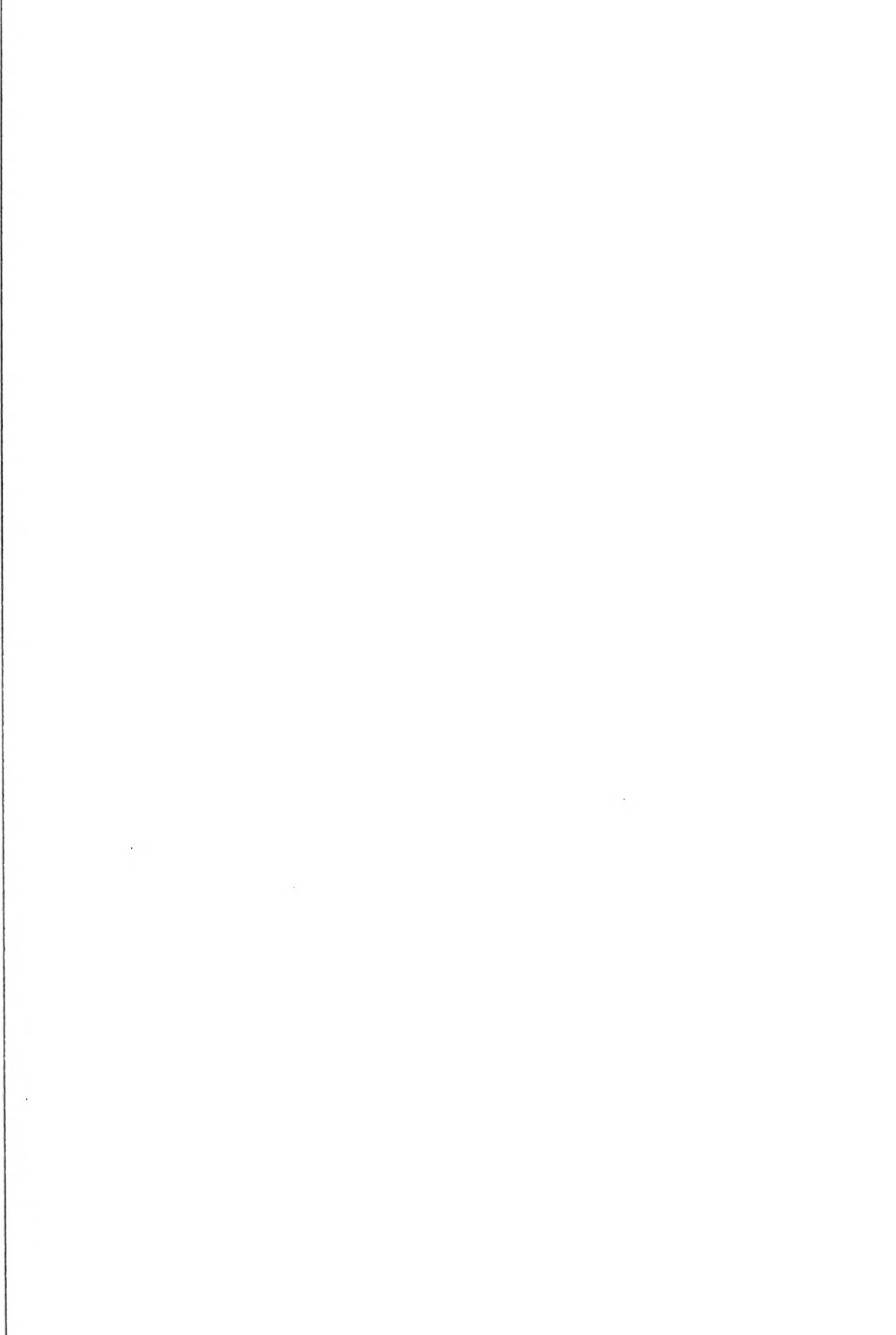
CAROTENOIDS



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CAROTENOIDS

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AUTHOR'S FOREWORD

The first monograph on carotenoids was written in 1922 by L. S. PALMER (*Carotenoids and Related Pigments*, New York). In view of the state of the subject at the time, this author could say relatively little about the chemistry of these natural pigments and his work therefore consisted mainly of a summary of the knowledge then available concerning the distribution and the biological significance of the carotenoids.

