.*, 27, 414-422 (1950).

^b Values following semicolon are for the sample on which the results of fractionation of tocopherol are based. ^c Sum of γ - and δ -tocopherols.

^d J. L. Jensen, K. C. D. Hickman, and P. L. Harris, *Proc. Soc. Exptl. Biol. Med.*, 54, 294-296 (1943).

α -form as of the β -type, and only traces of γ -tocopherol.⁵⁹ There is sometimes as much γ - as α -tocopherol in cottonseed oil,⁵⁹ but usually there is less, while the tocopherol in palm oil is largely, and in corn oil exclusively, of the γ -type.⁶⁰ On the other hand, lettuce oil contains only the α -variety.

δ -Tocopherol is found in soybean oil⁶¹ to the extent of 30% of the total tocopherols; there is also evidence that δ -tocopherol is present in cottonseed and peanut oils.

⁵⁹ O. H. Emerson, *J. Am. Chem. Soc.*, 60, 1741-1742 (1938).

⁶⁰ O. H. Emerson, G. A. Emerson, and H. M. Evans, *Science*, 89, 183 (1939).

⁶¹ L. Weisler, C. D. Robeson, and J. G. Baxter, *Ind. Eng. Chem., Anal. Ed.*, 19, 906-909 (1947).

(2) *Distribution in Animal Tissues*

With the possible exception of the crustacean *Daphnia magna* (water-flea), there is no evidence that the tocopherols are required by the invertebrates, or that they are stored in this group of animals. Fishes may contain these vitamins, as some liver oils have appreciable amounts. Mason⁴⁹ states that it is generally agreed that cod liver oil is devoid of vitamin E.

TABLE 2

ESTIMATED α -TOCOPHEROL IN FRESH TISSUES OF RATS ON LOW OR HIGH VITAMIN E DIETS^a

Tissue	Milligrams α -tocopherol per kilogram tissue		
	Low vit. E (4 \times minimal)	High vit. E (100 \times minimal)	Excess vit. E (10,000 \times minimal)
Liver	8.3	118	1,000
Muscle	16.6	67	200
Kidney	16.6	77	—
Body fat	20	59	125*
Pancreas and thymus	22	100*	
Heart	28.6	111	
Lung	28.6	125	
Spleen	33.3	100*	
Testis	16.6	50*	
Epididymis	18*	67*	
Prostate and seminal vesicle	16.6*	50*	
Uterus	—	125	
Placenta	—	77	
Mammary gland	28.6*	250	
Newborn	5	20	
Suckling young (24–48 hours)	20	67	

^a K. E. Mason, *Vitamins and Hormones*, 2, 107–153 (1944), p. 117.

* Values based upon a limited number of assay tests.

However, Quackenbush *et al.*⁶² reported an appreciable quantity of this vitamin in cod liver oil (26 mg. per 100 grams of oil). Vitamin E has been found in tuna liver oil,⁶³ and Robeson and Baxter⁶⁴ have reported minimal quantities in liver oils of *Mangona* (shark) and of the soup-fin shark (*Galeorhinus zyopterus*) (10 and 4 mg. per 100 grams of oil, respectively).

The distribution of vitamin E in the rat has been carefully studied by Mason.⁶⁵ Although the concentration of vitamin E is reported by Cuth-

⁶² F. W. Quackenbush, H. L. Gottlieb, and H. Steenbock, *Ind. Eng. Chem.*, 33, 1276–1278 (1941).

⁶³ L. Bocchi, *Ateneo parmense*, 10, 107–126 (1938); *Chem. Abst.*, 33, 2945 (1939). Cited by K. E. Mason, *Vitamins and Hormones*, 2, 116 (1944).

⁶⁴ C. D. Robeson and J. G. Baxter, *J. Am. Chem. Soc.*, 65, 940–943 (1943).

⁶⁵ K. E. Mason, *J. Nutrition*, 23, 71–81 (1942).

bertson *et al.*⁶⁶ and by Moore *et al.*⁶⁷ to be low in the livers of rats, Mason⁶⁵ demonstrated that this value is dependent upon the intake of vitamin E. On a diet containing 4 times the minimal daily requirement, liver storage was at a level of one-fourth to one-half of that of the skeletal muscles, visceral organs, and body fat; however, when the vitamin E intake was at a level of 100 times the minimal daily requirement, the liver content was increased 14 times, in contrast to a rise in most other tissues of only 3 to 4.5 times. When the dosage was increased to 10,000 times the daily minimal requirement, the liver storage was 150 times and that in the skeletal muscle was 12 times the amount present when the minimal dosage was given. These results are summarized in Table 2.

TABLE 3
DISTRIBUTION OF TOCOPHEROL IN TISSUES OF AN ADULT RAT^a

Tissue	Weight, g.	Tocopherol, mg./100 g.	Concn., mg./g. fat	Total tocopherol in tissue, mg.
Blood cells.....	—	—	1.1	0.047
Suprarenals.....	0.04	34.0	0.7	0.014
Lungs.....	1.38	3.24	0.74	0.045
Spleen.....	0.58	5.1	1.1	0.030
Liver.....	10.6	2.52	0.51	0.269
Blood plasma.....	—	0.70	—	0.070
Gut.....	4.64	3.69	0.42	0.171
Kidneys.....	2.04	1.18	0.24	0.024
Thymus.....	0.49	1.7	0.7	0.008
Diaphragm.....	0.66	2.5	0.7	0.016
Heart.....	0.90	3.42	0.96	0.031
Penis.....	0.24	4.5	1.0	0.011
Seminal vesicles.....	0.72	2.6	0.7	0.019
Residual (skeleton, head, etc.)....	91.0	3.55	0.57	3.23
Mesentery fat.....	5.85	6.0	0.071	0.349
Pelt.....	58.5	3.32	0.39	1.94
Muscle.....	86.0	1.33	0.54	1.15
Testes.....	3.18	2.26	1.05	0.072
Pancreas.....	1.62	5.48	0.20	0.089
Pituitary.....	0.01	90.0	1.2	0.009
Central nervous system.....	2.49	1.62	0.16	0.040
<i>Total</i>				<i>7.634</i>

^a M. L. Quaife, W. J. Swanson, M. Y. Dju, and P. L. Harris, *Ann. N. Y. Acad. Sci.*, 52, 300-305 (1949).

The liver thus provides a site for the storage of the tocopherols. The level of vitamin E present in this organ is to a considerable extent indicative

⁶⁶ W. F. J. Cuthbertson, R. R. Ridgeway, and J. C. Drummond, *Biochem. J.*, 34, 34-39 (1940).

⁶⁷ T. Moore, A. J. P. Martin, and K. R. Rajagopal, *Vitamin E Symposium*, April 22, 1939, *Soc. Chem. Ind. Food Group*, 1940, 41-43.

of the quantity of this vitamin present in the previous diet. It would seem that the liver serves to replenish the need of other tissues for tocopherol when the intake of vitamin E is low.

Further information on the distribution of tocopherol in the tissues of the adult rat can be obtained from the recent report of Quaife and her collaborators.⁶⁸ When 1 mg. of α -tocopherol was fed daily to male rats on a vitamin-E-deficient diet, the highest concentrations were found in the pituitary and adrenal glands (90 and 34 mg. per 100 grams, respectively). Tocopherol in the other tissues varied between 5.85 mg. per 100 grams in the mesenteric fat to 0.70 mg. per 100 milliliters in the blood plasma. The data are summarized in Table 3.

Apparently vitamin E is required by the bird. A considerable amount exists in the yolk of eggs; this quantity varies with the intake in the food.⁶⁹ Quaife *et al.*⁶⁸ have shown that the hen, like the cow and the human subject, absorbs and deposits α -tocopherol in preference to the other members of the vitamin E group. It was found that, when pure natural α -, γ -, or δ -tocopherol was administered to hens in the form of capsules in doses of 100 to 4,000 mg. per week, the tocopherol content of the eggs increased linearly with the logarithm of the dose of the supplement fed. The relative amounts of the γ - and δ -tocopherol found in the eggs were much less than that of the α -tocopherol deposited in this product. When α -, γ -, or δ -tocopherols were administered to the hens at a level of 400 mg. per week, the relative concentrations of the tocopherols were 24.2, 5.7, and 2.3 mg., respectively, per 100 milligrams of the fresh egg. The relative efficiency of transfer of α -tocopherol to the egg was shown to be 22.1%, compared with figures of 3.6 and 2.0% for the γ - and δ -tocopherols, respectively.

There is no evidence of an *in vivo* transformation of one tocopherol to another type. Thus, the hens fed steadily increasing doses of δ -tocopherol produced eggs with a correspondingly increased proportion of δ -tocopherol. In the case of the hens fed γ -tocopherol at higher levels, eggs containing more than 90% of the total tocopherols as γ -tocopherol were reported.

The livers of normal cattle and horses are rich in vitamin E,⁷⁰ while appreciable amounts have also been found in the livers of monkeys and of man.⁶⁵ According to Abderhalden,⁷¹ cow milk is an exceedingly poor source of vitamin E. The average value reported was 0.061 milligram per cent. When the cow's fodder was supplemented with α -tocopherol,⁶⁸ increased amounts of tocopherol were secreted in the milk; on the other hand, when the supplement was made up of 90% of γ - and δ -tocopherol, only slight increases in milk tocopherol were noted. In the first case, the milk tocopherol

⁶⁸ M. L. Quaife, W. J. Swanson, M. Y. Dju, and P. L. Harris, *Ann. New York Acad. Sci.*, 52, 300-305 (1949).

⁶⁹ G. L. Barnam, *J. Nutrition*, 9, 621-635 (1935).

⁷⁰ P. Karrer, W. Jaeger, and H. Keller, *Helv. Chim. Acta*, 23, 464-465 (1940).

⁷¹ R. Abderhalden, *Biochem. Z.*, 318, 47-53 (1948).

was increased from 0.025 to 0.068 milligram per cent, while in the second series the rise in tocopherol concentration was only from 0.022 to 0.034 milligram per cent.

Tissues from a normal man were found to contain a total of 3.4 g. of tocopherols,⁷² while the total from a 50-kg. woman was calculated to be 8.1 g. Table 4 shows the estimated distribution of tocopherol in man.

TABLE 4
ESTIMATED CONTENT OF TOTAL TOCOPHEROLS IN HUMAN SUBJECTS^a

Tissue	Woman, mg.	Man, mg.
Fat.....	6180	1885
Muscle.....	269	285
Blood.....	45	64
Liver.....	33	45
Pancreas.....	10	7
Spleen.....	7	4
Heart.....	4	3
Kidney.....	10	2
Uterus.....	2	—
Lung.....	—	12
Testis.....	—	2
<i>Total.....</i>	<i>6560</i>	<i>2309</i>
<i>Total on basis of 50 kg.....</i>	<i>8120</i>	<i>—</i>
<i>Total on basis of 70 kg.....</i>	<i>—</i>	<i>3440</i>

^a M. L. Quaife and M. Y. Dju, *J. Biol. Chem.*, 180, 263-272 (1949).

The tocopherol concentration showed a 50-fold range in human tissues on the wet basis, and less than an 8-fold variation when the comparison was based upon the amount per unit quantity of fat. In the latter case, the values varied from 0.2 to 1.2 mg. per gram of lipid. The tocopherol in the tissues of the 2 subjects consisted of approximately 90% of α -tocopherol (91% in the man and 88% in the woman). Since the average diet contains about equal proportions of α - and of non- α -tocopherol, it is thus evident that there is a marked preferential storage of the α -form. Kaunitz and Beaver⁷³ have reported a total tocopherol concentration in rat and human muscle of 17 to 30 mg. per kilogram of wet muscle; this figure is in line with the results of Quaife *et al.*^{68,72} Human milk samples were found to contain much higher proportions of tocopherol than did cow milk. While mature human milk was shown to average about 0.14 milligram per cent, the composition of colostrum obtained during the first week following parturition had a tocopherol content as high as 3.6 mg. per 100 milliliters.⁷⁴

⁷² M. L. Quaife and M. Y. Dju, *J. Biol. Chem.*, 180, 263-272 (1949).

⁷³ H. Kaunitz and J. J. Beaver, *J. Biol. Chem.*, 166, 205-217 (1946).

⁷⁴ M. L. Quaife, *J. Biol. Chem.*, 169, 513-514 (1949).

The body of the newborn rat contains only a small amount of vitamin E, in spite of a large intake of tocopherol by the mother. Although the uterus and placenta are able to take up a considerable amount of vitamin E, apparently the latter cannot be transmitted to the fetus. The deficiency in vitamin E is rapidly corrected in the offspring after birth; this is probably due to the high content in the milk, although the vitamin E content of rat colostrum is not known. The exceedingly high tocopherol value of mammary tissue is of considerable interest in this connection.

Preferential absorption of *d*- α -tocopherol as compared with that of *d*- γ -tocopherol has been demonstrated by Quaife *et al.*⁶⁸ in the human subject. After 500-mg. doses of α -tocopherol, the serum tocopherol reached a maximum level of approximately 1.75 mg. per 100 milliliters of serum after 4 hours; when γ -tocopherol was given, the maximum was reached after about the same interval, and amounted to only 1.35 mg. per 100 milliliters of serum.⁶⁸ The normal value for tocopherol in man is 1.00 milligram per cent⁷⁵; 75% is α -, and the balance non- α -tocopherol.

The relatively low content of tocopherol in the body fat, muscles, urine, feces, and blood of rats indicates that the absorption must be very inefficient, or that the substance is readily susceptible to destruction in the gastrointestinal tract, or both. It is also possible that the destruction within the tissues is rapid; the low content may be the result of inefficient storage.^{66,76}

The absorption of the tocopherols requires the intermediation of bile, just as fat and carotene do. Thus, it has been demonstrated that rats⁷⁷ and dogs^{65,78} with bile fistulas are unable to absorb vitamin E; Mason⁴⁹ believes that this deficiency is related to the failure of such animals to utilize this fat-soluble component from dietary sources.

The tocopherol content of several animal fats which have been studied is exceedingly low.⁷⁹ The values reported vary between 0.2 and 4.2 mg. per 100 grams of fat.⁷⁹ This level is exceedingly low as compared with a figure of 400 mg. per gram of wheat germ oil or 100 mg. per gram of cottonseed oil. Oleo oil⁷⁹ contains only 2 mg. of the vitamin per 100 grams of fat; according to Kofler,^{79a} the tocopherol content of beef tallow is only 1 milligram per cent. In the case of butter, a range of 1.7 to 4.2 mg. per 100 grams has

⁷⁵ M. L. Quaife, N. S. Scrimshaw, and O. H. Lowry, *J. Biol. Chem.*, **180**, 1229-1235 (1949).

⁷⁶ T. Moore and K. R. Rajagopal, *Biochem. J.*, **34**, 335-342 (1940).

⁷⁷ J. D. Greaves and C. L. A. Schmidt, *Proc. Soc. Exptl. Biol. Med.*, **37**, 40-42 (1937).

⁷⁸ K. M. Brinkhous and E. D. Warner, *Am. J. Path.*, **17**, 81-86 (1941).

⁷⁹ H. J. Deuel, Jr., in A. E. Bailey, *Cottonseed and Cottonseed Products*, Interscience, New York, 1948, p. 772.

^{79a} M. Kofler, *Helv. Chim. Acta*, **26**, 2166-2176 (1943).

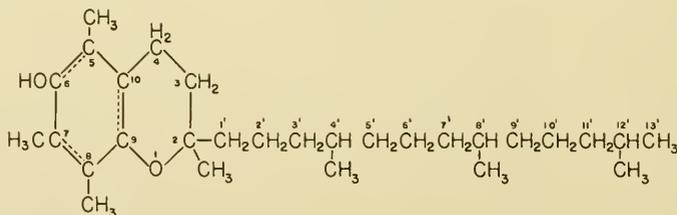
been reported by several authors.^{74, 79b-79e} Similar low values have been noted for lard^{79a, 79c}; in one sample with a total of 2.7 milligram per cent, 2.3 mg. was α -tocopherol, and 0.4 mg. consisted of the γ -form.^{79c} A compilation of the tocopherol content of a wide variety of animal and vegetable products, and of many animal tissues, has recently been prepared by Lange.^{79f}

The tocopherol content of animal fat can be increased when a generous amount of this vitamin is included in the diet. Lundberg and associates⁸⁰ found that, whereas rendered body fats of rats which had been raised from weaning to 100 days on a diet free from vitamin E were abnormally susceptible to oxidation,⁸¹ the addition of a single dose of α -tocopherol to the diet restored the stability of body fats to their normal value. The maximum deposition of α -tocopherol in the abdominal fats is not reached until 7 to 10 days after the feeding of a single 50-mg. dose. The quantities of α -tocopherol deposited in the abdominal fats increase in proportion to the amount fed, up to a maximum single dosage of 500 mg. Barnes *et al.*⁸¹ were unable to cause the deposition of antioxidants other than tocopherol when such substances were introduced in the diet.

3. Structure of the Tocopherols

(1) α -Tocopherol

α -Tocopherol, or better 5,7,8-trimethyltolcol, has the empirical formula of $C_{29}H_{50}O_2$, and has now been proved to have the structure shown in (I).



I

^{79b} A. Emmerie and C. Engel, *Z. Vitaminforsch.*, **13**, 259-266 (1943); *Chem. Zentr.*, **1943**, II, 1974. Cited by W. Lange, *J. Am. Oil Chemists' Soc.*, **27**, 414-422 (1950).

^{79c} P. L. Harris, M. L. Quaife, and W. J. Swanson, *J. Nutrition*, **40**, 367-381 (1950).

^{79d} P. L. Harris, W. J. Swanson, and K. C. D. Hickman, *J. Nutrition*, **33**, 411-427 (1947).

^{79e} H. Lieck and H. Willstaedt, *Svensk Kem. Tid.*, **57**, 134-139 (1945); *Chem. Abst.*, **40**, 4759 (1946).

^{79f} W. Lange, *J. Am. Oil Chemists' Soc.*, **27**, 414-422 (1950).

⁸⁰ W. O. Lundberg, R. H. Barnes, M. Clausen, and G. O. Burr, *J. Biol. Chem.*, **153**, 265-274 (1944).

⁸¹ R. H. Barnes, W. O. Lundberg, H. T. Hanson, and G. O. Burr, *J. Biol. Chem.*, **149**, 313-322 (1943).

tone group, one deduces that the alcohol must be tertiary. This is supported by the fact that it is esterified only with difficulty. When these facts were assessed in the light of knowledge of the empirical formula of the α -tocopherol, as well as of the structure of the C_{13} ketone (IX) and C_{16} acid (X), the structure of the whole molecule could be established. The C_{16}

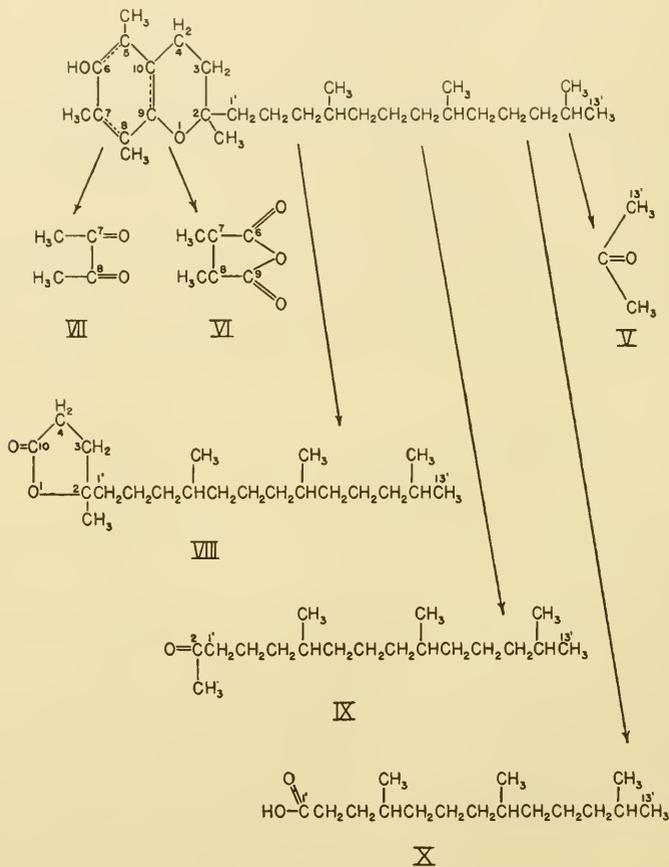
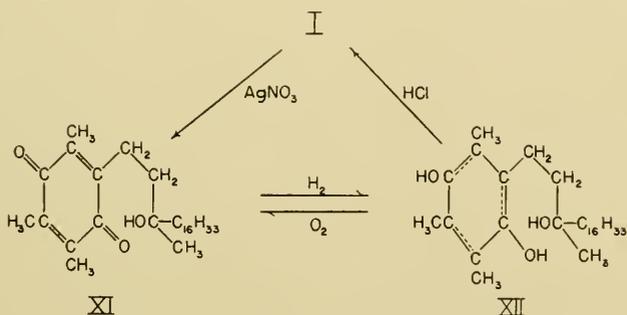


Fig. 1. Decomposition products of α -tocopherol on treatment with chromic acid. Superscript numbers indicate the source of the atoms in the tocopherol molecules.

acid was used to determine the number of methyl groups in the side chain, which proved to be 3; from this information the position of these methyl groups was assumed by analogy to other similar structures in naturally occurring members of the terpene family which follow the isoprene rule. Finally, Fernholz was able to show that the structure which he postulated for α -tocopherol would permit the production of all of these decomposition

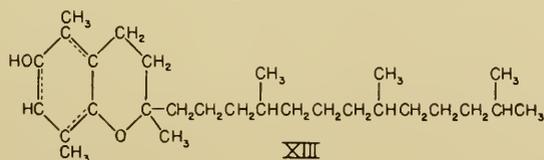
products on pyrolysis or chromic acid oxidation. The aromatic portion of the chroman ring accounts for the nucleus of duroquinone; the 3 methyls in the above compound represent groups present as such on the α -tocopherol molecules. The fourth methyl group originates from the $-\text{CH}_2$ group in position 4. Both diacetyl and dimethylmaleic anhydride also represent fragments of the aromatic ring; a rupture occurs between positions 6,7 and 8,9 in the first case, and between positions 5,6 and 9,10 in the second case. Acetone (V) obviously originates from the isopropyl group at the end of the side chain. The origin of the C_{21} lactone, C_{18} ketone, and C_{16} acid represents the side chain with 5, 2, and 0 additional carbons, respectively.