In 1934, L. ZECHMEISTER'S excellent book *Carotinoide* (Berlin) appeared, in which the great advances which had been made in the chemistry of these polyene pigments during the period 1927–1934 were described. Since then, the unravelling of the chemical nature of the carotenoids has further advanced and progress has also been made in the elucidation of their biological significance. A great deal of material has thus accumulated during a relatively short period.

It was the desire to sift and collate the extensive literature on carotenoids which led to the writing of the present monograph on this class of natural pigments. Special attention has been paid not only to the chemistry but also to the distribution and biological significance of the carotenoids. It is hoped that the numerous tables will help to clarify the relationships between the different pigments.

P. KARRER, E. JUCKER

ZÜRICH, August 1948

TRANSLATOR'S FOREWORD

In the present English edition of Professor P. KARRER'S and Dr E. JUCKER'S book, a number of corrections have been made, and a certain amount of new material, covering some of the more important investigations published since the appearance of the Swiss edition, has been added.

I am indebted to my wife for assistance in preparing the translation, and to Dr B. C. L. WEEDON for help in checking the proofs.

E. A. BRAUDE

LONDON, January 1950

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GENERAL PART

Introduction

The term *carotenoids* refers to a group of pigments, yellow to red in colour, which are widely distributed in the vegetable and animal kingdoms, and are distinguished by the following features: they are generally composed of isoprene residues, usually eight, arranged in such a way that in the middle of the molecule two methyl groups are present in 1:6 positions, while all other side-chain methyl groups occupy 1:5 positions. The general structure of the carotenoids is of the aliphatic or aliphatic-alicyclic type and their chromophoric systems contain numerous conjugated carbon-carbon double bonds.

All carotenoids are soluble in fats and lipoids; the term *lipochromes* is derived from this property. The only water-soluble carotenoids are those which, owing to the presence of acidic groups (e.g. carboxyl or enol groups), are able to form water-soluble alkali salts, or have acquired lyophilic properties by esterification with sugar residues (e.g. in crocin).

In view of their general chemical structure, carotenoids may be regarded as a sub-group of the polyene pigments. However, the latter also include pigments not composed of isoprene residues, but containing an unbranched aliphatic chain of conjugated double bonds (e.g. the diphenylpolyenes).

A revised nomenclature for carotenoids has recently been proposed*, but in the present monograph the individual pigments are mostly referred to by the names given to them by their discoverers.

The great interest which the carotenoids have aroused during the last twenty years is conditioned not only by their interesting chemical structure but also by their biological and physiological importance. Several of these pigments are pro-vitamins of vitamin A and thus play an essential part in the animal and human organism. Their significance in the vegetable kingdom has so far been less thoroughly investigated but there can be little doubt that here also they fulfil important functions.

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* The Nomenclature of the Carotenoid Pigments (Report of the Committee on Biochemical Nomenclature of the National Research Council, accepted by the Nomenclature, Spelling and Pronunciation Committee of the American Chemical Society — Chem. Eng. News *24* (1946) 1235. — Report of the 'Commissions de Réforme de la Nomenclature de Chimie organique et de Chimie biologique'. London, July 1947).

As the following chapters will show, between 70 and 80 carotenoids have been found in nature up to the present time. They can all be related to one parent substance, lycopene. By means of simple chemical changes such as cyclisation, double bond migration, partial hydrogenation, introduction of hydroxyl-, keto-, or methoxyl-groups, or introduction of an oxygen bridge, etc., the whole range of pigments can be derived from this parent substance. Their visible light absorption comprises a range of about 300 mμ (ca. 400–700 mμ). The carotenoids thus represent one of the most striking examples of the manifold variation of a parent substance by the vegetable and animal cell.

CHAPTER I

Mode of occurrence of carotenoids in plants and animals Detection and estimation of carotenoid pigments

1. MODE OF OCCURRENCE IN PLANTS

Relatively little information is available regarding the mode of occurrence of carotenoids in plants. In view of their non-polar nature, the majority of natural polyene pigments are insoluble in water and do not normally occur dissolved in the cell fluid. An exception is provided by crocetin which occurs in the cell fluid in the form of its water-soluble gentiobiose ester, crocin. Water solubility can be conferred not only by esterification with sugars, as in the case of crocin, but also by combination with proteins. Esterification can occur with the carotenoid carboxylic acids (e.g. crocetin, bixin and azafrin), while combination with proteins has so far been observed mainly with polyene pigments (e.g. astacene) occurring in animals. (Compare MENKE[1]).

The majority of vegetable carotenoids occur in the chromatophores. They seldom occur crystalline, but are usually present in colloidal suspension in the cell lipoids or in admixture with solid or semi-solid fats. MENKE[1] has recently found that certain carotenoids in the plastids are combined with proteins; this is in accord with the observations of JUNGE[2] regarding the mode of occurrence of carotenoids in animals. According to GOLDOWSKI and PODOLSKAJA[3], the carotenoids of the sunflower seeds occur in the aqueous and not in the oily phase. This is in contrast to the findings of SAVELLIS[4], according to whom the carotenoids are present in separate lipoid droplets in the chloroplasts. It will be clear from these partly contradictory results that this question has not yet been fully clarified. For further data and examples, reference should be made to the original literature[5].

2. MODE OF OCCURRENCE IN ANIMALS

Many attempts have been made in recent years to determine the fate of carotenoids taken up with the vegetable food by the animal organism. It has

References p. 8–9.

been found that part of the pigments is excreted unchanged, while the remainder is absorbed. The absorbed carotenoids are either deposited in the fat tissues, nerve tissues, inner organs, etc., or converted into other substances which often fulfil important physiological functions in the animal organism (e.g. vitamin A). In the animal organism, the carotenoids either occur dissolved in fats or combined with protein in the aqueous phase. Colloidal solutions are also observed[6].

A typical example of a carotenoid-protein complex is the astaxanthin proteid, ovoverdin. This water-soluble chromoproteid occurs, for example, in the green eggs of the lobster and in many other crustacea (cf. p. 230). Astaxanthin occurs as the fat-soluble carboxylic acid ester in the red hypodermis of the lobster, and the retina of the chicken also contains at least two different esters of this pigment[7].

JUNGE[8] has recently carried out investigations on the pigments of insects and has observed that many of these appear to be carotenoid-protein complexes. Thus, phytoxanthins (e.g. xanthophyll) as well as epiphasic carotenoids (e.g. β-carotene) have been found to be constituents of such chromoproteids.

The mode of occurrence of carotenoids in blood serum is also of importance. The present view is that the carotenoids here occur in the aqueous phase combined with lipoids and proteins[9].

VON EULER and ADLER[10] have established the occurrence of carotene in the retina. According to experiments by BRUNNER and collaborators[11], the pigment here occurs in the colloidal state.

3. DETECTION AND ESTIMATION

In order to establish the presence of carotenoids in natural sources, the dried materials (e.g. leaves or blossoms) are treated with certain reagents (e.g. concentrated sulphuric acid), which produce characteristic colourations. According to MOLISCH[12], the polyene pigments are best detected by first destroying the surrounding substances, e.g. fats, and only then applying the colour tests. In practice, the material is first treated with concentrated aqueous alcoholic alkali, which dissolves the fats and sets free the carotenoids. At the same time, the phytoxanthin esters* are hydrolysed and the phytoxanthins are liberated. In this way, crystalline carotenoids are often obtained and can be recognised under the microscope. Their presence can also be shown by colour reactions. Recently, however, it has become increasingly usual to isolate the carotenoids first and to characterise them subsequently. For this purpose the micromethod of KUHN and BROCKMANN[13] is often employed. It must be emphasised,

* In nature, phytoxanthins often occur esterified as colour waxes, e.g. physalien, helenien.

References p. 8–9.

however, that for the complete identification of a carotenoid it is necessary to carry out a chromatographic purification and to isolate the pigment in the crystalline state.

4. COLOUR REACTIONS

The polyene pigments are well known to give blue or violet solutions with a variety of strong acids such as concentrated sulphuric acid, hydrochloric acid, perchloric acid, trichloracetic acid, and with acid chlorides, such as antimony trichloride or arsenic trichloride. These colourations, although not specific, can be used as qualitative tests[14].

(a). Reaction with concentrated sulphuric acid: This reaction is carried out by carefully forming a layer of concentrated sulphuric acid under an ethereal solution of the pigment. The sulphuric acid layer acquires an intense dark blue to blue-violet or, occasionally, greenish-blue colour which disappears on the addition of water[15].

(b). Other strong acids: Fuming nitric acid produces a transient blue colouration. A number of observations have also been published recently regarding the blue colouration produced by concentrated aqueous hydrochloric acid. It appears that the following carotenoids colour concentrated aqueous hydrochloric acid blue:

(i). Aldehydes, e.g. β-citraurin, β-apo-2-carotenal.