John and co-workers⁵⁵ also proved that a chroman rather than a coumaran structure obtains in the ring portion of the molecule. This could be deduced from the fact that, on careful oxidation with silver nitrate⁵⁵ or ferric chloride, a yellow quinone, tocopherylquinone (XI), originates.^{55,56} This can be reduced to a hydroquinone (XII), the so-called "tocopherylquinol." One can readily esterify the 2 phenolic groups so formed. An attempt to esterify the third hydroxyl group indicated great resistance to this reaction; moreover, it could not be oxidized to a ketone. This is taken as proof that the alcoholic group is a tertiary one, as would be the case if the ring were a chroman ring. Had it been a coumaran ring, the third hydroxyl would have been a secondary alcohol group. On this basis, the chroman ring is assumed for the cyclic part of α -tocopherol, rather than the coumaran ring.

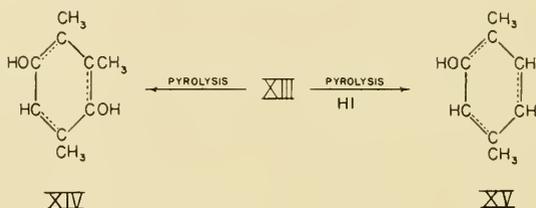


(2) β -Tocopherol

β -Tocopherol is 5,8-dimethyltocol. It has an empirical formula of $\text{C}_{23}\text{H}_{42}\text{O}_2$, which indicates that it contains one less methyl group than does α -tocopherol. The structure assigned to it is shown here (XIII).



The differences between α - and β -tocopherol can readily be proved by comparing the products formed on pyrolysis and as a result of chromic acid oxidation. In the case of β -tocopherol, the main product is trimethylhydroquinone^{55,83-87} or ψ -cumoquinol (XIV), instead of duroquinone. When pyrolysis occurs in the presence of hydrogen iodide, *p*-xylenol (XV) is formed in place of trimethylhydroquinone.

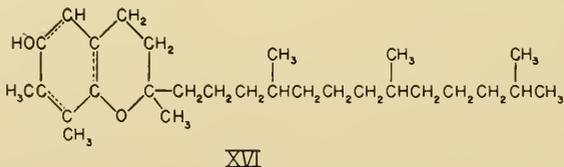


Pyrolysis of β -Tocopherol

On the other hand, treatment with chromic acid produces the same C_{21} lactone (VIII) which had been obtained with α -tocopherol.⁵⁹ These data would seem to indicate that β - and α -tocopherols are identical, except that only 2 methyl groups instead of 3 are attached to the aromatic portion of the chroman nucleus. Since these are in the paraposition to each other, they are obviously in positions 5 and 8. The final proof of the structure of β -tocopherol has been obtained by synthesis.^{82,88}

(3) γ -Tocopherol

γ -Tocopherol, or 7,8-dimethyltolcol, has the same empirical formula as β -tocopherol, namely $C_{28}H_{48}O_2$. The structural formula which has been proved by synthesis is shown in (XVI). On pyrolysis, the same trimethylhydroquinone (XIV) originates as with β -tocopherol. The same trimethylhydroquinone would originate from 5,8-, 5,7, or 7,8-dimethyltolcol.



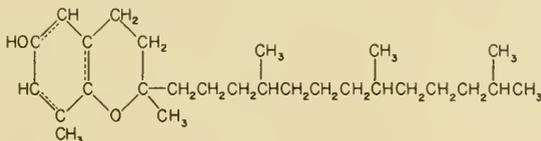
The fact that γ -tocopherol represents the 7,8-dimethyltolcol rather than 5,7-dimethyltolcol can best be proved by synthesis. Thus, when *o*-xylohydroquinone is used as the starting material in the synthesis of Karrer and Fritzsche,^{82,88} γ -tocopherol is the isomer which results. Since the methyl groups are on adjacent carbons in *o*-xylohydroquinone, they can be only in positions 7 and 8.

⁸⁷ F. Bergel, A. R. Todd, and T. S. Work, *J. Soc. Chem. Ind.*, 56, 1054 (1937).

⁸⁸ P. Karrer and H. Fritzsche, *Helv. Chim. Acta*, 22, 260-263 (1939).

(4) δ -Tocopherol

δ -Tocopherol or 8-methyltocol, has been found to have an empirical formula of $C_{27}H_{46}O_2$.³⁵ The structural formula (XVII) has been proved by synthesis.



XVII

Because of its composition, it was suspected that δ -tocopherol was a non-methylated or monomethyl chroman. The fact that its elimination maximum (170°C .), when it is subjected to molecular distillation with a constant yield oil, is 10° lower than that of γ -tocopherol also supports this conclusion.

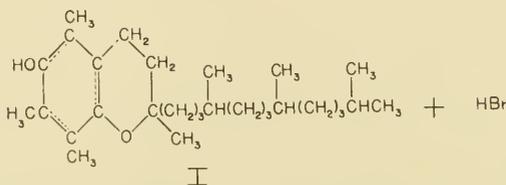
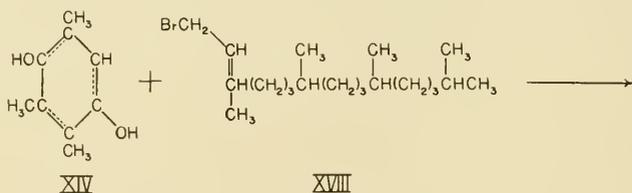
When δ -tocopherol was subjected to pyrolysis by the method of Fernholz,⁴⁰ it was found that the end product was 2,6-dimethylhydroquinone.³⁵ This was proved on the basis of melting point (found 144 – 147.5°C ., theory 147 – 149°C .) and of conversion to a diacetate which had a melting point of 89 – 91°C . (theory 91 – 92°C .). Mixed melting points of the two hydroquinones and diacetates (prepared from δ -tocopherol and synthetically) showed no depression. The infrared spectrum was also identical for the two hydroquinones. On the other hand, the pyrolysis product was shown not to be toluhydroquinone (m.p., 124 – 125°C .), which is monomethylhydroquinone. This was proved on the basis of melting point and mixed melting point. The latter hydroquinone would be expected if δ -tocopherol were non-methylated on the aromatic ring. The melting points of the hydroquinones which would be formed from 5-methyl-, 7-methyl-, or 5,7-dimethyltocol were so much higher than that found that they could be excluded from consideration. Stern *et al.*³⁵ completed their proof of the structure of the natural δ -tocopherol by demonstrating its identity with a product synthesized from toluhydroquinone monobenzoate and natural phytol by the method of Jacob *et al.*³⁹ and of Karrer and Fritzsche.³⁸

4. Synthesis of the Tocopherols

The most practical synthesis, which serves equally well for α -, β -, γ -, and δ -tocopherol, has been discovered by Karrer and his associates.^{41–43} This involves the condensation of the appropriate alkylated hydroquinone with phytol halide in the presence of a catalyst. In one reaction, the desired tocopherol is formed, usually in excellent yield. Thus, when trimethylhy-

³⁹ A. Jacob, F. K. Sutcliffe, and A. R. Todd, *J. Chem. Soc.*, 1940, 327–332.

droquinone (XIV) is condensed with phytyl bromide (XVIII) in the presence of zinc chloride, *dl*- α -tocopherol (I) results.



Synthesis of *dl*- α -Tocopherol

Bergel *et al.*^{90,91} have carried out the synthesis satisfactorily by employing phytol:



in place of phytyl bromide. Smith *et al.*⁴⁴ have also successfully used phytadiene:



in place of the phytyl bromide. The synthesis can be accomplished with the phytyl derivatives⁴² without the employment of catalysts. The resulting racemic α -tocopherol yields an ester with bromocamphorsulfonic acid which can be resolved into the *d*- and *l*- α -tocopherol.^{41,43,92}

For the synthesis of β -, γ -, and δ -tocopherols, the trimethylhydroquinone is replaced by *p*-xylohydroquinone, *o*-xylohydroquinone, or toluhydroquinone.³⁵ One of the difficulties which is encountered in these reactions is the condensation of the dimethyl- or monomethylquinone with 2 molecules of phytyl bromide. This side reaction does not occur with the trimethylhydroquinone, since only one space is available on the benzene ring for the condensation to take place. More by-products result when zinc chloride is used as a catalyst⁴³ than when formic acid⁹² is employed.^{55,87} Better yields

⁹⁰ F. Bergel, A. Jacob, A. R. Todd, and T. S. Work, *Nature*, 142, 36 (1938).

⁹¹ F. Bergel, A. M. Copping, A. Jacob, A. R. Todd, and T. S. Work, *J. Chem. Soc.*, 1938, 1382-1384.

⁹² P. Karrer, H. Koenig, B. H. Ringier, and H. Salomon, *Helv. Chim. Acta*, 22, 1139-1145 (1939).

of the tocopherols are obtained when esters such as monobenzoate are used for the condensation instead of the free quinols.⁹³

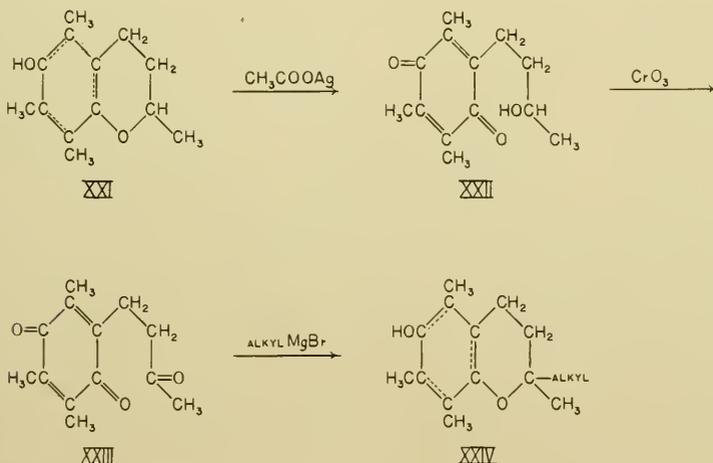
A second synthesis which can be employed for the tocopherols but which is more satisfactory for certain of their homologues involves the Grignard reaction on the dimethoxyketone (XIX) derivative. For example, the ethyl group can be inserted on the side chain and the open ring changed to the chroman ring (XX) by the accompanying reaction.^{94,95}



Synthesis of the Chroman Ring

The side chain can be varied by employing the appropriate alkyl magnesium bromide for the Grignard reagent.

A third partial synthesis suggested by John and Schmeil⁹⁶ involves the use of 2-methyl-6-hydroxychroman (XXI) as the starting material. The open-chain hydroxyquinone (XXII) is produced; this is converted to the corresponding ketone (XXIII) by means of chromic acid. Treatment with alkyl magnesium bromide causes a closing of the chroman ring with the introduction of the appropriate alkyl group on position 2 (XXIV).



Synthesis of Tocopherol Homologues by the Introduction of a Second Alkyl Group into the Chroman Ring at Position 2

⁹³ A. Jacob, M. Steiger, and A. R. Todd, *J. Soc. Chem. Ind.*, 57, 1188 (1938).

⁹⁴ W. John and P. Günther, *Ber.*, 72, 1649-1653 (1939).

⁹⁵ L. I. Smith, H. E. Ungnade, J. W. Opie, W. W. Prichard, R. B. Carlin, and E. W. Kaiser, *J. Org. Chem.*, 4, 323-333 (1939).

⁹⁶ W. John and M. Schmeil, *Ber.*, 72, 1653-1656 (1939).

5. Properties of the Tocopherols

The tocopherols and their esters are soluble in fat solvents and insoluble in water. Although such esters as the allophanates, the *p*-nitrophenyl-

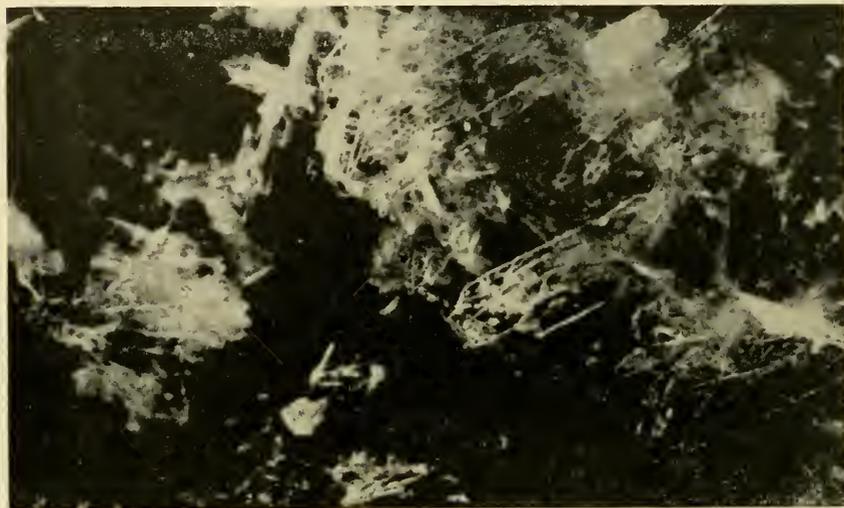


Fig. 2. Crystalline natural α -tocopherol ($\times 30$).⁴⁹



Fig. 3. Crystalline natural γ -tocopherol ($\times 10.5$).⁴⁹

urethans, and the 3,5-dinitrobenzoates were the only crystalline forms which had been obtained over a number of years, Robeson⁴⁶ has recently been

able to produce crystalline α - and γ -tocopherols from two highly purified preparations of natural tocopherols.⁴⁵ He was unsuccessful, however, in obtaining crystalline natural β -tocopherol. The method employed for crystallizing the α - and γ -tocopherols was by the use of a 2.5% methanol solution at -35°C . followed by drying at -10°C . under a high vacuum. The α - and γ -tocopherol crystals are pictured in Figures 2 and 3. δ -Tocopherol has been obtained only as a yellow oil, although the *p*-phenylazobenzoyl ester is crystalline.³⁵

In the absence of oxygen, the tocopherols are quite stable when heated alone to 200°C . or to 100°C . in the presence of sulfuric or hydrochloric acids. They are acted on by alkalis only slowly, and therefore may be obtained by alkaline hydrolysis. The tocopherols are reasonably resistant to destruction in visible light but are quite susceptible to alteration in ultraviolet light.^{31,97} They are all highly sensitive to oxidation, which causes a disappearance of their biological activity. In general, such esters as the succinates and acetates are more stable than are the free alcohols.

The four tocopherols have similar characteristic absorption spectra in the ultraviolet with maxima in the neighborhood of $295\text{ m}\mu$.⁴⁵ Olcott^{51,98} was the first investigator to call attention to this property. He placed the band at $294\text{ m}\mu$. The latter figure was confirmed shortly thereafter by Martin and associates,⁹⁹ as well as by Drummond *et al.*^{31,97} This maximum is displaced to approximately $285\text{ m}\mu$ for such esters as the acetates. The extinction coefficient, E (1%, 1 cm.), for the free α -tocopherol is reduced from 77 to 42 when changed to the allophanate ester.¹⁰⁰ The absorption maximum of δ -tocopherol was reported to be $298\text{ m}\mu$. E (1%, 1 cm.) was found³⁵ to be 91.2. The absorption spectrum of α -tocopherol in iso-octane is given in Figure 4. A similar absorption pattern obtains for β -tocopherol.¹⁰¹ Data on the characteristic absorption of α -tocopherol are also given in Table 5.

TABLE 5
ABSORPTION CHARACTERISTICS OF α -TOCOPHEROL IN ISO-OCTANE^a

Maxima			Minima		
λ , A.	ϵ	E (1%, 1 cm.)	λ , A.	ϵ	E (1%, 1 cm.)
2230	8080	188	2550	300	7
2980	3140	73	3160	0	0

^a O. H. Emerson, G. A. Emerson, A. Mohammad, and H. M. Evans, *J. Biol. Chem.*, **122**, 99-107 (1938).

⁹⁷ J. C. Drummond, E. Singer, and R. J. Mac Walter, *Biochem. J.*, **29**, 2510-2521 (1935).

⁹⁸ H. S. Olcott, *J. Biol. Chem.*, **105**, lxx (1934).

⁹⁹ A. J. P. Martin, T. Moore, M. Schmidt, and F. P. Bowden, *Nature*, **134**, 214 (1934).

¹⁰⁰ H. Rudy, "Chemie der Vitamine," in W. Stepp, *Ernährungslehre*, Springer, Berlin, 1939, pp. 108-166.

¹⁰¹ F. Bergel, A. Jacob, A. R. Todd, and T. S. Work, *Nature*, **141**, 646 (1938).

The tocopherols exhibit characteristic absorption spectra in the infrared spectrum. Absorption bands occur in the neighborhood of 3.0, 6.3, and 8.0 μ ; additional unassigned absorption bands near 8.6 and 10.9 μ are characteristic of the tocopherol structure.¹⁰² The maximum which occurs at 2.9 to 3.0 μ is due to the effect of the hydroxyl group, while the intense

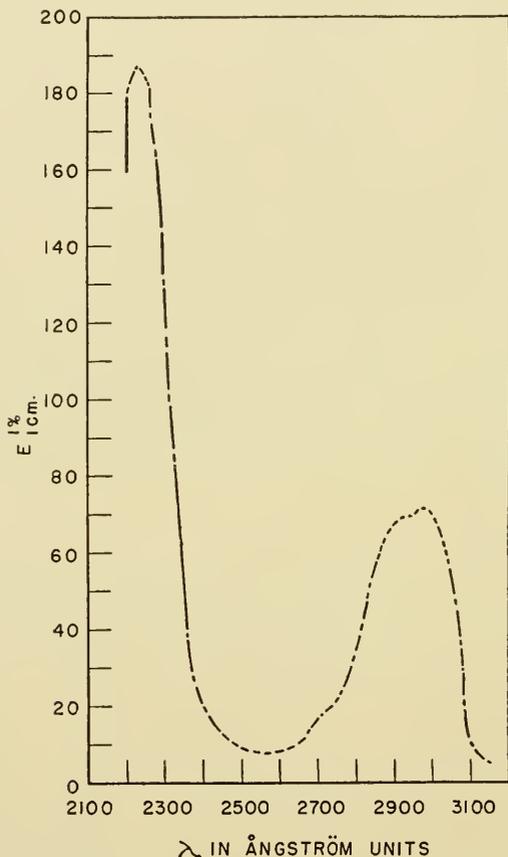


Fig. 4. Absorption spectrum of α -tocopherol in iso-octane.³⁴

absorption at 8.0 μ is probably to be traced to the C—O vibration of the phenolic hydroxyl. The conjugated C=C system of the benzene portion of the molecule accounts for the strong band in the 6.15 to 6.35 μ region. Phenol itself has a strong absorption maximum at 6.27 μ .¹⁰³ Some absorption also originates near 3.25 μ from the C—H groups of benzene, while the

¹⁰² H. Rosenkrantz, *J. Biol. Chem.*, 173, 439-447 (1948).