(ii). Some carotenoids containing several hydroxyl groups, e.g. fucoxanthin, azafrin.

(iii). Carotenoid epoxides and their furanoid transformation products, e.g. violaxanthin, auroxanthin, xanthophyll epoxide, flavoxanthin, β-carotene diepoxide, aurochrome.

In practice, the hydrochloric acid reaction is carried out in the following way: The pigment is dissolved in a little ether and concentrated aqueous hydrochloric acid is added to the solution. After shaking, the acid layer is coloured blue. With a number of hydrocarbon epoxides, such as α-carotene monoepoxide, the blue colouration is very weak and only persists for a short time. For further details, reference should be made to the description of this reaction in the sections on individual carotenoids.

(c). Antimony trichloride in chloroform solution (Carr-Price reagent):

Similarly to vitamin A, carotenoids give dark blue colourations with the Carr-Price reagent[16]. These blue colourations often have characteristic absorption maxima, and can be used for the quantitative estimation of carotenoids[17].

References p. 8–9.

5. SPECTROSCOPY

An important part of the characterisation of a carotenoid is the determination of its absorption maxima. (With regard to the relationships between constitution and colour, and extinction curves, cf. page 53). On examining the solution of a carotenoid, usually in carbon disulphide or petroleum ether, in a spectrometer, two or three sharp absorption bands can usually be observed. Their positions can be accurately determined (approximately to within 0.5 mμ) and represent the wavelengths of the absorption maxima. These data are characteristic for each carotenoid and together with other physical constants are used for its identification. (Detailed light absorption data will be quoted in the description of the individual pigments in later sections). By means of the photographic method of determining solution spectra, as developed, for instance, by von Halban, Kortüm and Szigeti[18], or by other suitable means, the complete absorption curves can also be determined[19].

6. COLORIMETRY

There are numerous methods for the colorimetric determination of a carotenoid, all of which have in common the comparison of an unknown quantity of the pigment with a standard solution. Potassium dichromate[20], azobenzene[21], bixin[22] and β-carotene[23] have been used as standard substances. Instruments not requiring standard solutions have also been employed[24]. It is necessary to separate the carotenoids before their colorimetric determination, otherwise misleading results are obtained.

7. FLUORESCENCE SPECTRA

Following the determination of the fluorescence spectra of different diphenylpolyenes by Hausser and collaborators[25], Dhéré[26] examined vitamin A, β-carotene and lycopene at —180° in this respect. The determination of fluorescence spectra has not, however, found general application.

REFERENCES

1. W. Menke, *Naturwissenschaften 28* (1940) 31.
2. H. Junge, *Z. physiol. Chem. 268* (1941) 179.
3. A. M. Goldowski and M. S. Podolskaja, *Chem. Centr. 1939* II 2437.
4. R. Savelli, *Protoplasma 29* (1938) 601; *C. 1938* II 2126.
5. H. Molisch, *Ber. deut. Botan. Ges. 36* (1918) 281. — K. Noack, *Biochem. Z. 183* (1927) 135. — A. Guilliermond, *Compt. rend. 164* (1917) 232. — Courchet, *Ann. Sci. nat.* (7) (1888) 263. — R. Kuhn and H. J. Bielig, *Ber. 73* (1940) 1080.
6. Cf. W. Straus, *Dissertation*, Zürich, (1939) p. 50.

7. R. Kuhn, J. Stene and N. A. Sörensen, *Ber. 72* (1939) 1688.
8. H. Junge, *Z. physiol. Chem. 268* (1941) 179.
9. L. Palmer, *J. Biol. Chem. 23* (1915) 261. — H. van den Bergh, P. Muller and J. Broek-Meyer, *Biochem. Z. 108* (1920) 279. — Bendien and Snapper, *Biochem. Z. 261* (1933) 1. — E. Lehnartz, *Einführung in die chemische Physiologie*, 1931, p. 353. — E. v. Dániel and T. Béres, *Z. physiol. Chem. 238* (1936) 160. — S. P. L. Sørensen, *Kolloid-Z. 53* (1930) 306. — W. Kraus, *Dissertation*, Zürich 1939.
10. H. v. Euler and E. Adler, *Arkiv. Kemi Mineral. Geol. B 11* (1933) No. 20.
11. O. Brunner and co-workers, *Z. physiol. Chem. 236* (1935) 257.
12. H. Molisch, *Mikrochemie der Pflanzen*, Jena, 1923, p. 253.
13. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
14. Cf. also: H. Molisch, *Mikrochemie der Pflanzen*, 1923. — C. van Wisselingh, *Flora 7* (1915) 371. — L. Zechmeister, *Carotinoide*, Berlin (1934), pp. 82, 126, 235.
15. R. Kuhn and A. Winterstein, *Helv. Chim. Acta 11* (1928) 87, 116, 123, 144.
16. F. H. Carr and E. A. Price, *Biochem. J. 20* (1926) 497.
17. P. Karrer and H. Wehrli, *25 Jahre Vitamin-A Forschung*, Nova Acta Leopoldina, New series, *1* (1933) 175. — A. Winterstein and C. Funk, *G. Kleins Handbuch der Pflanzenanalyse 4* (1933) 1041. — L. Zechmeister and L. v. Cholnoky, *Ann. 465* (1928) 288. — B. v. Euler and P. Karrer, *Helv. Chim. Acta 15* (1932) 496.
18. H. v. Halban, G. Kortüm and B. Szigeti, *Z. Elektrochem. 42* (1936) 628.
19. Cf. F. Zscheile, *Botan. Rev. 7* (1941) 587.
20. R. Willstätter and A. Stoll, *Untersuchungen über Chlorophyll*, 1913. — G. Fraps and co-workers, *J. Agr. Research 53* (1936) 713. — K. Sjöberg, *Biochem. Z. 240* (1931) 156. — S. W. Clausen and A. B. McCoord, *J. Biol. Chem. 113* (1936) 89.
21. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
22. H. N. Holmes and W. H. Bromund, *J. Biol. Chem. 112* (1936) 437.
23. H. R. Bruins, J. Overhoff and L. K. Wolff, *Biochem. J. 25* (1931) 430.
24. Ferguson and Bishop, *Analyst 61* (1936) 515. — Munsey, *J. Assoc. Offic. Agr. Chemists 21* (1938) 331. — H. v. Euler, H. Hellström and M. Rybdom, *Mikrochemie, Pregl-Festschrift* 1929, p. 69.
25. K. W. Hausser and co-workers, *Z. physik. Chem. (B) 29* (1935) 391; *29* (1935) 363.
26. C. Dhéré, *Fortschr. Chemie Org. Nat.* II (1939) 301.

CHAPTER II

The formation of carotenoids in plants and their physiological significance

1. MODE OF FORMATION

No experimental evidence, only hypotheses, exist at present with regard to the mode of formation of carotenoids in plants. It therefore appears premature to come to any definite conclusions on this subject.

KARRER, HELFENSTEIN, WEHRLI and WETTSTEIN[1] consider the possibility that lycopene may be formed from phytyl aldehyde by a benzoin condensation or by a pinacol reduction, followed by dehydrogenation.

Carotenoids containing fewer than 40 carbon atoms in the molecule may be formed by oxidation of C_{40} carotenoids[2].

Several investigations have been made concerning the morphological changes which take place in fruit during the ripening process. Some of these are referred to on p. 119[3].

2. THE FUNCTION OF CAROTENOIDS IN PLANTS

Although numerous investigations have been carried out within recent years concerning the significance of carotenoids in the vegetable organism, our present knowledge of this subject is still very scanty and no final opinion can be formed. The first studies in this field by WILLSTÄTTER and his school[4] attempted to demonstrate a possible influence of carotenoids on the processes of respiration and assimilation, but with negative results. More recently a number of workers have sought to determine the influence of carotenoids on sexual reproduction. The following is a brief summary of the results so far obtained. For details the original literature should be consulted.

WILLSTÄTTER and STOLL[4] examined the function of carotene and xanthophyll in green leaves, in which the two carotenoids occur in fairly constant proportion to chlorophyll. They were unable to detect any definite influence of the two pigments on respiration. According to NOACK[5], carotene and xanthophyll fulfil the role of light filters for chlorophyll, whereas WENT[6] has expressed

References p. 17–19.

the opinion that they are more likely to function as protectors for sensitive enzymes in the cell. WARBURG and NEGELEIN[7] considered that carotene and xanthophyll are photochemically active in assimilation. FODOR and SCHOENFELD[8] have recently reported that colloidal carotene solutions can act as hydrogen acceptors, and consider the possibility that they fulfil a similar function in the respiratory process.