¹⁰³ R. B. Barnes, R. C. Gore, U. Liddel, and V. Z. Williams, *Infrared Spectroscopy. Industrial Applications and Bibliography*, Reinhold, New York, 1944.

same group in the aliphatic linkages causes an absorption at 3.41μ . A series of bands centering around 7μ (6.7 to 7.15) can likewise be ascribed to the angular vibration of the C—H linkages. Finally, there are a number of "unassigned" bands between 10 and 12μ , some of which arise from C—C vibrations. Both Stern *et al.*³⁵ and Rosenkrantz¹⁰² consider that the infrared

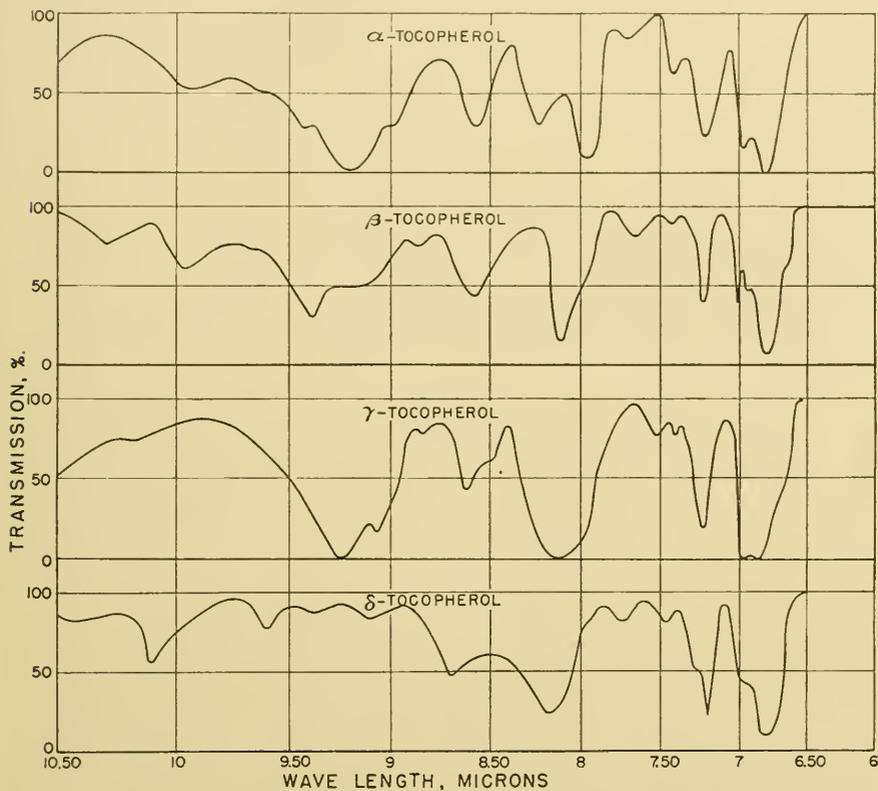


Fig. 5. Infrared transmission spectra of natural tocopherols.^{35,102}

spectrum offers much promise for the detection and determination of the individual tocopherols. Figure 5 illustrates the infrared absorption pattern of the four common tocopherols.

Table 6 records the absorption maxima of some of the tocopherols and their derivatives as determined in the infrared area of the spectrum.

All natural tocopherols have been shown to be optically active. They have three centers of asymmetry,⁹² which are on carbons 2, 4', and 8'. Thus, 8 potential isomers of each are possible. Stern *et al.*³⁵ have reported that, when dissolved in ethyl alcohol, all natural tocopherols show a dextro-

rotation. δ -Tocopherol has the highest specific rotation, while the lowest value obtains with α -tocopherol. These data are summarized in Table 7.

TABLE 6
PROBABLE ABSORPTION MAXIMA IN THE INFRARED SPECTRUM OF SOME TOCOPHEROLS
AND THEIR DERIVATIVES^a

Compound	Hydroxy absorption		Wave length (phenolic C—O linkage), μ
	Type	Wave length, μ	
α -Tocopherol.....	Phenolic	3.02	7.85
γ -Tocopherol.....	Phenolic	3.02	8.17
δ -Tocopherol.....	Phenolic	3.01	8.28
α -Tocopherol acetate.....	—	—	8.18
α -Tocopherol palmitate.....	—	—	8.06
α -Tocopherol succinate.....	Acid OH	3?	8.16
γ -Tocopherol palmitate.....	—	—	8.14
α -Tocopherolhydroquinone triacetate..	—	—	8.08
2,5,6-Trimethylhydroquinone.....	Phenolic	3.03	<i>b</i>
α -Tocopherylquinone.....	Alcoholic	2.93	—

^a Data adapted from H. Rosenkrantz, *J. Biol. Chem.*, 173, 439-447 (1948).

^b Split band at 7.6 μ and another band at 8.23 μ . It is uncertain which is due to phenolic C—O linkage.

TABLE 7
SPECIFIC ROTATIONS OF TOCOPHEROLS AND ESTERS IN ETHYL ALCOHOL AND BENZENE^a

Tocopherol	$[\alpha]_{546.1}^{25}$	Tube length, dm.	Soln. concn., g./100 ml.
Ethyl alcohol			
α -	0.32	1	14.8
γ -	2.2	1	9.32
β -	2.9	1	7.15
δ -	3.4	1	15.48
Benzene			
α -	-3.0	1	13.50
γ -	-2.4	1	8.59
β -	0.9	1	8.00
δ -	1.1	1	10.86

^a M. H. Stern, C. D. Robeson, L. Weisler, and J. G. Baxter, *J. Am. Chem. Soc.*, 69 869-874 (1947), p. 872.

One of the most important uses of the tocopherols in commerce results from their ready susceptibility to oxidation. They are thus able to protect other less vulnerable compounds from destruction by breaking up the chain of oxidation reactions. Such antioxidant activity continues until all of the tocopherol has been exhausted. δ -Tocopherol is the best antioxidant of the group, followed in order by the γ -, β -, and α -isomers. This has been demonstrated by *in vitro* tests on the protective action upon

vitamin A and β -carotene.³⁵ The sparing action of natural tocopherol concentrates on the utilization of carotene and vitamin A *in vivo* has been demonstrated by Hickman, Kaley, and Harris.¹⁰⁴⁻¹⁰⁶ The relative position of the γ -, β -, and α -tocopherols as antioxidants is the same as that demonstrated by Olcott and Emerson¹⁰⁷ and by Hove and Hove,¹⁰⁸ using markedly different systems. A typical result obtained when vitamin A acetate was

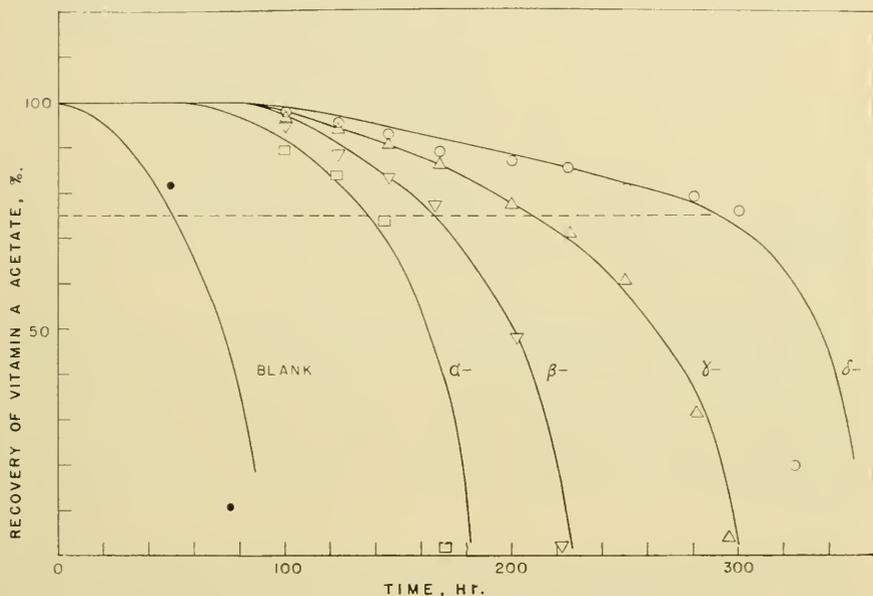


Fig. 6. The relative activity of 0.1% tocopherols as antioxidants for vitamin A acetate in olive oil (50,000 U.S.P. units per gram) at 39°C.³⁵

employed as the test substance is shown in Figure 6. The relative effectiveness of the α -, β -, γ -, and δ -tocopherols expressed as percentage increase in induction period over unprotected blanks was found to be 1:1.3:1.8:2.7, respectively, at 39°C., and 1:1.1:1.6:1.7 at 55°C.³⁵

Another property closely associated with the antioxidant activity of tocopherol is the resistance to oxidation. Stern and associates³⁵ have compared this property by determining the amount of the several tocopherols

¹⁰⁴ K. C. D. Hickman, M. W. Kaley, and P. L. Harris, *J. Biol. Chem.*, 152, 303-311 (1944).

¹⁰⁵ P. L. Harris, M. W. Kaley, and K. C. D. Hickman, *J. Biol. Chem.*, 152, 313-320 (1944).

¹⁰⁶ K. C. D. Hickman, M. W. Kaley, and P. L. Harris, *J. Biol. Chem.*, 152, 321-328 (1944).

¹⁰⁷ H. S. Olcott and O. H. Emerson, *J. Am. Chem. Soc.*, 59, 1008-1009 (1937).

¹⁰⁸ E. L. Hove and Z. Hove, *J. Biol. Chem.*, 156, 611-621, 623-632 (1944).

recovered from an olive oil solution kept in an oven at 55°C. in contact with oxygen for periods of 88 and of 108 hours. These data are summarized in Table 8. The results of this test parallel the behavior of these tocopherols as antioxidants. The tocopherols which can best protect themselves are also the most effective as antioxidants.

TABLE 8
RELATIVE RESISTANCE OF THE TOCOPHEROLS TO OXIDATION IN OIL AT 55°C.^a

Olive oil containing 0.1% tocopherol	Per cent of added tocopherol recovered	
	88 hour	108 hour
δ-	94.2	93.5
γ-	93.5	92.5
β-	83.8	76.8
α-	81.2	75.5

^a M. H. Stern, C. D. Robeson, L. Weisler, and J. G. Baxter, *J. Am. Chem. Soc.*, **69**, 869-874 (1947), p. 871.

On oxidation of the tocopherols with gold chloride, *p*-quinones are formed which apparently result from the rupture of the 1:2 linkage. These products are readily distinguishable by a maximum absorption band at about 260 m μ . Stern *et al.*³⁵ have reported a similar behavior for δ -tocopherol, in which case the maximum absorption band is at 257 m μ . When the quinone is reduced with sodium hydrosulfite, a hydroquinone is formed:

E (1%, 1 cm., 294 m μ) = 81.6 for δ -tocopherol.³⁵ This can be condensed with sulfonic acid in ethyl alcohol to produce the original tocopherol. The gold chloride method has been used by Karrer *et al.*^{109,110} for the quantitative determination of tocopherol.

Vitamin E also develops a characteristic red color when treated with silver nitrate and nitric acid. The product obtained by such treatment of δ -tocopherol has the spectral absorption properties of an *o*-quinone; the corresponding compounds from α -, β -, and γ -tocopherol have likewise been identified as *o*-quinones.^{111,112} The yellow color which is produced has an intensity proportional to the amount of tocopherol present; this can be used for the quantitative determination of vitamin E. The maximum absorption is from 460 to 480 m μ ¹¹¹ for α -, β -, and γ -tocopherols, and the extinction coefficient values are from 11 to 15; in the case of δ -tocopherol, the maximum absorption is at 435 m μ and the E (1%, 1 cm.) is about 15.

¹⁰⁹ P. Karrer, R. Escher, H. Fritzsche, H. Keller, B. H. Ringier, and H. Salomon, *Helv. Chim. Acta*, **21**, 939-953 (1938).

¹¹⁰ P. Karrer and H. Keller, *Helv. Chim. Acta*, **21**, 1161-1169 (1938).

¹¹¹ L. I. Smith, W. B. Irwin, and H. E. Ungnade, *J. Am. Chem. Soc.*, **61**, 2424-2429 (1939).

¹¹² L. I. Smith, W. B. Irwin, and H. E. Ungnade, *Science*, **90**, 334-335 (1939).

When the tocopherols are treated with nitric acid alone, a different result obtains for δ -tocopherol, as compared with the other tocopherols. Both natural and synthetic δ -tocopherol show a maximum absorption band at $373\text{ m}\mu$ with extinction coefficients³⁵ of 59 and 48, respectively. The divergencies are explained as being due to the fact that the oxidation products were not purified. On the other hand, the α -, β -, and γ -tocopherols yield the same results with nitric acid as with silver nitrate, namely, *o*-quinones with maxima between 460 and 480 $\text{m}\mu$.³⁵ The intensity of the color production at $373\text{ m}\mu$ should furnish a quantitative method for the determination of δ -tocopherol in the presence of the other tocopherols.

There are several color reactions which are more or less specific for the tocopherols. One of these is based upon the oxidation of vitamin E in alcoholic solution by ferric chloride,¹¹³ or by the quantitative reduction of ferric to ferrous chloride by the tocopherols.¹¹⁴ When tocopherol is mixed with the ferric chloride- α, α' -dipyridyl solution, a characteristic red color develops due to the formation of ferrous dipyridyl.¹¹⁴ Since the amount of ferrous ions formed depends upon the amount of tocopherols present, the measurement of the red color is an index of that factor. This is the basis of the Emmerie-Engel method for the determination of tocopherol.^{114, 115} While this procedure is quite satisfactory for α -, β -, and γ -tocopherols, it can be used only under controlled conditions with δ -tocopherol.³⁵ If the reaction is allowed to proceed for 10 minutes instead of for $2\frac{1}{2}$ minutes, a 22% excess of color develops over that produced by the isomeric tocopherols.

An especially satisfactory reaction for γ - and δ -tocopherol has been described by Weisler, Robeson, and Baxter.⁶¹ This involves the reaction between diazotized *o*-anisidine and γ - or δ -tocopherol.

6. Standards for Vitamin E

The international standard for vitamin E which has been adopted by the League of Nations is synthetic racemic α -tocopherol acetate. One International Unit is defined as the specific activity of 1 mg. of a standard preparation. The international standard contains the 1 mg. of α -tocopherol in 100 mg. of olive oil. This is the amount which, when administered orally, prevents resorption-gestation in rats deprived of vitamin E.¹¹⁶

The Rat Unit or "fertility dose" is more widely employed. This is the smallest amount of vitamin E which, when given *per os* daily to resorption-

¹¹³ J. Waddell and H. Steenbock, *J. Biol. Chem.*, **80**, 431-442 (1928).

¹¹⁴ A. Emmerie and C. Engel, *Nature*, **142**, 873 (1938).

¹¹⁵ A. Emmerie and C. Engel, *Rec. trav. chim.*, **57**, 1351-1355 (1938).

¹¹⁶ E. M. Hume, *Nature*, **148**, 472-473 (1941).

sterile female rats for the full 21 days of gestation, brings about the birth of at least one living young in 50% of the animals. This corresponds to about 2 to 3 mg. of α -tocopherol. Another standard is the Pacini-Linn Unit, which equals about one-tenth of the Rat Unit⁵⁵; the Bromskov Rat Unit¹¹⁷ is the amount of vitamin E which, when administered once during the first 8 days of pregnancy, prevents resorption of the fetuses. These standards apply to vitamin E only in relation to its biological activity, and are not used in considering its antioxidant behavior.

7. Chemical Structure in Relationship to Biological Activity of Vitamins E and of Vitamin-E-Like Compounds

Something over 130 compounds have been studied for their vitamin E activity.¹¹⁸ Of these about 40 have shown some biological potency, although none compares with the tocopherols in this respect. Whereas different tocopherols are active in amounts varying from 3 to 20 mg., from 50 to 100 mg. of the other substances showing vitamin E activity are required to produce a positive effect. In several cases, the amount in which the compound must be fed to produce biological activity approaches the level at which toxicity develops.

Any change in the groups on the aromatic nucleus or on the long aliphatic side chain markedly alters the vitamin E activity. It is also necessary that the hydroxyl group on the benzene ring be in para position to the oxygen in the bridge. The effectiveness can be masked by any of several carboxylic esters without marked reduction in activity,^{119,120} although the allophanates⁵⁵ and the ethers are completely without any antisterility effect.⁶⁰

(1) Tocopherols

α -Tocopherol possesses the greatest potency of any of the natural or synthetic products which have a vitamin E activity. It is effective when administered in doses of 2 to 3 mg. per day. Although the *dl*-mixture of α -tocopherol has generally been considered to have a biological activity identical with that of natural *d*- α -tocopherol,^{41,43,121} recent reports of Harris and his collaborators indicate that the *d*-form has considerably greater potency than the synthetic *dl*-mixture (1.36:1).¹²²⁻¹²⁴

¹¹⁷ C. Bromskov, *Arch. exptl. Path. Pharmacol.*, **190**, 627-647 (1938).

¹¹⁸ H. M. Evans, O. H. Emerson, G. A. Emerson, L. I. Smith, H. E. Ungnade, W. W. Prichard, F. L. Austin, H. H. Hoehn, I. W. Opie, and S. Wawzonek, *J. Org. Chem.*, **4**, 376-388 (1939).

¹¹⁹ V. Demole, O. Isler, B. H. Ringier, H. Salomon, and P. Karrer, *Helv. Chim. Acta*, **22**, 65-68 (1939).

¹²⁰ O. Isler, *Helv. Chim. Acta*, **21**, 1756-1759 (1938).

¹²¹ P. Karrer and B. H. Ringier, *Helv. Chim. Acta*, **22**, 610-616 (1939).

¹²² P. L. Harris, J. L. Jensen, M. Joffe, and K. E. Mason, *J. Biol. Chem.*, **156**, 491-498 (1944).

¹²³ P. L. Harris and M. I. Ludwig, *J. Biol. Chem.*, **179**, 1111-1115 (1949).

¹²⁴ P. L. Harris and M. I. Ludwig, *J. Biol. Chem.*, **180**, 611-614 (1949).

β -Tocopherol (*p*-xylocopherol) and γ -tocopherol (*o*-xylocopherol) are only about one-half as efficacious as the α -isomer. *m*-Xylocopherol (5,7-dimethyltolcol), which is not a natural product, is intermediate between α - and β -tocopherols in potency.^{88,89,92,118} δ -Tocopherol (8-methyltolcol) has only about 1% of the physiologic activity of α -tocopherol.⁸⁵ However, Karrer and Fritzsche⁸⁸ found that tolucopherol, which has a single methyl group at an undetermined location, was ineffective in 20 times the dosage (40 mg.) which was active in the case of α -tocopherol.⁸⁹

Most of the esters of α -tocopherol have a high potency, with the exception of the allophanates which, as has already been pointed out, are biologically inactive.⁵⁵ As regards the esters, Demole *et al.*¹¹⁹ have reported that the acetate, propionate, and *n*-butyrate have an even greater effect than has free α -tocopherol. Harris and his collaborators^{123,124} have recently confirmed the fact that the esters have a higher biological potency than do the free tocopherols. α -Tocopherol acetate was shown to have a 62% greater potency than the free tocopherol, irrespective of whether comparisons were made between the ester and free forms of the *d*-tocopherol or of the synthetic *dl*-tocopherol.

Inorganic esters such as the phosphate have also been shown to be biologically active,¹²⁵ but the ethers are wholly inactive. α -Tocopherol and its acetate are completely non-toxic, since they may be administered to mice in amounts of 50 g. per kilogram of body weight without deleterious effects.¹²⁶ They are also non-carcinogenic.¹²⁷

The replacement of the methyl groups on the aromatic ring by one or more ethyl groups profoundly depresses the potency. Thus, 5,7-dimethyl-8-ethyltolcol^{92,128} is only about 20 to 25% as effective as the trimethyl derivative (1 Rat Unit = 10–16 mg.), while diethylmethyltolcol¹²⁹ is potent in 10-mg. doses. 5,7-Diethyltolcol and monomethyltolcol (δ -tocopherol?) have been reported to be inactive in doses as high as 40–50 mg. daily.^{88,89} Tocol itself⁸⁹ in doses up to 50 mg., and 6-desoxy- α -tocopherol¹³⁰ in the amount of 100 mg. per day, were found to be completely ineffective. A number of tocopherol quinones have been prepared, but these fail to show any vitamin-E-like action.^{131–134}

One compound which is of interest because it plays a dual role as a vitamin is naphthotocopherol. This compound shows vitamin E activity

¹²⁵ P. Karrer and G. Bussman, *Helv. Chim. Acta*, **23**, 1137–1138 (1940).

¹²⁶ V. Demole, *Z. Vitaminforsch.*, **8**, 338–341 (1939).

¹²⁷ V. Demole, *Z. Vitaminforsch.*, **8**, 341–347 (1939).

¹²⁸ P. Karrer and O. Hoffmann, *Helv. Chim. Acta*, **22**, 654–657 (1939).

¹²⁹ P. Karrer and O. Hoffmann, *Helv. Chim. Acta*, **23**, 1126–1131 (1940).

¹³⁰ F. v. Werder, T. Moll, and F. Jung, *Z. physiol. Chem.*, **257**, 129–139 (1939).

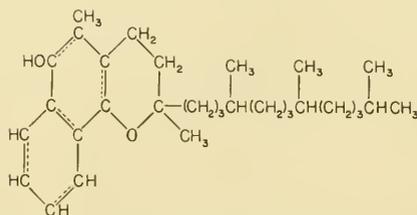
¹³¹ P. Karrer and A. Geiger, *Helv. Chim. Acta*, **23**, 455–459 (1940).

¹³² P. Karrer, H. Salomon, and H. Fritzsche, *Helv. Chim. Acta*, **21**, 309–313 (1938).

¹³³ W. John, E. Dietzel, and W. Emte, *Z. physiol. Chem.*, **257**, 173–189 (1939).

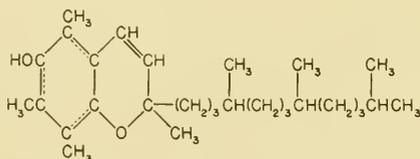
¹³⁴ M. D. Wright, and J. C. Drummond, *Biochem. J.*, **34**, 32–33 (1940).

at a 25-mg. dosage and a vitamin K effect when given in amounts of 300 to 600 μg .¹³⁵



Naphthotocopherol

Although the replacement of the methyl groups attached to the chroman ring with ethyl radicals profoundly alters the physiological activity, just as does the complete removal of such side groups, it is possible to introduce a double bond into the chroman ring without exerting a marked effect upon biopotency. Thus, Karrer, Legler, and Schwab¹³⁶ found that *dl*-3,4-dehydro- α -tocopherol is active in amounts of about 6 mg.



dl-3,4-Dehydro- α -tocopherol

The side chain on carbon 2 probably has the most specific requirements of any part of the molecule. When this is shortened by one isoprene unit of 5 carbons, $-\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{CH}_3)\text{CH}_3$, the resulting compound is inactive when administered in 20-mg. doses; the effect of larger quantities has not been tested.¹³⁷ When the side chain is shortened by 2 isoprene units, no biological activity can be detected in doses as high as 40 mg. daily.¹³⁸ The introduction of a double bond in the long side chain likewise causes a loss of physiological activity.¹³⁷ When the side chain is completely removed, or is replaced by a methyl group, the resulting compound is devoid of any antisterility effect¹³⁷ when fed in doses of 30 mg. However, John and co-workers¹³⁹ have prepared 2-dodecyl-2,5,7,8-tetramethyl-6-oxychroman, which Rosenberg⁵⁵ states is active when fed in 60-mg. doses.

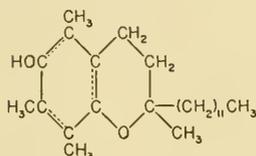
¹³⁵ M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 1982-1991 (1940).

¹³⁶ P. Karrer, R. G. Legler, and G. Schwab, *Helv. Chim. Acta*, **23**, 1132-1137 (1940).

¹³⁷ P. Karrer and K. A. Jensen, *Helv. Chim. Acta*, **21**, 1622-1624 (1940).

¹³⁸ P. Karrer and K. S. Yap, *Helv. Chim. Acta*, **23**, 581-584 (1940).

¹³⁹ W. John, P. Günther, and M. Schmeil, *Ber.*, **71**, 2637-2649 (1938).



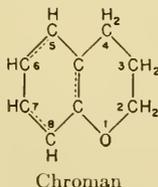
2-Dodecyl-2,5,7,8-tetramethyl-6-oxychroman

Karrer and Stähelin¹⁴⁰ have recently prepared two homologues of α -tocopherol in which the methyl group on position 2 is replaced by an ethyl or propyl side chain. The acetates of both of these compounds have been found to be active in 10-mg. but not in 5-mg. daily doses.

The natural tocopherols behave in an inverse manner as antioxidants and as antisterility agents.^{35,107} Thus, α -tocopherol is the most potent and δ -tocopherol the least effective in vitamin E activity. On the other hand, δ -tocopherol is the member which is most efficient as an antioxidant, followed in order by γ -, β -, and α -tocopherol.

(2) Chromans and Chromene Derivatives

Chroman itself has been shown to possess some biological potency.¹³⁰ This is true also for 2,2-diethyl- and 2,2-di-*n*-butylchroman.¹¹⁸ On the other hand, 2,2-dimethyl- and 2,2-dipropylchromans have no activity.¹¹⁸



Chroman

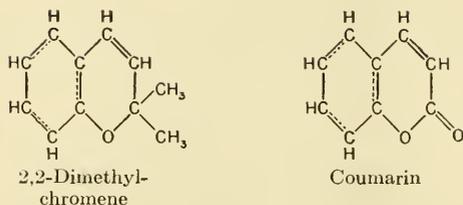
This alteration in activity is a very curious one, and may be related to variations in end products resulting from the oxidation of the hydrocarbon chains in position 2. The only other chroman reported as active is 2,5,7,8-tetramethylchroman.¹¹⁸ Evans *et al.*¹¹⁸ proved that 2,2,3-trimethyl-, 2-methyl-4-ethyl-, and 2,2,5,7-tetramethylchromans are all inactive.

Although 6-hydroxychroman is inactive, two of its derivatives, namely, 2,2,5,7,8-pentamethyl¹¹⁷ and 2,5,7,8-tetramethyl-2-dodecyl-6-hydroxychroman,¹⁴¹ have been found to be active when given in 100-mg. and 60-mg. daily doses, respectively. Other compounds studied which were found to be inactive included 2,5,7,8-tetramethyl-,^{130,137,139} 2,3,5,7,8-pentamethyl-,¹³⁹ and 2,5,7,8-tetramethyl-2-isohexyl-6-hydroxychroman.¹¹⁸

Three chromene derivatives have been reported as ineffective.¹¹⁸ These are 2,2-dimethyl-, 2,2-diethyl-, and 2,2-di-*n*-butylchromenes.

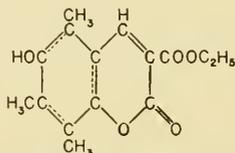
¹⁴⁰ P. Karrer and M. Stähelin, *Helv. Chim. Acta*, **28**, 438-443 (1945).

¹⁴¹ W. John, *Angew. Chem.*, **52**, 413-419 (1939).

(3) *Coumarins*

Coumarin, like chromene, has a double bond between carbons 3 and 4; it also has a carbonyl group on carbon 2. Although no chromenes were found which have biological activity, the presence of the double bond at the 3,4 position does not exclude activity, since 3,4-dehydro- α -tocopherol is active.

Coumarin and its dihydroderivative (dihydrocoumarin) are both inactive.¹¹⁸ Coumarin, in fact, was shown to be toxic at a 100-mg. level. 5,7,8-Trimethyl-6-hydroxy-3,4-dihydrocoumarin is also without activity.¹¹⁸ However, 3-carbethoxy-5,7,8-trimethyl-6-hydroxycoumarin is exceedingly potent (1 Rat Unit = 20 mg.), although the corresponding 3-carboxy compound was inactive at this dosage. It is the compound, aside from the tocopherols, which for some mysterious reason possesses the highest vitamin E activity. Even more strange is the fact that the free acid and the isoamyl ester are both inactive.¹¹⁸



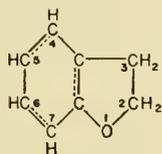
3-Carbethoxy-5,7,8-trimethyl-
6-hydroxycoumarin

(4) *Coumarans and Coumarones*

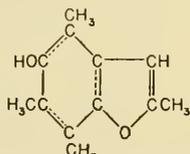
Despite the profound change in the heterocyclic structure of the coumarans, several active derivatives have been reported. 2-Methylcoumaran was found, in one assay, to possess considerable potency when fed in 50-mg. doses; however, in 3 other assays at 25, 50, and 100 mg., it was found to be inactive.¹¹⁸ Further work is needed to clear up this discrepancy. However, two other coumarans, namely, 2,2,7-trimethylcoumaran and 2,3,4,6,7-pentamethyl-5-hydroxycoumaran, were found to be active.¹¹⁸ On the other hand, 3-methyl-,¹¹⁸ 2,4,6,7-tetramethyl-,¹¹⁸ 2,4,6,7-tetramethyl-5-hydroxy-,^{118,130} and 4,6,7-trimethyl-2-*n*-heptadecyl-5-hydroxycoumarans,¹⁴² are all entirely inactive. Coumaran itself is without activity.¹³⁰

¹⁴² F. Bergel, A. Jacob, A. R. Todd, and T. S. Work, *J. Chem. Soc.*, 1938, 1375-1382.

The only coumarone which has been studied is 2,4,6,7-tetramethyl-5-hydroxycoumarone. It is devoid of vitamin E action.¹¹⁸



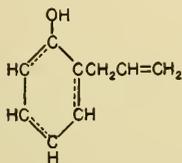
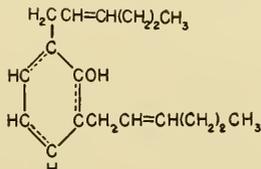
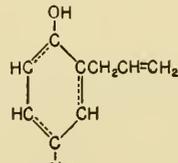
Coumaran



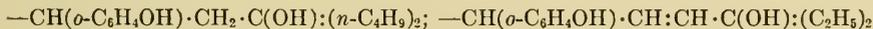
2,4,6,7-Tetramethyl-5-hydroxycoumarone

(5) Phenols

Although phenol and most of its derivatives fail to possess vitamin E activity, several substituted phenols have given positive results. This is true of *o*-allylphenol, which is a possible intermediate in the synthesis of chroman. Although it is inactive in 25-mg. doses, it is effective when administered in the amount of 50 mg.¹¹⁸ Di-*o*-hexenylphenol and *p*-amino-*o*-allylphenol¹¹⁸ are also active when fed in relatively large doses.


o-Allylphenol

 Di-*o*-hexenylphenol

p-Amino-*o*-allylphenol

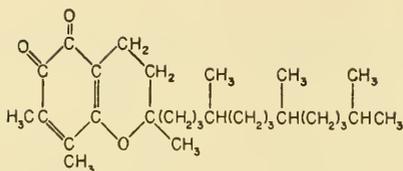
In spite of the fact that *o*-allylphenol is active, its isomer—*o*-propenylphenol—is inactive. A large number of other phenols have also been found to be devoid of activity. This list includes *o*-[α -methylallyl]-, *o*-hexenyl-, 2,3,5-trimethyl-, 6-allyl-, *p*-capryl-, *p*-*tert*-octyl-, *o*-allyl *p*-carboxy-, and *o*-allyl-*p*-carbomethoxy-phenols,¹¹⁸ as well as two *o*-phenols with the following side chains:



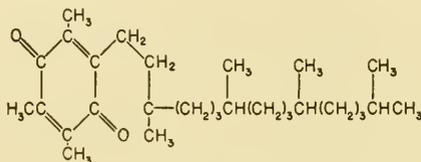
Evans *et al.*¹¹⁸ found that phenylhexenyl ether, phenylcinnamyl ether, and *p*-carboxyphenylallyl ether are also inactive.

(6) Quinones

Practically all the quinones which have been studied are ineffective. However, the red *o*-quinone oxidation product of α -tocopherol is active in doses of 12 mg., although it is inactive when given in the amount of 3 or 6 mg. daily.¹¹⁸

Red *o*-quinone from α -tocopherol

The only *p*-quinone which has been reported to be efficacious is α -tocopherylquinone (also called α -tocoquinone) in which, according to Emerson, Emerson, and Evans,¹⁴³ the activity is substantially identical with that of α -tocopherol. The formula for α -tocoquinone was worked out by John, Dietzel, and Emte,¹³³ who reported it to be inactive, as have several other groups of workers.¹³⁰⁻¹³²

 α -Tocoquinone

All the other quinones tested have given negative results. These include the following: durohydroquinone,^{118,144,145} tetraethylquinone (also toxic),¹¹⁸ thymoquinone (also toxic),¹¹⁸ trimethylquinone,¹¹⁸ 1,4-naphthoquinone,¹¹⁸ 1,2-naphthoquinone (also toxic),¹¹⁸ 2,3-dimethyl-1,4-naphthoquinone,¹³⁰ 2-methyl-1,4-naphthoquinone,¹¹⁸ 2-hydroxy-1,4-naphthoquinone,¹¹⁸ 2-methoxy-1,4-naphthoquinone,¹¹⁸ anthraquinone,¹¹⁸ and β -methyl-anthraquinone.¹¹⁸

(7) Hydroquinones

Although hydroquinone lacks vitamin E activity,¹⁴⁴ as is also the case with *m*-xylohydroquinone,^{118,130} both *o*-xylohydroquinone and *p*-xylohydroquinone¹¹⁸ are active (in 100-mg. doses but not in 50-mg. amounts). Trimethylhydroquinone has been reported both as possessing vitamin E activity⁵⁵ and as devoid of such potency,¹¹⁸ when given in 100-mg. quantities. Durohydroquinone (tetramethyl),¹⁴⁵ 2,3-dimethyl-5,6,7,8-tetrahydro-1,4-dioxynaphthalene,¹⁴⁶ as well as its mono-*n*-decyl^{50,145} ether, have been cited as active. On the other hand, trimethylethyl-, and trimethyl-5-acetohydroquinone gave negative results.¹³⁰ A list of the biological activity

¹⁴³ O. H. Emerson, G. A. Emerson, and H. M. Evans, *J. Biol. Chem.*, **131**, 409-412 (1939).

¹⁴⁴ H. M. Evans, G. A. Emerson, and O. H. Emerson, *Science*, **88**, 38-39 (1938).

¹⁴⁵ W. John and P. Günther, *Z. physiol. Chem.*, **254**, 51-56 (1938).

¹⁴⁶ F. v. Werder and T. Moll, *Z. physiol. Chem.*, **254**, 39-50 (1938).

of esters and ethers of trimethylhydroquinone is given in Table 9, while the corresponding data on the derivatives of tetramethylhydroquinone are summarized in Table 10.

TABLE 9

VITAMIN E ACTIVITY OF ETHERS AND ESTERS OF TRIMETHYLHYDROQUINONE^a

Active compounds	Inactive compounds
Monobenzoate ^b	Bis- β -iodopropionate ^b
Mono- <i>n</i> -hexyl ether ^c	Di- <i>n</i> -dodecyl ether ^c
Mono- <i>n</i> -dodecyl ether ^c	
Mono- <i>n</i> -dodecyl ether acetate ^c	
Monodihydrochaulmoogryl ether ^c	

^a Adapted from L. I. Smith, *Chem. Revs.*, 27, 287-329 (1940).

^b F. v. Werder, T. Moll, and F. Jung, *Z. physiol. Chem.*, 257, 129-139 (1939).

^c F. v. Werder and T. Moll, *Z. physiol. Chem.*, 254, 39-50 (1938).

TABLE 10

VITAMIN E ACTIVITY OF ETHERS AND ESTERS OF TETRAMETHYLHYDROQUINONE^a

Active compounds	Inactive compounds
Mono- <i>n</i> -butyl ether ^b	Mono- <i>n</i> -heptyl ether ^b
Di- <i>n</i> -butyl ether ^b	Dibenzyl ether ^b
Mono- <i>n</i> -hexyl ether ^b	
Di- <i>n</i> -hexyl ether ^b	
Mono- <i>n</i> -octyl ether ^b	
Di- <i>n</i> -octyl ether ^b	
Monocetyl ether ^c	Monocetyl ether ^c
Mono- <i>n</i> -dodecyl ether palmitate ^b	Mono- <i>n</i> -dodecyl ether <i>n</i> -propionate ^b
Monodecyl ether ^{b,c}	Di- <i>n</i> -dodecyl ether ^b
Mono- <i>n</i> -nonadecyl 2-ether ^c	Mono- <i>n</i> -octadecyl ether ^c
Mono- <i>n</i> -nonadecyl ether ^d	Mono-2-methyloctadecyl ether ^c
	Di- <i>n</i> -nonadecyl ether ^d
Monodihydrochaulmoogryl ether ^b	Mono-3-methyl-5-(1',1',3'-trimethyl-2'-cyclohexyl)pentyl-1 ether ^d
Monobenzyl ether ^b	

^a Adapted from L. I. Smith, *Chem. Revs.*, 27, 287-329 (1940).

^b F. v. Werder and T. Moll, *Z. physiol. Chem.*, 254, 39-50 (1938).

^c H. M. Evans, G. A. Emerson, and O. H. Emerson, *Science*, 88, 38-39 (1938).

^d F. v. Werder, T. Moll, and F. Jung, *Z. physiol. Chem.*, 257, 129-139 (1939).

Phytol, when fed alone or in conjunction with trimethylhydroquinone¹¹⁸ has been found to be inactive as a source of vitamin E. Although these two components readily condense in the laboratory to form α -tocopherol in excellent yield, a synthesis of this nature apparently does not take place *in vivo*.

CHAPTER X

DISTRIBUTION, PROPERTIES, AND CHEMISTRY OF THE VITAMIN K GROUP

I. Introduction

The newest recognized member of the fat-soluble vitamin group is vitamin K. The pioneer work leading to the discovery of this vitamin was not begun until as recently as 1929. Dam¹ was the first to report the development of certain symptoms, such as subcutaneous and intramuscular hemorrhages, which appear in chicks on an artificial diet low in lipids. The deficiency failed to respond to treatment with any of the vitamins known at that time. Dam stated shortly thereafter² that the disease was associated with a reduction in the clotting capacity of the blood. Hemorrhages occurred chiefly in areas exposed to injury.

Several years later, Dam and Schönheyder³ came to the conclusion that the hemorrhagic symptoms produced in chicks are the result of an avitaminosis. The vitamin which was able to prevent these pathological symptoms was found to be present in green leaves and in certain vegetables.⁴ It was named *vitamin K*,^{4,5} inasmuch as Dam and others had most frequently referred to it in the German and Danish literature as *koagulationsvitamin*, because it was obviously concerned primarily with the blood-clotting mechanism. Dam, Schönheyder, and Tage-Hansen^{6,7} demonstrated that vitamin K is of importance in regulating and maintaining the normal level of prothrombin in the blood. In vitamin K avitaminosis, subnormal levels of prothrombin occur which can obviously account for the retardation or complete absence of blood clotting. In 1938, the importance of vitamin K therapy in certain types of hemorrhagic conditions in man was

¹ H. Dam, *Biochem. Z.*, 215, 475-492 (1929).

² H. Dam, *Biochem. Z.*, 220, 158-163 (1930).

³ H. Dam and F. Schönheyder, *Biochem. J.*, 28, 1355-1359 (1935).

⁴ H. Dam, *Biochem. J.*, 29, 1273-1285 (1935).

⁵ H. Dam, *Nature*, 135, 652-653 (1935).

⁶ H. Dam, F. Schönheyder, and E. Tage-Hansen, *Biochem. J.*, 30, 1075-1079 (1936).

⁷ F. Schönheyder, *Nature*, 135, 653 (1935).

demonstrated by several groups of workers in the United States,^{8,9} as well as by Dam and Glavind^{10,11} in Denmark.

Vitamin K was first shown to be present in lettuce. It was later found in hog-liver fat, hempseed, tomatoes, kale,⁴ and to a small extent in a number of cereals.⁵ Halbrook¹² reported, as early as 1935, that the deficiency in chicks could be prevented when 5% of dehydrated alfalfa was included in the diet, or when fish meal which had been kept moistened for a period of time was employed as a source of protein. Almquist and Stokstad¹³ isolated the active principle from the non-saponifiable fraction of alfalfa lipid. It was proven to be stable to heating in an oven at 120°C. for 24 hours. Neither chlorophylls, sterols, carotene, nor xanthophylls were shown to have any antihemorrhagic effect. Moreover, the active component possessed no acidic or basic properties, nor was it found to be an ester.

Pure vitamin K was first isolated in 1939, almost simultaneously by the Dam-Karrer group¹⁴ and by Doisy and co-workers.¹⁵ The products prepared from alfalfa by Dam *et al.*,¹⁴ and also by MacCorquodale *et al.*, differed both in physical and in chemical properties from that separated from putrefied fish meal by McKee and associates.^{16,17} Both preparations had approximately the same activity as antihemorrhagic agents. It therefore became evident that more than one product possesses antihemorrhagic activity. The compounds isolated from alfalfa and from putrefied fish were designated as vitamins K₁ and K₂, respectively.

The chemical nature of both of these vitamins was elucidated very soon after their separation in pure form. The structure of vitamin K₁, with proof of its synthesis, was announced simultaneously by Almquist and Klose,¹⁸ Binkley *et al.*¹⁹ of the Doisy group, and by Fieser.²⁰ The follow-

⁸ E. D. Warner, K. M. Brinkhous, and H. P. Smith, *Proc. Soc. Exptl. Biol. Med.*, **37**, 628-630 (1938).

⁹ H. R. Butt, A. M. Snell, and A. E. Osterberg, *Proc. Staff Meetings Mayo Clinic*, **13**, 74-80 (1938).

¹⁰ H. Dam and J. Glavind, *Lancet*, **234**, 720-721 (1938).

¹¹ H. Dam and J. Glavind, *Acta Med. Scand.*, **96**, 108-128 (1938).

¹² E. R. Halbrook, *Thesis*, Univ. Calif. (1935). Cited by H. J. Almquist and E. L. R. Stokstad, *J. Biol. Chem.*, **111**, 105 (1935).

¹³ H. J. Almquist and E. L. R. Stokstad, *J. Biol. Chem.*, **111**, 105-113 (1935).

¹⁴ H. Dam, E. Geiger, J. Glavind, P. Karrer, W. Karrer, E. Rothschild, and H. Salomon, *Helv. Chim. Acta*, **22**, 310-313 (1939).

¹⁵ D. W. MacCorquodale, S. B. Binkley, R. W. McKee, S. A. Thayer, and E. A. Doisy, *Proc. Soc. Exptl. Biol. Med.*, **40**, 482-483 (1939).

¹⁶ R. W. McKee, S. B. Binkley, D. W. MacCorquodale, S. A. Thayer, and E. A. Doisy, *J. Am. Chem. Soc.*, **61**, 1295 (1939).

¹⁷ R. W. McKee, S. B. Binkley, S. A. Thayer, D. W. MacCorquodale, and E. A. Doisy, *J. Biol. Chem.*, **131**, 327-344 (1939).

¹⁸ H. J. Almquist and A. A. Klose, *J. Am. Chem. Soc.*, **61**, 2557-2558 (1939).

¹⁹ S. B. Binkley, L. C. Cheney, W. F. Holcomb, R. W. McKee, S. A. Thayer, D. W. MacCorquodale, and E. A. Doisy, *J. Am. Chem. Soc.*, **61**, 2558-2559 (1939).

²⁰ L. F. Fieser, *J. Am. Chem. Soc.*, **61**, 2559-2561 (1939).

ing year the structure of vitamin K₂ was demonstrated by Binkley, McKee, Thayer, and Doisy.²¹

Even before the structure of the vitamins K had been established, Ansbacher and Fernholz²² discovered the antihemorrhagic activity of 2-methyl-1,4-naphthoquinone. This preceded an extensive study of a wide variety of naphthoquinones, naphthohydroquinones, esters and ethers of the hydroquinones, and of many related compounds, which was carried out by Fieser, Tishler, and Sampson.²³ Excellent reviews of the subject are those of Wöhlisch,²⁴ Riegel,²⁵ Grossman,²⁶ and Brinkhous.²⁷ The reader is particularly referred to the more recent comprehensive reviews of three authorities in the field: Almquist,²⁸ Doisy *et al.*,²⁹ and Dam.³⁰ Considerable information is to be obtained from the articles in *Annual Reviews of Biochemistry* by Dam,³¹ Morton,³² and Hickman.³³ A complete review of vitamin K up to 1945 is included in the monograph of Rosenberg.³⁴

2. Occurrence of the K Vitamins

The primary sources of the natural vitamin K compounds are plants and microorganisms. It is believed that vitamin K₁ occurs chiefly in green leaves, while vitamin K₂ is mainly a product of the metabolism of bacteria. Vitamin K₁ has been reported in the green leafy tissues of alfalfa¹³ and spinach,²⁴ in rice bran,¹³ and in cabbage,^{4,5,35,36} as well as in kale, cauliflower, nettle, and chestnut.³⁷ Dam and Glavind³⁷ also found that vitamin K occurs in tomatoes, hempseed, and seaweed, while Almquist and Stokstad³⁸ noted that it is a component of soybean oil. Oat shoots contain this vitamin, and it is present in the cereals in limited amounts.^{24,37,39-41}

²¹ S. B. Binkley, R. W. McKee, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, **133** 721-729 (1940).

²² S. Ansbacher and E. Fernholz, *J. Am. Chem. Soc.*, **61**, 1924-1925 (1939).

²³ L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941).

²⁴ E. Wöhlisch, *Ergeb. Physiol. biol. Chem. exptl. Pharmacol.*, **43**, 174-370 (1940).

²⁵ B. Riegel, *Ergeb. Physiol. biol. Chem. exptl. Pharmacol.*, **43**, 133-173 (1940).

²⁶ A. M. Grossman, *J. Pediat.*, **16**, 239-253 (1940).

²⁷ K. M. Brinkhous, *Medicine*, **19**, 329-416 (1940).

²⁸ H. J. Almquist, *Physiol. Revs.*, **21**, 194-216 (1941).

²⁹ E. A. Doisy, S. B. Binkley, and S. A. Thayer, *Chem. Revs.*, **28**, 477-517 (1941).

³⁰ H. Dam, *Advances in Enzymol.*, **2**, 285-324 (1942).

³¹ H. Dam, *Ann. Rev. Biochem.*, **9**, 353-382 (1940).

³² R. A. Morton, *Ann. Rev. Biochem.*, **11**, 365-390 (1942).

³³ K. Hickman, *Ann. Rev. Biochem.*, **12**, 353-396 (1943).

³⁴ H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945.

³⁵ H. J. Almquist and E. L. R. Stokstad, *Nature*, **136**, 31 (1935).

³⁶ W. F. Holst and E. R. Halbrook, *Science*, **77**, 354 (1933).

³⁷ H. Dam and J. Glavind, *Biochem. J.*, **32**, 485-487 (1938).

³⁸ H. J. Almquist and E. L. R. Stokstad, *J. Nutrition*, **14**, 235-240 (1937).

³⁹ H. Dam, *Angew. Chem.*, **50**, 807-811 (1937).

⁴⁰ E. M. Nelson and C. D. Tolle, *Ann. Rev. Biochem.*, **8**, 415-434 (1939).

⁴¹ Members of Mayo Staff, *Proc. Staff Meetings Mayo Clinic*, **13**, 65-80 (1938).

One unusual plant source which has recently been reported is the berry of the European mountain ash or rowan (*Sorbus aucuparia* L.).⁴² The concentration of vitamin K₁ in alfalfa is sufficiently high so that a potent preparation has been made commercially by oil extraction of the dried leaves.⁴³

Most bacteria contain vitamin K₂.⁴⁴ On the other hand, molds, yeasts, and fungi have practically no vitamin K of either type. Putrefied animal and plant materials usually possess abundant amounts of vitamin K₂, due to bacterial growth. Intestinal bacteria of most of the higher animals can synthesize this vitamin readily. For this reason the feces have been shown to contain abundant quantities of vitamin K. This has been demonstrated in the case of chick feces,⁴⁵ and of dried human feces. Vitamin K₂ can be separated from human feces by extraction with alcohol or petroleum ether.³⁹⁻⁴¹ Vitamin K₂ appears in the rumen of the cow, even on vitamin-K-deficient diets.⁴⁶

Most animal products contain very little of this vitamin; when present the type of vitamin depends upon the previous diet. Vitamin K₁ predominates when abundant amounts of this type of vitamin K are ingested; however, definite proportions of vitamin K₂ may occur. One would expect the latter when the bulk of the vitamin K stored can be traced to that synthesized by the intestinal bacteria. Hog liver⁴⁷ seems to be the most abundant animal source, while chicken livers^{45,47} and the livers of rats⁴⁷ contain very little of this vitamin. Egg-yolk has considerable amounts of vitamin K when sufficient vitamin is present in the diet.⁴⁵ None can be demonstrated in egg albumen. There is also an appreciable amount of the antihemorrhagic vitamin in cow milk if the cow has received an adequate amount of it in her food.³⁴

3. Structure of the K Vitamins

(1) Constitution of Vitamin K₁

Karrer and Geiger⁴⁸ proved, on the basis of elementary composition and molecular weight, that the empirical formula of vitamin K₁ is C₃₁H₄₆O₂. The molecular weight was found to be 450 as determined by potentiometric titrations with sodium hydrosulfite. Vitamin K₁ was proved to be 2-

⁴² G. Y. Shinowara, J. De Lor, and J. W. Means, *J. Lab. Clin. Med.*, **27**, 897-907 (1942).

⁴³ S. Musher, *U. S. Patent* No. 2,282,796 (May 12, 1942).

⁴⁴ J. Almquist, C. F. Pentler, and E. Mecchi, *Proc. Soc. Exptl. Biol. Med.*, **38**, 336-338 (1938).

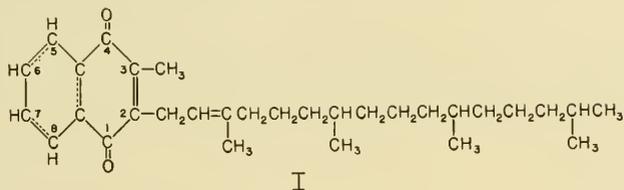
⁴⁵ H. J. Almquist and E. L. R. Stokstad, *J. Nutrition*, **12**, 329-335 (1936).

⁴⁶ L. W. McElroy and H. Goss, *J. Nutrition*, **20**, 527-540 (1940).

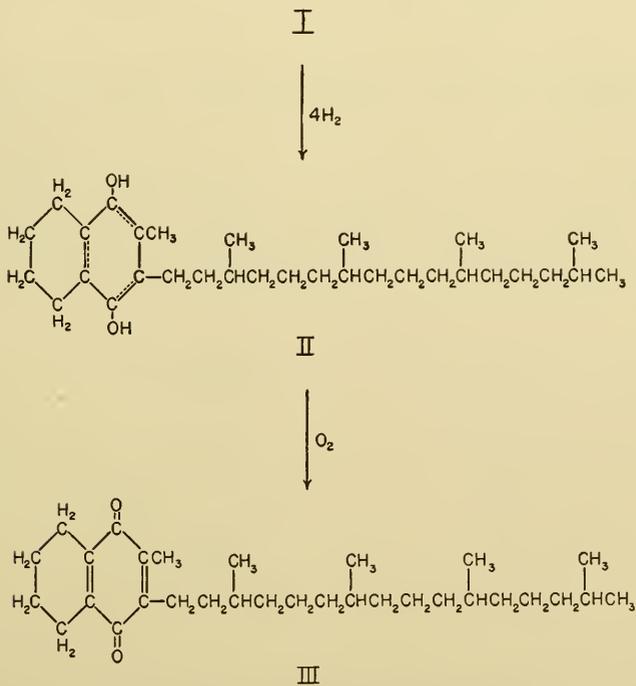
⁴⁷ H. Dam and F. Schönheyder, *Biochem. J.*, **30**, 897-901 (1936).

⁴⁸ P. Karrer and A. Geiger, *Helv. Chim. Acta*, **22**, 945-948 (1939).

methyl-3-phytyl-1,4-naphthoquinone (I), largely as a result of the experimental work of the Doisy group.



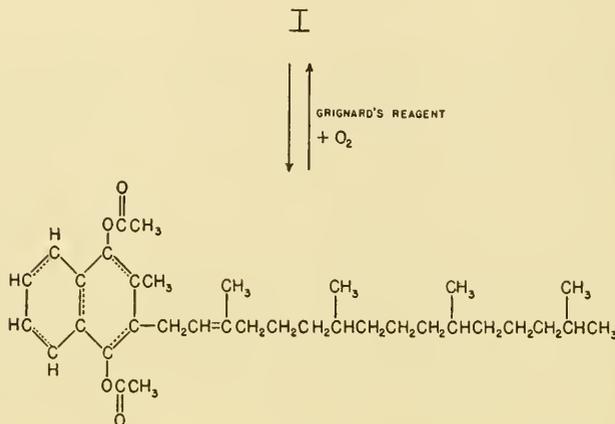
A number of circumstances indicate that vitamin K_1 contains a naphthoquinone residue. In the first place, the redox potential is found to be similar to that of many known 1,4-quinones⁴⁹ such as the anthraquinone-hydroanthraquinone system.^{46,48} Second, the ultraviolet absorption spectrum corresponds closely to that given by 2,3-disubstituted-1,4-naphthoquinones.¹⁶ Still another circumstance which points to the quinone structure is the instability of the vitamin toward light and alkali. Furthermore, McKee *et al.*¹⁶ found that vitamin K_1 absorbs 8 atoms of hydrogen to form a colorless compound (II) on catalytic hydrogenation; in the light this reoxidizes to give a yellow compound (III) similar in color to that of the original vitamin. The quinone, after reduction, may be oxidized as shown.



Autoxidation of Octahydrovitamin K_1 in Light

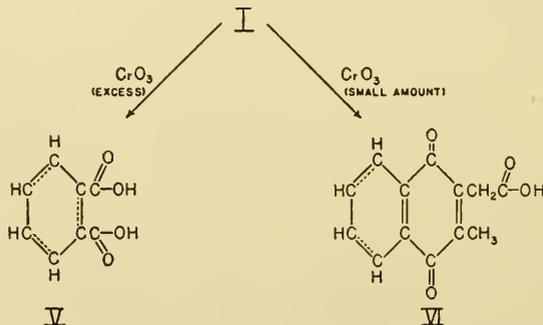
⁴⁹ S. B. Binkley, D. W. MacCorquodale, L. C. Cheney, S. A. Thayer, R. W. McKee, and E. A. Doisy, *J. Am. Chem. Soc.*, **61**, 1612-1613 (1939).

Another indication of the quinone nature of this vitamin can be deduced by reductive acetylation, which yields a crystalline diacetate (IV).⁴⁹ The vitamin can be recovered by means of a Grignard reaction, after which the product is oxidized in the air. The color of the quinone produced is yellow; this indicates that the oxygens are in the 1,4 position in the vitamin. If the oxygen had been present in the 1,2 position, the resulting compound would have been red.



Reversible Reaction of Vitamin K₁ in the Formation of a Diacetate Hydroquinone or a Quinone

Additional proof of the nature of the cyclic portion of the molecule can be obtained by investigation of the products of chromic acid oxidation. When an excess of chromic acid is employed, phthalic acid (V) originates.⁵⁰ If the original ring structure is a 1,4-naphthoquinone, substitution can be

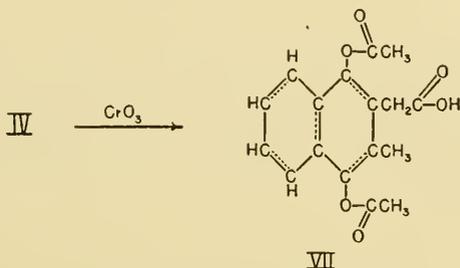


Oxidation Products on Strenuous or Mild Oxidation of Vitamin K₁ with Chromic Acid

⁵⁰ D. W. MacCorquodale, S. B. Binkley, S. A. Thayer, and E. A. Doisy, *J. Am. Chem. Soc.*, 61, 1928-1929 (1939).

only in the 2,3 positions, since the aromatic portion of the naphthoquinone is shown to have no side chains attached.

The position of the side chains was determined by Binkley *et al.*⁵⁰ on the basis of the products obtained by mild oxidation with chromic acid. Under such treatment, 2-methyl-1,4-naphthoquinone-3-acetic acid was isolated (VI); this was identified by comparing it with the same product prepared synthetically.¹⁹ An analogous reaction was obtained by chromic acid oxidation of the diacetate of dihydrovitamin K₁. The end product in this case, as determined by comparison with the synthetic methyl ester, is 1,4-diacetoxy-2-methylnaphthalene-3-acetic acid (VII).¹⁹

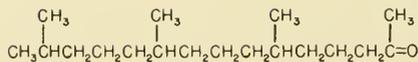


Oxidation of Dihydrovitamin K₁ with Chromic Acid

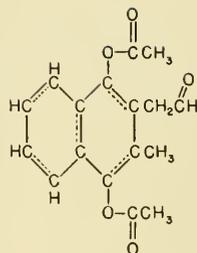
The nature of the side chain was readily elucidated as the result of the hydrogenation and the identification of the products of ozonolysis of dihydrovitamin K₁ diacetate. It was shown that, as a result of hydrogenation, 4 molecules of hydrogen were taken up. Since only 3 molecules can be absorbed in the saturation of the naphthoquinone and conversion to the naphthohydroquinone, it was indicated that there is one double bond in the side chain. The location of the double bond in the side chain, and the nature of the side chain, were deduced by the isolation of a ketone, C₁₈H₃₆O, on oxidation of the diacetate of dihydrovitamin K₁. This was found to be identical with the ketone formed when phytol is oxidized,⁵¹ which is 2,6,10-trimethylpentadecan-14-one (VIII). Further indication of the structure of the side chain and position of attachment to the naphthoquinone nucleus was obtained by the demonstration of the simultaneous formation of 1,4-diacetoxy-2-methylnaphthalene-3-acetaldehyde (IX), which Binkley *et al.*^{21,52} characterized from its semicarbazone. This compound can be isolated when the product formed following ozonolysis of dihydrovitamin K₁ diacetate in glacial acetic acid is decomposed by zinc in ether. These reaction products definitely establish vitamin K₁ as 2-methyl-3-phytyl-1,4-naphthoquinone.

⁵¹ F. G. Fischer and K. Löwenberg, *Ann.*, 464, 69-90 (1928).

⁵² S. B. Binkley, R. W. McKee, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, 133, xii-xiii (1940).



VIII

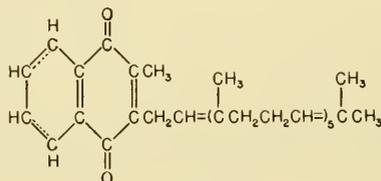


IX

Oxidation Products of Dihydrovitamin K₁

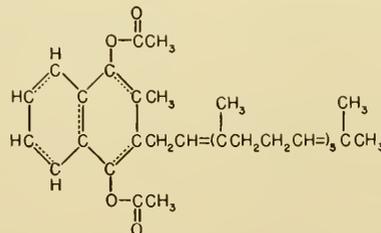
(2) Constitution of Vitamin K₂

Vitamin K₂ has been shown⁵² to have the empirical formula of C₄₁H₅₆O₂. On the basis of reactions similar to those employed for the establishment of the formula for vitamin K₁, Binkley *et al.*²¹ and Karrer and Epprecht⁵³ have assigned formula (X) to vitamin K₂, which is 2-methyl-3-difarnesyl-1,4-naphthoquinone.



X

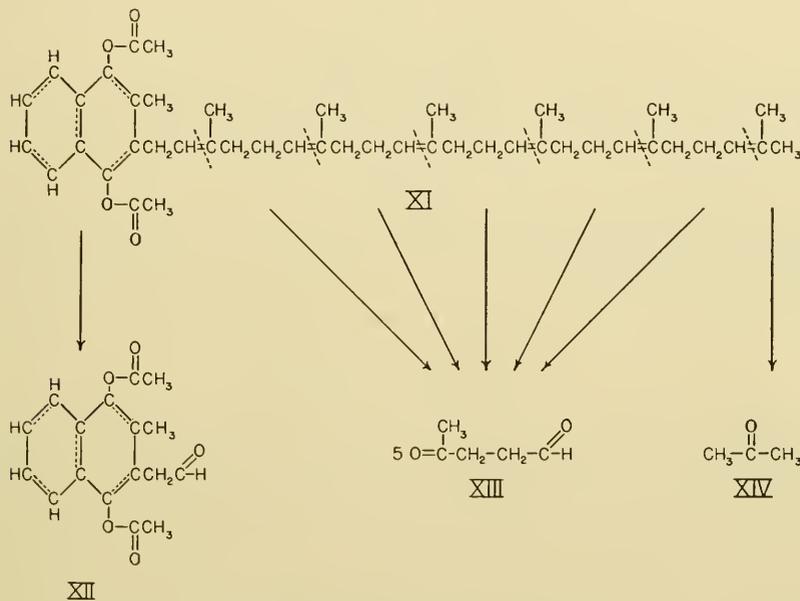
The nature of the cyclic portion of the molecule has been established by methods similar to those employed in the case of vitamin K₁. Upon reductive acetylation, dihydrovitamin K₂ diacetate (XI) is formed in a manner analogous to that which occurs in the case of vitamin K₁. This indicates the presence of the 2 oxygens in the quinoid structure. The absorption spectrum, which is similar to that for vitamin K₁, also confirms the presence of a quinone structure.⁴⁹



XI

⁵³ P. Karrer and A. Epprecht, *Helv. Chim. Acta*, 23, 272-283 (1940)

The nature of the side chain attached to the naphthoquinone nucleus can readily be determined. McKee *et al.*¹⁶ found that 9 molecules of hydrogen were absorbed on catalytic hydrogenation. Since only 3 molecules are involved in the reduction of the naphthoquinone to the naphthohydroquinone structure, there must be 6 double bonds in the side chain. This is further confirmed by the proof that dihydrovitamin K₂ diacetate, which cannot add bromine to the naphthohydroquinone ring, adds 6 molecules of bromine. Moreover, it was shown that the double bonds are not in conjugation, since no addition product obtains on treatment with maleic anhydride; further support for the absence of conjugated double bonds (other than in the quinone ring) can be adduced from the ultraviolet absorption curve.



Oxidation of Dihydrovitamin K₂ Diacetate

The nature of the side chain is at once evident when the products of ozonolysis are known.^{21,52} These consist of 1,4-diacetoxy-2-methylnaphthalene-3-acetaldehyde (XII), levulinaldehyde (XIII), and acetone (XIV). The 1,4-diacetoxy-2-methylnaphthalene-3-acetaldehyde was characterized by forming the semicarbazone, as was the case when this product was identified as a decomposition product of vitamin K₁. Levulinaldehyde was obtained in 93% yield calculated on the basis of 5 moles per molecule of vitamin K₂; it was identified as the bis-2,4-dinitrophenylhydrazone, prepared earlier by Strain.⁵⁴ Acetone was also detected as its 2,4-dinitro-

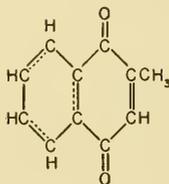
⁵⁴ H. H. Strain, *J. Biol. Chem.*, 102, 151-155 (1933).

phenylhydrazone, and by iodometric titration. Assuming that one molecule of acetone originates on the oxidation of one molecule of vitamin K₂, the recovery amounted to only 53% of the theoretical value.²¹ The origin of the fragments is pictured on page 837.

These data on degradation products are consistent with the formula assigned to vitamin K₂, which pictures it as 2-methyl-3-difarnesyl-1,4-naphthoquinone.

(3) Constitution of Menadione

Although 2-methyl-1,4-naphthoquinone, or menadione, does not occur naturally, it is the most powerful of all antihemorrhagic agents. The struc-



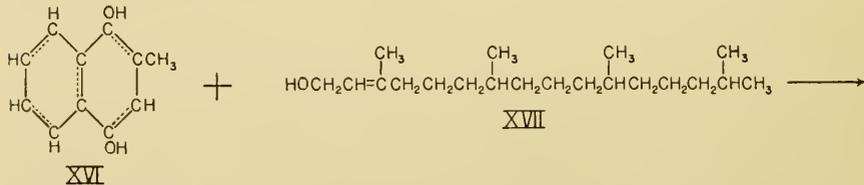
XV

ture of this compound (XV), which is shown here, can readily be ascertained by synthesis.^{55,56}

4. Synthesis of the K Vitamins

(1) Synthesis of Vitamin K₁

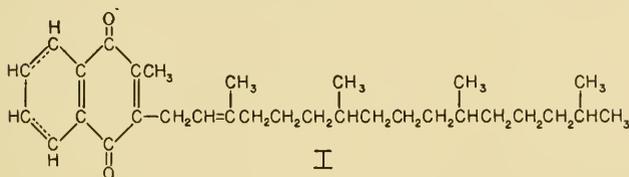
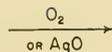
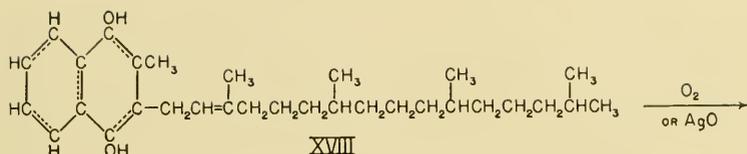
Vitamin K₁ was synthesized by Almquist and Klose¹⁸ by the simple expedient of condensing 2-methyl-1,4-naphthoquinone with the alcohol phytol (XVII). The dihydrovitamin K₁ (XVIII) so produced was readily oxidized to vitamin K₁. Somewhat better results appear to occur when 2-methyl-1,4-naphthohydroquinone (XVI) is used instead of the naphthoquinone for condensation with phytol in the presence of such catalysts as oxalic acid or trichloroacetic acid in dioxane.²⁰ The Doisy group^{19,57} condensed the monosodium salt of 2-methyl-1,4-naphthohydroquinone with phytyl bromide to produce vitamin K₁.



⁵⁵ P. P. T. Sah, W. Brüll, and H. Holzen, *Ber.*, 73, 762 (1940).

⁵⁶ P. P. T. Sah, *Rec. trav. chim.*, 59, 461-470 (1940).

⁵⁷ D. W. MacCorquodale, L. C. Cheney, S. B. Binkley, W. F. Holcomb, R. W. McKee, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, 131, 357-370 (1939).



Synthesis of Vitamin K₁

The best yields of the vitamin were obtained when the reaction was carried out in a slightly acid medium. In the presence of strong acids or mineral acids, a further condensation occurs, with the result that tocopheryl-like compounds arise. In the presence of alkali, the yield is low, and it is difficult to separate vitamin K₁ from the resulting brown mixture.

Another synthesis involves the use of the alcohols, phthiocol and phytol, according to the procedure of Tishler, Fieser, and Wendler.⁵⁸ The yield is not as satisfactory as that obtained by the procedures described previously.

(2) *Synthesis of Vitamin K₂*

In view of the fact that the most potent antihemorrhagic agent is 2-methyl-1,4-naphthoquinone, and that no advantage is to be gained by the use of the natural vitamins K₁ or K₂, the synthesis of vitamin K₂ is solely of academic interest. As yet, no synthesis of vitamin K₂ has been reported.

(3) *Synthesis of Menadione*

Menadione, which is the recent name coined for 2-methyl-1,4-naphthoquinone, is made most simply by oxidation of 2-methylnaphthalene by means of chromic acid in acetic acid⁵⁹⁻⁶¹ at room temperatures below 50°C.,⁶² by hydrogen peroxide,⁶³ or by air. As much as 30 to 60% of the

⁵⁸ M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 1982-1991 (1940).

⁵⁹ K. Fries and W. Lohmann, *Ber.*, **54**, 2912-2924 (1921).

⁶⁰ R. J. Anderson and M. S. Newman, *J. Biol. Chem.*, **103**, 405-412 (1933).

⁶¹ L. I. Smith and I. M. Webster, *J. Am. Chem. Soc.*, **59**, 662-667 (1937).

⁶² L. F. Fieser, W. P. Campbell, E. M. Fry, and M. D. Gates, *J. Am. Chem. Soc.*, **61**, 2559, 3216-3223 (1939).

⁶³ R. T. Arnold and R. Larson, *J. Org. Chem.*, **5**, 250-252 (1940).

theoretical yields can be obtained by these syntheses. 2-Methylnaphthalene is available as a by-product of the coal-tar industry.⁶⁴

A simple synthesis has been proposed by Sah *et al.*⁵⁵ and by Sah alone,⁵⁶ starting with naphthalene. On sulfonation, naphthalene- β -sulfonic acid is formed, which is changed to the nitrile upon treatment with potassium ferrocyanide. The nitrile is readily converted to β -naphthoic acid on hydrolysis, and this is reduced to the corresponding aldehyde by distillation

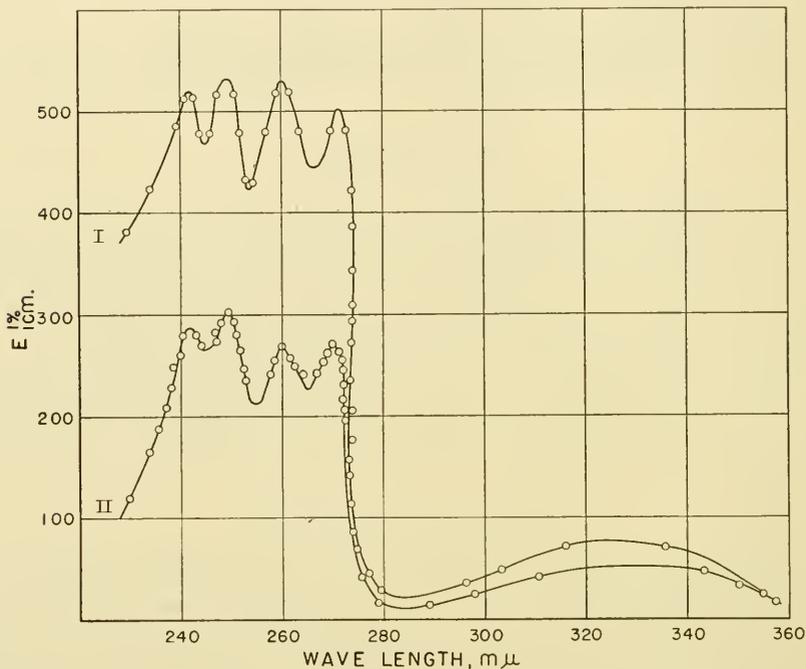


Fig. 1. Absorption curves of vitamin K₁ (I), and vitamin K₂ (II) in hexane.⁶⁵

with barium formate in a 60% yield. 2-Methylnaphthalene results when a Clemmensen reduction is applied to the aldehyde. The 2-methylnaphthalene is readily converted to the corresponding 1,4-naphthoquinone by the oxidation methods described above.

A total synthesis of menadione from benzene has been accomplished by Sah and Brüll.⁶⁶ These workers condensed benzene with the anhydride of methylsuccinic acid, using aluminum chloride as the catalyst. The resulting product, α -methyl- β -benzoylpropionic acid, is first reduced to α -methyl- γ -phenylbutyric acid by the use of the Clemmensen reaction;

⁶⁴ E. A. Coulson, *J. Soc. Chem. Ind.*, 60, 123-126T (1941).

⁶⁵ D. T. Ewing, J. M. Vanderbilt, and O. Kamm, *J. Biol. Chem.*, 131, 345-356 (1939).

⁶⁶ P. P. T. Sah and W. Brüll, *Ber.*, 73, 1430-1432 (1940).

ring closure occurs on treatment of the chloride with aluminum chloride, with the resultant formation of 2-methyl- α -tetralone. The latter product yields 2-methyl-1,2,3,4-tetrahydronaphthalene on reduction; this can be dehydrogenated to 2-methylnaphthalene on treatment with sulfur or selenium; this product is readily oxidized to the quinone.

5. Properties of the K Vitamins

In common with other fat-soluble vitamins, the natural members of the vitamin K group dissolve only in fat solvents, and are insoluble in aqueous

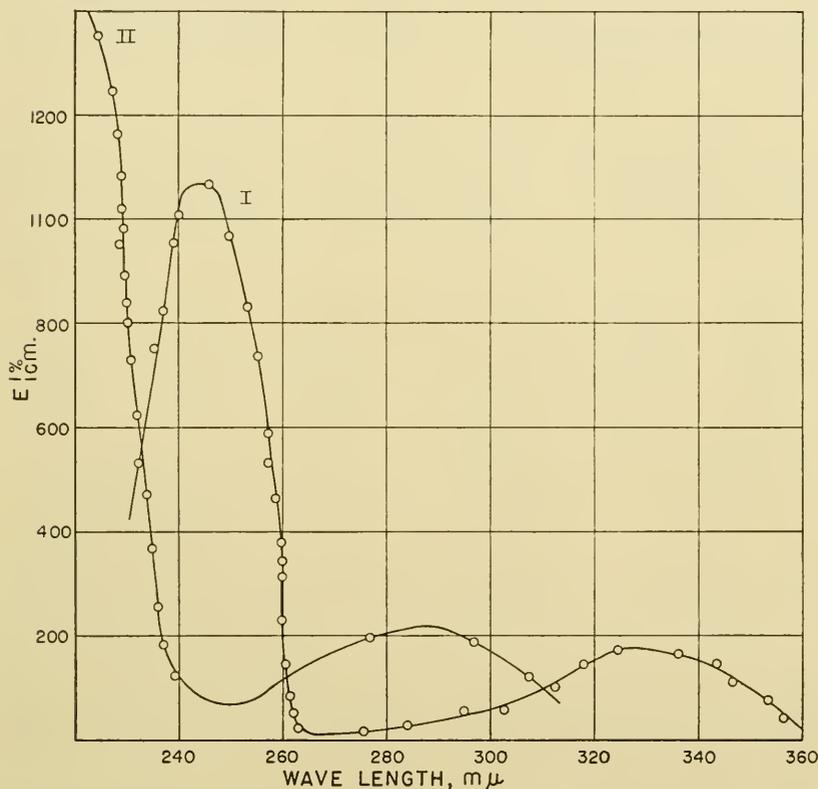


Fig. 2. Absorption curves of 1,4-naphthoquinone (I) and of the diacetate of naphthoquinone (II) in hexane.⁶⁵

media. They are especially soluble in petroleum ether, diethyl ether, hexane, acetone, and chloroform, but only sparingly soluble in methyl alcohol. The synthetic members of the group which no longer have the long aliphatic hydrocarbon chain attached to the naphthoquinone nucleus are

more readily soluble in water. Thus, although menadione dissolves to the extent of only 0.013% in water, the esters of the hydroquinones are quite soluble in this solvent.

Although the K vitamins are fairly thermostable,⁴¹ they are readily destroyed by alkali,^{34,41} alcohol,^{67,68} and by various kinds of light, including sunlight,^{17,34} light from ordinary Mazda bulbs,¹⁷ and, especially, ultraviolet light.⁶⁸

One of the most characteristic properties of the vitamins K which is useful in their identification is the ultraviolet absorption pattern. Maximum absorption bands occur at 243, 249, 260, 269, and 325 m μ .⁶⁵ The absorption curves for vitamins K₁ and K₂ are reproduced in Figure 1, and those of the 1,4-naphthoquinones in Figure 2. The extinction coefficients are recorded in Table 1.

TABLE 1
ABSORPTION COEFFICIENTS OF VITAMIN K₁ IN HEXANE AT MAXIMUM AND MINIMUM WAVE LENGTHS^a

Maximum points			Minimum points		
λ , A.	ϵ	E (1%, 1 cm.)	λ , A.	ϵ	E (1%, 1 cm.)
2400	16,000	355	2410	15,600	344
2430	18,700	412	2460	16,100	355
2490	20,000	440	2550	12,800	282
2600	18,000	398	2655	15,000	330
2700	18,000	398	2870	700	15.5
3250	3,200	70.8	—	—	—

^a Adapted from J. R. Loofbourow, *Vitamins and Hormones*, 1, 109-155 (1943).

The positions for the maxima and minima are similar for vitamins K₁ and K₂,²⁹ but the ϵ values are somewhat lower for vitamin K₂. Ewing *et al.*⁶⁵ report the maxima on the synthetic vitamin K₁ prepared by Mac-Corquodale *et al.*⁵⁷ to be λ 243, 249, 260, 269, and 325; the corresponding E (1%, 1 cm.) values were 410, 425, 395, 395, and 75, respectively. Thus, the data on the synthetic product correspond within experimental limits to those of the natural vitamin.

Vitamin K₁ is a yellow oil melting at about -20°C ., while vitamin K₂ is a yellow crystalline solid melting at 53.5 – 54.5°C .²¹ Menadione is also a crystalline lemon-yellow powder, which melts at 106°C . It has a faint but characteristic odor. Vitamin K₁ has a redox potential, E_m , of $+0.005$ volt. According to Almquist and Klose,¹⁸ its solutions exhibit a white fluorescence when exposed to the light of the argon lamp. The latter

⁶⁷ H. J. Almquist, *J. Nutrition*, 14, 241-245 (1937).

⁶⁸ H. J. Almquist, *J. Biol. Chem.*, 117, 517-523 (1937).

property is not displayed by naphthoquinones without a side chain on position 3.

6. Standards for the K Vitamins

There are no national or international standards for vitamin K, and this is probably unnecessary, in view of the ready availability of pure 2-methyl-1,4-naphthoquinone. However, both 2-methyl-1,4-naphthoquinone^{69,70} and 2-methyl-1,4-naphthohydroquinone diacetate^{65,71} have been suggested as possible reference standards, as their physical constants are well known and they are quite stable.

Although it is obvious that vitamin K activity can now best be expressed in relation to the biological effect of a given amount of 2-methyl-1,4-naphthoquinone, it is important to understand the earlier methods of expressing biological activity before the nature of the vitamin K was discovered. These relationships are given in Table 2, which has been worked out by Ansbacher.⁷² By definition, the activity of 1 mg. of vitamin K₁ is equivalent to 1000 Thayer-Doisy (1939) Units; since 2-methyl-1,4-naphthoquinone has 3.3 times the efficacy of natural vitamin K₁, it is equivalent to 3300 Thayer-Doisy (1939) Units.

TABLE 2

BIOLOGICAL ACTIVITY OF VARIOUS METHODS OF EXPRESSION OF VITAMIN K EFFECT IN TERMS OF ACTIVITY OF 2-METHYL-1,4-NAPHTHOQUINONE (MENADIONE) AND THAYER-DOISY (1939) UNITS

Unit	Number of units given by 1 mg. menadione	Number of Thayer-Doisy (1939) Units ^{b,c}
Thayer-Doisy (1939) ^a	3,300	1
Ansbacher Unit ^b	6,600	0.5
Thayer Unit (1938) ^e	6,600	0.5
Dam Unit ^d	330	10
Dann Unit (1938) ^f	13,200	0.25
Dann Unit (1939) ^f	5,280	0.625
Almquist Reference Standard	—	0.08 ml.

^a S. A. Thayer, S. B. Binkley, D. W. MacCorquodale, E. A. Doisy, A. D. Emmett, R. A. Brown, and O. D. Bird, *J. Am. Chem. Soc.*, **61**, 1932, 2563 (1939).

^b S. Ansbacher, *J. Nutrition*, **17**, 303-315 (1939); *J. Biol. Chem.*, **133**, iii-iv (1940).

^c H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York 1945, p. 504.

^d H. Dam, *Z. Vitaminforsch.*, **8**, 248-257 (1938).

^e F. P. Dann, *Am. J. Physiol.*, **123**, 48-49 (1938).

^f F. P. Dann, *Proc. Soc. Exptl. Biol. Med.*, **42**, 663-668 (1939).

⁶⁹ S. A. Thayer, S. B. Binkley, D. W. MacCorquodale, E. A. Doisy, A. D. Emmett, R. A. Brown, and O. D. Bird, *J. Am. Chem. Soc.*, **61**, 2563 (1939).

⁷⁰ E. Fernholz, S. Ansbacher, and H. B. MacPhillamy, *J. Am. Chem. Soc.*, **62**, 430-432 (1940).

⁷¹ H. Dam, J. Glavind, and P. Karrer, *Helv. Chim. Acta*, **23**, 224-233 (1940).

⁷² S. Ansbacher, *J. Biol. Chem.*, **133**, iii-iv (1940).

7. Chemical Structure of the K Vitamins and Vitamin-K-Like Compounds in Relationship to Biological Activity

A very large number of substances, other than the natural K vitamins, possess antihemorrhagic activity. Many of these are equally potent, or even more potent than the naturally occurring vitamins. This is because their relative effectiveness seems to be related to their capacity for conversion into 2-methyl-1,4-naphthoquinone in the animal organism.

A large number of compounds with structures related to that of vitamin K have been investigated to determine their ability to promote prothrombin production. This was especially studied by Fieser *et al.*,²³ who employed a modification of the 18-hour test of Thayer *et al.*^{73,74} for prothrombin formation in chicks. The minimum amounts necessary to produce an effect comparable with that brought about by 0.3 μg . of 2-methyl-1,4-naphthoquinone were determined. When no appreciable effect results at a dosage of 1000 μg . (1 mg.), it is considered that the substance possesses little importance as an antihemorrhagic agent. The effects of variations within each group have been systematically investigated.

(1) Effect of Substitution of 2-Methyl-1,4-naphthoquinone in Position 3

The side chain on position 3 of the naturally occurring K vitamins has been found to be of no value in contributing to prothrombin production, although it is important in augmenting the solubility of these vitamins in organic solvents. In fact, the hydrocarbon side chains in this position reduce the potency of the vitamins in accordance with the proportion to which they contribute to the increase in molecular weight over that of menadione.

The greatest biological effectiveness is obtained with menadione. The generally accepted minimal dose of this quinone, using deficient chicks, is 0.3 μg .²³ By a similar test, the dose of vitamin K₁ which gives the same activity is 1 μg . Thus, menadione is 3.3 times as active as is vitamin K₁,²³ which corresponds to a ratio of activity of 3.8:1 reported by Almquist and Klose,⁷⁵ one of 2.2:1 by Emmett, Brown, and Kamm,⁷⁶ one of 2.1:1 by Dam *et al.*,⁷¹ and finally a ratio of 4:1 given by Ansbacher, Fernholz, and MacPhillamy.⁷⁷

The antihemorrhagic effect of a large number of compounds structurally

⁷³ S. A. Thayer, R. W. McKee, S. B. Binkley, D. W. MacCorquodale, and E. A. Doisy, *Proc. Soc. Exptl. Biol. Med.*, **40**, 478-481 (1939).

⁷⁴ S. A. Thayer, R. W. McKee, S. B. Binkley, D. W. MacCorquodale and E. A. Doisy, *Proc. Soc. Exptl. Biol. Med.*, **41**, 194-197 (1939).

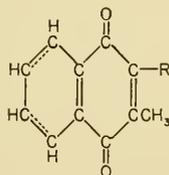
⁷⁵ H. J. Almquist and A. A. Klose, *J. Biol. Chem.*, **130**, 787-789 (1939).

⁷⁶ A. D. Emmett, R. A. Brown, and O. Kamm, *J. Biol. Chem.*, **132**, 467-468 (1940).

⁷⁷ S. Ansbacher, E. Fernholz, and H. B. MacPhillamy, *Proc. Soc. Exptl. Biol. Med.*, **42**, 655-658 (1939).

related to vitamin K has been investigated by Fieser, Tishler, and Sampson.²³ The data on the response are summarized in Tables 3 to 11. References to the synthesis and characterization of the individual compounds are included in each table.

Table 3 shows the relative effectiveness of various substituents when present in the place of R in 2-methyl-1,4-naphthoquinone.



R-substituted-2-methyl-
1,4-naphthoquinone

Vitamin K₂ has been shown to have between 62¹⁵ and 67⁷¹ per cent of the activity of vitamin K₁, while its molecular weight is only 1.3 times as great. On this basis, it has an activity of 82% of that of vitamin K₁. The maximum effect on the side chain is reached with a 20-carbon residue of isoprene groupings; little further change occurs on further elaboration of the side chain. The branched chain on carbon 3 seems to favor the antihemorrhagic effect, as the octadecyl compound is practically inactive.⁷⁰ Moreover, when the farnesyl or geranyl group is introduced in the 3-position, the resulting derivatives are surprisingly potent. Although such compounds have not been reported as occurring naturally, the corresponding alcohols—farnesol and geraniol—are widely distributed in nature.

(2) *Effect of Substitution of 1,4-Naphthoquinone in Position 2 with Alkyl and β-Alkenyl Residues*

Fieser and his co-workers⁷⁸⁻⁸⁰ have been able to prepare a series of mono-substituted naphthoquinones by direct condensation of the naphthohydroquinone with such alcohols as farnesol, phytol, and geraniol; these compounds are similar to those studied earlier (see page 844), except that the methyl group at position 2 is absent. Table 4 summarizes the results on these monosubstituted naphthoquinones of the presence of various substituents in the place of R in 2-R-1,4-naphthoquinone.

⁷⁸ L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Am. Chem. Soc.*, 62, 996 (1940).

⁷⁹ L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Am. Chem. Soc.*, 62, 1628-1629 (1940).

⁸⁰ L. F. Fieser, M. Tishler, and N. L. Wendler, *J. Am. Chem. Soc.*, 62, 2861-2866 (1940).

TABLE 3
EFFECT OF SUBSTITUTION IN THE 3-POSITION OF 2-METHYL-1,4-NAPHTHOQUINONE

Name of substituent on 3-position of 2-methyl-3-R-1,4-naphthoquinone	Formula for R	Effective dose, μg .
2-Methyl-1,4-naphthoquinone ^a	H	0.3
-3-ptylyl ^{b,c} (Vitamin K ₁)	-CH ₂ ·CH·C(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·CH(CH ₃) ₂	1.0
-3-difarnesyl ^{a,d,e} (Vitamin K ₂)	-CH ₂ ·CH·C(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·C(CH ₃) ₂	1.6
-3-farnesyl ^{f,g}	-CH ₂ ·CH·C(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·C(CH ₃) ₂	5
-3-(β , γ -dihydrophytyl) ^{a,g,h}	-CH ₂ ·CH ₂ ·CH(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·C(CH ₃) ₂	8
-3-geranyl ^e	-CH ₂ ·CH·C(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·C(CH ₃) ₂	25
-3-cinnamyl ⁱ	-CH ₂ ·CH·CH·C ₆ H ₅	25
-3-(β , γ , γ -trimethylallyl) ^j	-CH ₂ ·C(CH ₃)·C(CH ₃) ₂	50
-3-dimethyl ^{k,l}	-CH ₃	50
-3-benzyl ^k	-CH ₂ ·C ₆ H ₅	200
-3-hydrocinnamyl ^b	-C ₁₂ ·CH ₂ ·CH ₂ ·C ₆ H ₅	300
-3-octadecyl ^{i,k}	-CH ₂ (CH ₂) ₁₆ ·CH ₃	1000 ⁱ

^a L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941), p. 662.

^b L. F. Fieser, *J. Am. Chem. Soc.*, **61**, 2559-2561 (1939).

^c L. F. Fieser, *J. Am. Chem. Soc.*, **61**, 3467-3475 (1939).

^d R. W. McKee, S. B. Binkley, D. W. MacCorquodale, S. A. Thayer, and E. A. Doisy, *J. Am. Chem. Soc.*, **61**, 1295 (1939); *J. Biol. Chem.*, **137**, 327-344 (1939).

^e H. Damm, J. Glavind, and P. Karrer, *Helv. Chim. Acta*, **23**, 224-233 (1940).

^f L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 996, 1628-1629 (1940).

^g L. F. Fieser, M. Tishler, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2861-2866 (1940).

^h L. F. Fieser, M. Tishler, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

ⁱ L. F. Fieser, W. P. Campbell, E. M. Fry, and M. D. Gates, *J. Am. Chem. Soc.*, **61**, 2559, 3216-3223 (1939).

^j E. Fernholz, S. Ansbacher, and H. B. MacPhillamy, *J. Am. Chem. Soc.*, **62**, 430-432 (1940).

^k P. Karrer and A. Epprecht, *Helv. Chim. Acta*, **23**, 272-283 (1940).

TABLE 4
EFFECT OF SUBSTITUTION OF ALKYL AND ALKENYL GROUPS IN THE 2-POSITION OF 1,4-NAPHTHOQUINONE^a

Name of substituent on 2-position of 2-R-1,4-naphthoquinone	Formula for R	Effective dose, $\mu\text{g.}$
2-Methyl-1,4-naphthoquinone ^b	-CH ₃	0.3
2-Phenyl- ^{c,d,e}	-CH ₂ ·CH: C(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·CH(CH ₃) ₂	50
2-Farnesyl- ^{c,d}	-CH ₂ ·CH: C(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH:] ₂ ·C(CH ₃) ₂	500
2-(β , γ -Dihydrophytyl)- ^f	-CH ₂ ·CH ₂ ·CH(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH:] ₃ ·CH(CH ₃) ₂	600
2-n-Hexadecyl- ^g	-CH ₂ ·(CH ₂) ₁₄ ·CH ₃	> 600 ^g
2-n-Octadecyl- ^g	-CH ₂ ·(CH ₂) ₁₆ ·CH ₃	> 600 ^g
2-Allyl- ^{h,i}	-CH ₂ ·CH: CH ₂	800 ^f
2-Geranyl- ^{c,d}	-CH ₂ ·CH: C(CH ₃)·CH ₂ ·CH ₂ ·CH: C(CH ₃) ₂	1000
2-Ethyl- ^{g,i,k}	-CH ₂ ·CH ₃	1000 ^f
2-n-Propyl- ^{f,g,h,i}	-CH ₂ ·CH ₂ ·CH ₃	1000 ^f

^a H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, p. 496.

^b S. Ansbacher and E. Fernholz, *J. Am. Chem. Soc.*, **61**, 1924-1925 (1939).

^c L. F. Fieser, M. Tishler, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2861-2866 (1940).

^d L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 996, 1628-1629 (1940).

^e H. Dam, J. Glavind, and P. Karrer, *Helv. Chim. Acta*, **23**, 224-233 (1940).

^f L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941).

^g E. Fernholz, S. Ansbacher, and H. B. MacPhillamy, *J. Am. Chem. Soc.*, **62**, 430-432 (1940).

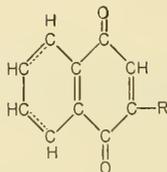
^h L. F. Fieser, W. B. Campbell, and E. M. Fry, *J. Am. Chem. Soc.*, **61**, 2206-2218 (1939).

ⁱ L. F. Fieser, D. M. Bowen, W. P. Campbell, M. Fieser, E. M. Fry, R. N. Jones, B. Riegel, C. E. Schweitzer, and P. G. Smith, *J. Am. Chem. Soc.*, **61**, 1925-1926 (1939).

^j M. Tishler and W. L. Sampson, *J. Am. Chem. Soc.*, **61**, 2563-2564 (1939).

^k B. Sjögren, *Z. physiol. Chem.*, **262**, I-III (1939).

^l Given as inactive at 1000 $\mu\text{g.}$



2-R-1,4-naphthoquinone

The most striking phenomenon evident from Table 4 is the profound importance of the methyl group in position 2. 2-Methyl-1,4-naphthoquinone occupies a unique position, since it is 170 times as potent as the next most active member of the series. Whereas menadione is biologically effective in doses of 0.3 μg ., the next two higher homologues, namely, 2-ethyl- and 2-*n*-propyl-1,4-naphthoquinone, are inactive in doses of 1000 μg .. The latter finding has been confirmed by Fernholz *et al.*,⁷⁰ as well as by Sjögren.⁸¹ Another interesting result is the fact that the order of activity for the hydrocarbon side chains on position 3 in 2-methyl-1,4-naphthoquinone is the same as it is when the methyl group is absent. Thus, phytyl > farnesyl > dihydrophytyl > geranyl, but these compounds are only 2, 1, 1.4, and 2.5%, respectively, as active as are the corresponding 2-methyl-3-R-1,4-naphthoquinones.

Fieser⁸² suggested that the commanding position occupied by menadione may not be due to the functioning of the compound as such but rather to its rapid transformation in the body to a quinone of the vitamin K type. Since the simple reduced quinone readily condenses with phytol, farnesol, and geraniol *in vitro*, it would presumably also react *in vivo* with other β -unsaturated isoprenoid alcohols to yield antihemorrhagic compounds. It is altogether possible that such an alcohol might be derived from the breakdown of the aliphatic side chain of vitamin A. Menadione would therefore give rise to 2.6 parts of vitamin K₁ or 3.4 parts of vitamin K₂, if such syntheses were quantitative. These relationships are approximately those which have actually been found.

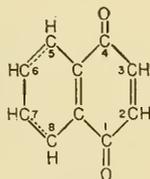
(3) Effect of High Alkylation on Activity of 1,4-Naphthoquinones

When several alkyl groups are substituted on the 1,4-naphthoquinones, profound changes may result, even if the methyl group is still attached to position 2. Thus, when the second methyl group is at positions 5, 6, 7, or 8, the resulting compound has been found to possess only slight activity, or to be completely devoid of any biopotency when fed in an amount as high as 1000 μg .. A summary of the data on such highly alkylated compounds is

⁸¹ B. Sjögren, *Z. physiol. Chem.*, **262**, I-III (1939).

⁸² L. F. Fieser, *J. Am. Chem. Soc.*, **61**, 3467-3475 (1939).

given in Table 5. The positions of the substituted groups can be deduced from the numbering of 1,4-naphthoquinone.



1,4-Naphthoquinone

TABLE 5

BIOLOGICAL ACTIVITY OF HIGHLY ALKYLATED DERIVATIVES OF 1,4-NAPHTHOQUINONES AS ANTIHEMORRHAGIC AGENTS^a

Compound	Effective dose, μg.
2,5-Dimethyl-1,4-naphthoquinone ^b	500
2,6-Dimethyl-1,4-naphthoquinone ^c	Inactive at 1000
2,7-Dimethyl-1,4-naphthoquinone ^c	1000
2,8-Dimethyl-1,4-naphthoquinone ^b	500
6,7-Dimethyl-1,4-naphthoquinone ^{d,e}	Inactive at 1000
2,6-Dimethyl-3-phytyl-1,4-naphthoquinone ^f	Inactive at 1000
2,3-Diallyl-1,4-naphthoquinone ^{d,e}	1000
2-Ethyl-3-phytyl-1,4-naphthoquinone ^f	1000
1,2,4-Trihydroxyanthraquinone ^g	100
1,1-Dimethyl-3- <i>tert</i> -butyl-1,4-dihydroanthraquinone ^h	Inactive at 1000
2-(δ -Methyl- γ -pentenyl)-1,4-dihydroanthraquinone ^h	Inactive at 1000

^a H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, p. 497.

^b M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

^c L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941).

^d L. F. Fieser, W. P. Campbell, and E. M. Fry, *J. Am. Chem. Soc.*, **61**, 2206-2218 (1939).

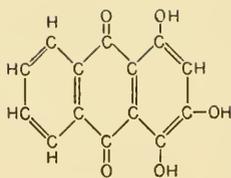
^e L. F. Fieser, D. M. Bowen, W. P. Campbell, E. M. Fry, and M. D. Gates, *J. Am. Chem. Soc.*, **61**, 1926-1927 (1939).

^f L. F. Fieser, *J. Am. Chem. Soc.*, **61**, 3467-3475 (1939).

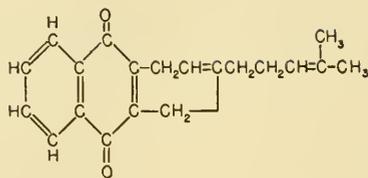
^g G. J. Martin and C. F. Lischer, *J. Biol. Chem.*, **137**, 169-171 (1941).

^h L. F. Fieser and C. W. Wiegand, *J. Am. Chem. Soc.*, **62**, 153-155 (1940).

The introduction of a second methyl group into positions 5 or 8 lowers the potency to 1/1500 as compared with that of menadione; when the second methyl group is on position 7, the product has only 1/3000 the potency of 2-methyl-1,4-naphthoquinone. Finally, when the second methyl group is placed in position 6, the resulting product is devoid of activity at 1000 μ g. The ethyl analogue of vitamin K₁ (2-ethyl-3-phytyl-1,4-naphthoquinone) has only 1/1000 the biological activity of vitamin K₁. It is interesting that 1,2,4-trihydroxyanthraquinone (XIX) is fairly potent, while 2-(δ -methyl- γ -pentenyl)-1,4-dihydroanthraquinone (XX) is completely inactive at a dose of 1000 μ g.



XIX



XX

(4) Effect of Replacement of Naphthoquinones with Carbethoxy or Hydroxy Groups

Practically all of the hydroxynaphthoquinones are inactive except when a methyl group is retained on the 2-position. Even in these cases, the effectiveness of such compounds in relieving hemorrhagic tendencies due to the lack of vitamin K₁ is greatly depressed. The relative biological activities of these various hydroxynaphthoquinones are summarized in Table 6.

TABLE 6
BIOLOGICAL ACTIVITY OF CARBETHOXY- AND HYDROXYNAPHTHOQUINONES

Compound	Effective dose, μg.
2-Methyl-3-carbethoxy-1,4-naphthoquinone ^a	25
2-Methyl-5-hydroxy-1,4-naphthoquinone (plumbagin) ^b	400
2-Methyl-3-hydroxy-1,4-naphthoquinone (phthiocol) ^c	500
2-β-Heptenyl-3-hydroxy-1,4-naphthoquinone ^d	Inactive at 1000
2-Farnesyl-3-hydroxy-1,4-naphthoquinone ^e	Inactive at 1000
2-Methyl-3-(γ-hydroxydihydrophytyl)-1,4-naphthoquinone ^e	Inactive at 1000
5-Hydroxy-1,4-naphthoquinone (juglone) ^f	Inactive at 1000, feebly positive at 10,000
2-Hydroxy-1,4-naphthoquinone (lawsone) ^{f,g}	Inactive at 1000, active at 10,000
2-Hydroxyl-3-dimethyl-allyl-1,4-naphthoquinone (lapachol) ^f	Inactive at 1000, active at 5000
Hydroquinone diacetate ^h	Inactive at 1000

^a C. F. Koelsch and D. J. Byers, *J. Am. Chem. Soc.*, **62**, 560-562 (1940).

^b L. F. Fieser and J. T. Dunn, *J. Am. Chem. Soc.*, **53**, 572-575 (1936).

^c H. J. Almquist and A. A. Klose, *J. Am. Chem. Soc.*, **61**, 1611 (1939).

^d L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941).

^e M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

^f R. Kuhn, K. Wallenfels, F. Weygand, T. Moll, and L. Hepding, *Naturwissenschaften*, **27**, 518-519 (1939).

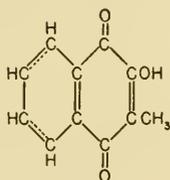
^g H. Dam, J. Glavind, and P. Karrer, *Helv. Chim. Acta*, **23**, 224-233 (1940).

^h M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 1982-1991 (1940).

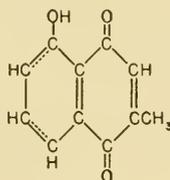
Phthiocol (XXI) was the first simple compound which was demonstrated to have an antihemorrhagic effect.⁸³ Although phthiocol⁸² can condense with the reduced form of phytol to give a low yield of vitamin K₁,⁸⁶ the reaction does not explain the activity of this compound, since its

⁸³ H. J. Almquist and A. A. Klose, *J. Am. Chem. Soc.*, **61**, 1611 (1939).

isomer plumbagin (XXII) also has some activity, in spite of the fact that it is impossible to convert it to vitamin K₁.



XXI



XXII

There appears to be some activity in the case of three drugs which have the hydroxyl but not the methyl group on 1,4-naphthoquinone. These include lapachol, active at a 5-mg. level, and juglone and lawsone, both of which respond when given at a 10-mg. level. These are massive doses, however, and many of the other compounds inactive at 1-mg. dosage might have shown some effectiveness if tested at these higher levels.

One of the most markedly depressing effects exerted by the hydroxyl group on antihemorrhagic activity has been noted in the case of 2-methyl-3-(γ -hydroxydihydrophytyl)-1,4-naphthoquinone. When 2-methyl-3- β,γ -dihydrophytyl-1,4-naphthoquinone was fed, it proved to be very active; the curative dose was found to be at a level of only 8 μ g. The simple introduction of a single hydroxyl group on the side chain 3 carbons removed from the naphthoquinone ring thus results in a practically complete loss of vitamin K effect. It should be noted that in the latter case the hydroxyl group is alcoholic, whereas the depressing effect cited earlier caused by the introduction of an hydroxyl group was noted in cases in which it was phenolic in nature.

The high potency of 2-methyl-3-carbethoxy-1,4-naphthoquinone (25 μ g.) may be a reflection of its ready conversion to the corresponding quinone. Koelsch and Byers⁸⁴ have suggested that esters of this type can easily revert to the 2-alkyl-1,4-naphthoquinones by saponification and oxidation.

(5) *Biological Activity of Naphthoquinone Oxides*

The colorless naphthoquinone oxides containing one additional oxygen present in the form of a bridge between carbons 2 and 3 have been prepared in almost quantitative yield from the yellow quinones, by Fieser and co-workers.⁶² They are readily susceptible to reduction; under mild reducing action, they are converted to the naphthohydroquinones, with the elimination of the oxygen bridge.^{79,85} Presumably, the oxides have a high biologi-

⁸⁴ C. F. Koelsch and D. J. Byers, *J. Am. Chem. Soc.*, **62**, 560-562 (1940).

⁸⁵ M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

cal potency, because they are able to change *in vivo* to their corresponding naphthoquinones or to their physiologically equivalent naphthohydroquinones. A summary of the present experimental data on compounds of this class is given in Table 7.

TABLE 7
BIOLOGICAL ACTIVITY OF NAPHTHOQUINONE OXIDES AND COMPARISON IN POTENCY WITH UNOXIDIZED FORMS^a

Compound	Effective dose, $\mu\text{g.}$	Effective dose of unoxidized form, $\mu\text{g.}^a$
Vitamin K ₁ oxide ^{b,c}	1.5	1.0
2-Methyl-1,4-naphthoquinone oxide ^b	5	0.3
2,3-Dimethyl-1,4-naphthoquinone oxide ^{b,c}	25	50
2-Methyl-3-cinnamyl-1,4-naphthoquinone oxide ^{b,c}	150	25
2-Phytyl-1,4-naphthoquinone oxide ^{b,c}	200	50
2-Farnesyl-1,4-naphthoquinone oxide ^{b,c}	1000	500
2,7-Dimethyl-1,4-naphthoquinone oxide ^d	Inactive at 1000	1000

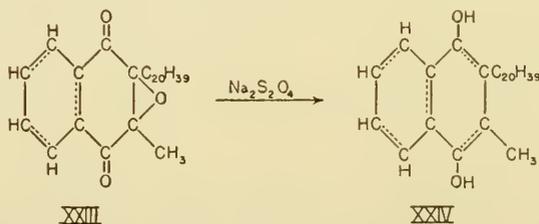
^a Adapted from H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, p. 498.

^b L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 996, 1628-1629 (1940).

^c M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

^d L. F. Fieser, W. P. Campbell, E. M. Fry, and M. D. Gates, *J. Am. Chem. Soc.*, **61**, 2559, 3216-3223 (1939).

Vitamin K₁ oxide (XXIII) has a biological activity almost equal to that of its unoxidized form. In addition it is much more stable to light⁸⁵ than is unoxidized vitamin K₁. It may occur naturally.⁸⁶ Fieser *et al.*²³ suggest the ready transformation of vitamin K₁ oxide to the reduced naphthohydroquinone (XXIV) by the accompanying reaction.²³ This form of



oxide is similar to the group of epoxides of the vitamins A and the carotenoids which have been extensively studied by the Karrer group. Just as the epoxides of vitamin A exhibit an antixerophthalmic effect, similarly the vitamin K oxides are antihemorrhagic. For a discussion of epoxides, see Chapter VI.

On the other hand, 2-methyl-1,4-naphthoquinone oxide falls far short in

⁸⁶ E. Fernholz, S. Ansbacher, and M. L. Moore, *J. Am. Chem. Soc.*, **61**, 1613-1614 (1939).

potency, below its unoxidized parent substance. It is believed that the transformation to the corresponding unoxidized hydroquinone may not proceed as efficiently as in the case of the vitamin K₁ oxide. It is also possible that the oxide of the 2-alkyl-1,4-naphthoquinones in which the 3-position is not protected may form the corresponding 3-hydroxy compound *in vivo*. Thus, the oxide of 2-methyl-1,4-naphthoquinone is converted by the action of sulfuric acid at a low temperature into phthiocol.⁸⁷ Furthermore, under the influence of alkali, the 2-methyl oxide may isomerize partly into phthiocol and partly into lawsone, with the loss of a methyl group.⁸⁵

The 2-alkyl-1,4-naphthoquinone oxides which have side chains longer than those of methyl on position 2 do not suffer such a marked decrease in potency as is the case with 2-methyl-1,4-naphthoquinone oxide. Fieser *et al.*²³ explain this paradox as due to the fact that the large isoprenoid groups manifest a protective influence in preventing hydrolytic cleavage of the oxide linkage.

(6) Biological Activity of Other Miscellaneous Quinones

Although most of the quinones other than the naphthoquinones were shown to be inactive at levels of 1000 $\mu\text{g.}$, two members in this series do respond in a positive manner. These include 2-methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone (XXV) and naphthotocopherol (XXVI).

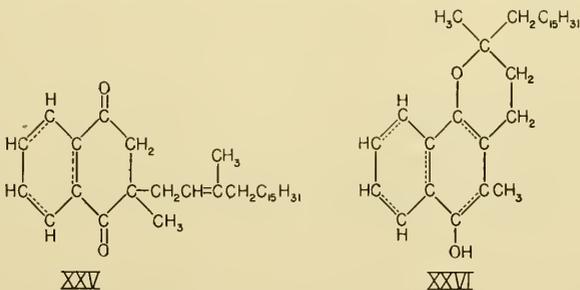


Table 8 lists some of the miscellaneous quinones tested, and the level at which the positively reacting members responded.

The high potency of 2-methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone is difficult to explain, although it is isomeric with vitamin K₁ hydroquinone. However, the Fieser group^{58,78,79} have shown that it can be converted to vitamin K₁ by pyrolysis and oxidation to the extent of 1 to 2%. Possibly, some such avenue of transformation is also available *in vivo*.

Naphthotocopherol occupies a unique position among the vitamins in that it has a dual effect. It can act both as an antisterility agent and as an antihemorrhagic vitamin. It is conceivable that the latter activity results

⁸⁷ L. F. Fieser, *J. Biol. Chem.*, 133, 391-396 (1940).

TABLE 8
ANTHEMORRHAGIC ACTIVITY OF SOME MISCELLANEOUS QUINONES^a

Compound	Effective dose, μg.
2-Methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone ^{b,c}	50
Naphthotocopherol ^{b,c}	500
Duroquinone ^a	Inactive at 1000
α-Tocopherylquinone ^a	" " "
9-Methylperinaphthenone-7 ^d	" " "
2,3,5-Trimethyl-1,4-benzoquinone ^a	" " "
2,3,5-Trimethyl-6-phytyl-1,4-benzoquinone ^{a,b,e}	" " "

^a Adapted from L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941), p. 677.

^b L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 996, 1628-1629 (1940).

^c M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 1982-1991 (1940).

^d M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

^e L. F. Fieser, M. Tishler, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2861-2866 (1940).

from the breakdown of the chroman ring, since β,γ-dihydrovitamin K₁ would result. This latter compound is effective when administered at a level of 8 μg.

(7) Biological Activity of Naphthohydroquinone Esters and Ethers

The naphthohydroquinones can be reversibly changed to the corresponding naphthoquinones in the body. Presumably, the formation of such an oxidation-reduction system may explain their physiological behavior in the animal. Although the naphthohydroquinones differ little in solubility from the corresponding quinones, they do form inorganic esters which readily dissolve in water. Such products are widely used for parenteral therapy. A number of sulfate and phosphate esters have been synthesized by Fieser and Fry,⁸⁸ who also investigated their biological activity.

The investigation of organic esters has been prompted by the demonstration by Binkley *et al.*⁴⁹ that the crystalline hydroquinone diacetates of vitamins K₁ and K₂ have about 50% of the potency which is exhibited by the corresponding quinones. The dibenzoate and dimethyl ethers have likewise been studied; Ansbacher, Fernholz, and Dolliver⁸⁹ were the first to report on these ethers. The results of these and of other investigators are summarized in Table 9.

⁸⁸ L. F. Fieser and E. M. Fry, *J. Am. Chem. Soc.*, **62**, 228-229 (1940).

⁸⁹ S. Ansbacher, E. Fernholz, and M. A. Dolliver, *J. Am. Chem. Soc.*, **62**, 155-158 (1940).

TABLE 9

ANTHEMORRHAGIC ACTIVITY OF SOME INORGANIC AND ORGANIC ESTERS AND OF SOME ETHERS OF NAPHTHOHYDROQUINONE^a

Compound	Effective dose, μg.
Inorganic esters	
Sodium 2-methyl-1,4-naphthohydroquinone diphosphate ^{b,c,d}	0.5
Sodium 2-methyl-1,4-naphthohydroquinone disulfate ^{b,d}	2
Vitamin K ₁ hydroquinone diphosphoric acid ^b	25
Sodium 2,3-dimethyl-1,4-naphthohydroquinone disulfate ^b	Inactive at 500 ^e
Potassium vitamin K ₁ hydroquinone disulfate ^b	Inactive at 500
Organic esters	
Diacetate of 2-methyl-1,4-naphthohydroquinone ^{f,g}	1
Dibenzoate of 2-methyl-1,4-naphthohydroquinone ^{b,g}	1
Dimesitoate of 2-methyl-1,4-naphthohydroquinone ^{b,h}	300
Vitamin K ₁ hydroquinone diacetate ⁱ	2
Vitamin K ₂ hydroquinone diacetate ⁱ	3.2
Ethers	
Monoethyl ether of 2-methyl-1,4-naphthohydroquinone ^k	1
Dimethyl ether of 2-methyl-1,4-naphthohydroquinone ^g	5
Dibenzyl ether of 2-methyl-1,4-naphthohydroquinone ^f	7

^a L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941).^b L. F. Fieser and E. M. Fry, *J. Am. Chem. Soc.*, **62**, 228-229 (1940).^c R. H. K. Foster, J. Lee, and U. V. Solmssen, *J. Am. Chem. Soc.*, **62**, 453-454 (1940).^d S. Ansbacher, E. Fernholz, and M. A. Dolliver, *Proc. Soc. Exptl. Biol. Med.*, **43**, 652-655 (1940).^e Reported as active at 500 μg. by L. F. Fieser, *et al.*, *J. Biol. Chem.*, **137**, 659-692 (1941).^f L. F. Fieser, W. P. Campbell, E. M. Fry, and M. D. Gates, *J. Am. Chem. Soc.*, **61**, 2559, 3216-3223 (1939).^g S. Ansbacher, E. Fernholz, and M. Dolliver, *J. Am. Chem. Soc.*, **62**, 155-158 (1940).^h M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).ⁱ M. Tishler, L. F. Fieser, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 1881-1882 (1940).^j S. B. Binkley, D. W. MacCorquodale, L. C. Cheney, S. A. Thayer, R. W. McKee, and E. A. Doisy, *J. Am. Chem. Soc.*, **61**, 1612-1613 (1939).^k M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 1982-1991 (1940).

The most striking finding in regard to the naphthohydroquinones is the fact that the diphosphate ester of the 2-methyl derivative is active in a 0.5-μg. dose, which corresponds to 0.2 μg. of the free 2-methyl-1,4-naphthoquinone. Since the minimum level at which the corresponding unesterified compound is effective is at 0.3 μg., it is apparent that an increased activity obtains. On the other hand, the diphosphoric acid ester of vitamin K₁ hydroquinone has only one-fiftieth of the activity of the free vitamin. Since it is believed that the vitamin K effect is obtained only following hydrolysis of the esters, the decreased potency of the vitamin K₁ diphosphoric acid ester would result from the difficulty of accomplishing this hydrolysis, due to the hindrance of the bulky isoprenoid side chain. Where only a methyl group is involved, as in the case of sodium 2-methyl-

1,4-naphthohydroquinone diphosphate, no such steric hindrance results, and hydrolysis of the ester can proceed at an augmented rate.

The organic ethers are very highly active, with the exception of the 2-methyl-1,4-naphthohydroquinone dimesitoate, which is only slightly effective. Since this is hydrolyzable only with difficulty, because of steric hindrance, it has been suggested by Fieser, Tishler, and Sampson²³ that this offers support for the hypothesis that acylated naphthohydroquinones undergo hydrolysis in the organism, and exert an antihemorrhagic effect by functioning as the liberated hydroquinone.

The ethers might be expected to be sufficiently resistant to breakdown so that they would function much less efficiently as vitamin-K-like compounds than the esters. However, they were found to be active in surprisingly small amounts; this fact led Ansbacher, Fernholz, and Dolliver⁸⁹ to the conclusion that in general the hydroquinone derivatives function as such rather than as components of the hydroquinone-quinone oxidation-reduction system. Another possibility is that the methylnaphthohydroquinone diethers may be readily converted to the corresponding quinones. Such a reaction has been demonstrated *in vitro* with methylnaphthohydroquinone dimethyl ether, which yielded methylnaphthoquinone on oxidation with chromic acid.⁹⁰ Whether such a transformation can be effected *in vivo* has not as yet been demonstrated.

(8) Biological Activity of Hydrides of Vitamin K and of Methylnaphthoquinones

Partial hydrogenation in the cyclic portion of vitamin K₁, or in the methylnaphthoquinone, may take place without a very great decrease in

TABLE 10
ANTIHEMORRHAGIC ACTIVITY OF HYDRIDES OF VITAMIN K₁ AND
METHYLNAPHTHOQUINONE^a

Compound	Effective dose, μg.
5,8-Dihydrovitamin K ₁ ^{b,c}	4
β,γ,5,6,7,8-Hexahydrovitamin K ₁ ^{b,d}	1000 (very slight)
2-Methyl-5,8-dihydro-1,4-naphthohydroquinone ^c	6
2-Methyl-5,8,9,10-tetrahydro-1,4-naphthoquinone ^a	8
2-Methyl-5,6,7,8-tetrahydro-1,4-naphthoquinone ^d	500

^a L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941).

^b L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 996 (1940).

^c L. F. Fieser, M. Tishler, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2861-2866 (1940).

^d M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

⁹⁰ M. Tishler, L. F. Fieser, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 1881-1882 (1940).

potency. Results of tests on such partially hydrogenated compounds are summarized in Table 10.

The 5,8-dihydrovitamin K₁ has 25% of the activity of vitamin K₁ while the isomer, β,γ -dihydrovitamin K₁, in which the hydrogens are added to the side chain, is only 12.5% as active as is the corresponding unreduced vitamin K. The surprisingly high activity of 5,8-dihydrovitamin K₁ is to be explained on the basis that such partially hydrogenated quinones can be readily aromatized by chemical oxidation, since the hydrogens at positions

TABLE 11

ANTIHEMORRHAGIC ACTIVITY OF METHYLNAPHTHOLS, METHYLTETRALONES, AND SOME RELATED COMPOUNDS

Compounds	Effective dose, $\mu\text{g.}$
2-Methyl-1,4-naphthohydroquinone ^a	0.5 ^a
2-Methyl-1-naphthol ^{b,c}	1 ^a
3-Methyl-1-naphthol ^{b,c}	0.6 ^a
4-Methyl-1-naphthol ^{b,c}	Inactive at 1000 ^a
1-Methyl-2-naphthol ^{b,c}	" " "
3-Methyl-2 naphthol ^{b,c}	" " "
1-Naphthol ^a	1000 (slight) ^a
2-Methyl-1-tetralone ^{a,b,c}	0.6 ^a
3-Methyl-1-tetralone ^{a,b,c}	Highly active ^b
β -Methylnaphthalene ^a	1000 (slight) ^{a,b}
2-Methyl-1-naphthylamine ^{b,c}	5
4-Amino-2-methyl-1-naphthol hydrochloride ^{d,e,f,g}	1 ^h
4-Amino-3-methyl-1-naphthol hydrochloride ^e	1 ^h
β -Methyl- γ -phenylbutyric acid ^c	Inactive at 1000 ^a

^a L. F. Fieser, M. Tishler, and W. L. Sampson, *J. Biol. Chem.*, **137**, 659-692 (1941).

^b M. Tishler, L. F. Fieser, and W. L. Sampson, *J. Am. Chem. Soc.*, **62**, 1881-1882 (1940).

^c M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, **62**, 2866-2871 (1940).

^d E. A. Doisy, D. W. MacCorquodale, S. A. Thayer, S. B. Binkley, and R. W. McKee, *Science*, **90**, 407 (1939).

^e H. J. Almquist and A. A. Klose, *Proc. Soc. Exptl. Biol. Med.*, **45**, 55-59 (1940).

^f D. Richtert, S. A. Thayer, R. W. McKee, S. B. Binkley, and E. A. Doisy, *Proc. Soc. Exptl. Biol. Med.*, **44**, 601-604 (1940).

^g A. D. Emmett, O. Kamm, and E. A. Sharp, *J. Biol. Chem.*, **133**, 285-286 (1940).

^h H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, Interscience, New York, 1945, pp. 491, 499, 500.

5 and 8 are highly active. On the other hand, the $\beta,\gamma,5,6,7,8$ -hexahydrovitamin K₁ shows practically no activity when given in the amount of 1000 $\mu\text{g.}$; in fact, Fernholz *et al.*⁹¹ observed no effect on a 2000- $\mu\text{g.}$ dosage. It should be recalled that the β,γ -dihydrovitamin K₁ is active in amounts of 8 $\mu\text{g.}$ The inactivity of such tetrahydro-naphthoquinones must be due

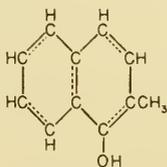
⁹¹ E. Fernholz, H. B. MacPhillamy, and S. Ansbacher, *J. Am. Chem. Soc.*, **62**, 1619-1620 (1940).

to the fact that they cannot be readily dehydrogenated in the same manner as can the compounds which possess an activating double bond. It is only when such compounds can be transformed to the naphthoquinones that they are able to exert activity as antihemorrhagic agents.

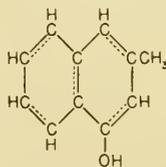
(9) *Biological Activity of Methylnaphthols, of Methyltetralones, and of Some Related Compounds*

Apparently it is not always necessary that a substance have the quinone or hydroquinone structure in order to exert a vitamin K effect. Fieser and his co-workers have demonstrated high activity on the part of certain naphthols and tetralones.^{58,90} A summary of their findings, as well as of the results of the Doisy group^{92,93} and others⁹⁴, on some related amines is given in Table 11.

Although 2-methyl-1-naphthol (XXVII) and 3-methyl-1-naphthol (XXVIII) are extremely potent antihemorrhagic agents, the corresponding 4-methylnaphthol is entirely ineffective. Moreover, the two methyl- β -naphthols tested (where the hydroxyl is on position 2) are entirely inactive. The only methylnaphthols which exhibit biological activity are those two which are directly convertible to 2-methyl-1,4-naphthoquinone; the three which cannot yield such a product are completely inactive. Fieser *et al.*²³ believe that the animal possesses an extremely efficient hydroxylation mechanism which acts on the carbon in para position to the original hydroxyl group.



XXVII



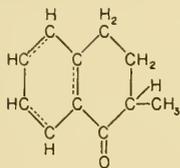
XXVIII

The methyltetralones also show activity comparable to that of the methylnaphthol. It is believed that 2-methyl-1-tetralone (XXIX) and 3-methyl-1-tetralone (XXX) can be effectively aromatized in the body to the corresponding naphthols. Their conversion to 2-methyl-1,4-naphthoquinones would follow, as has been assumed for the naphthols.

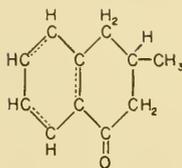
⁹² E. A. Doisy, D. W. MacCorquodale, S. A. Thayer, S. B. Binkley, and R. W. McKee, *Science*, 90, 407 (1939).

⁹³ D. Richtert, S. A. Thayer, R. W. McKee, S. B. Binkley, and E. A. Doisy, *Proc. Soc. Exptl. Biol. Med.*, 44, 601-604 (1940).

⁹⁴ H. J. Almquist and A. A. Klose, *Proc. Soc. Exptl. Biol. Med.*, 45, 55-59 (1940).

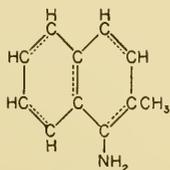


XXXIX

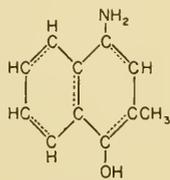


XXX

The activity of 2-methyl-1-naphthylamine (XXXI) presumably indicates its conversion to 2-methyl-1-naphthol in the body. That this transformation must be somewhat inefficient is indicated by the fact that it has only 20% of the potency of the latter compound. On the other hand, the 2-methyl-4-aminonaphthol (XXXII) possesses a potency almost as great as that of methylnaphthoquinone. The formation of the methylnaphthoquinone follows the formation of the quinonamine.



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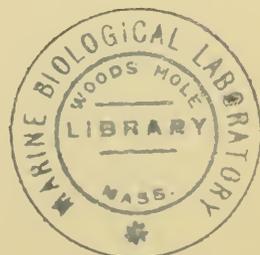
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PLANT AND ANIMAL SOURCES OF LIPIDS

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