It has also been suggested[9] that certain carotenoids (e.g. γ-carotene) play a part in the reproduction of algae. There appears to be no certain foundation for this hypothethis. Some investigators[10] assume that carotenoids influence the growth of plants, and BUENNING[11] has recently attempted to identify the pigment concerned in the phototropy of plants with β-carotene.

According to KUHN, MOEWUS and JERCHEL[12], crocin (crocetin digentiobiose ester), as well as *cis*- and *trans*-crocetin dimethyl ester play a part in the reproduction of the unicellular algae *chlamydomonas eugametos f. simplex.* It appears that crocin can induce the formation of flagella in the gametes, while mixtures of *trans*- and *cis*-crocetin methyl ester convert the motile, but still infertile gametes into male and female gametes. The ratio of *cis*- to *trans*-crocetin methyl ester determines whether male or female gametes are formed. These experiments require further confirmation.

As the result of recent investigations (cf. p. 66) which show that various carotenoid epoxides are widely distributed in plants, it has been suggested that these compounds play a part in the transport of oxygen or in oxidation reactions[13].

All these investigations are still at a preliminary stage and further researches will be required in order to elucidate the importance of carotenoids in plants.

3. THE FUNCTION OF CAROTENOIDS IN THE ANIMAL ORGANISM

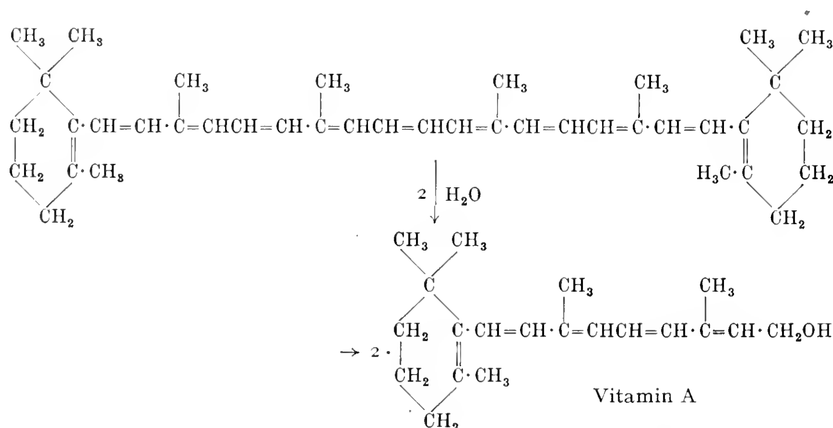
We are somewhat better informed regarding the significance of certain carotenoids in the animal organism, but again many important problems still require to be elucidated. A number of carotenoids are converted into vitamin A in the animal organism and therefore play the part of pro-vitamins. Furthermore, carotenoids play a part in the visual process, but their function is as yet incompletely understood.

a. Carotenoids as Pro-vitamins A

Numerous papers have been published dealing with the function of carotenoids as pro-vitamins A[14]. Only a brief summary of the definitely established facts will be given here. For further details, the original literature should be consulted.

References p. 17–19.

STEENBOCK and his collaborators were the first to suggest, about 30 years ago, that a connection exists between the yellow plant pigments (carotene) and vitamin A.[15] During the following years, the question of the growth-promoting properties of carotene was investigated by different workers. The results of these investigations were very contradictory and a definite solution of this problem was only achieved in 1929 by B. v. EULER, H. v. EULER and KARRER[16]. The results of their investigations definitely proved that carotene possesses qualitatively the same biological effect (resumption of arrested growth) as vitamin A, and was therefore probably related to the latter. This result at first appeared difficult to understand as carotene is a deeply coloured crystalline substance, quite different from the pale yellow vitamin A*. Later experiments by KARRER and collaborators elucidated the constitution of vitamin A[17] and of β-carotene[18] and the relationship between the two compounds. The chemical structure of β-carotene is such that by the uptake of water it can be converted into two molecules of vitamin A, and the growth-promoting properties of β-carotene thus find their explanation.



In agreement with this view, experiments reported by T. Moore showed that rats kept on a vitamin A-free diet, and whose livers contained practically no vitamin A, again accumulated vitamin A in the liver after being given β-carotene[19]. γ-Carotene, which contains only *one* β-ionone ring in the molecule, and can therefore only form one molecule of vitamin A by the addition of water, exhibits much weaker growth-promoting properties than β-carotene[20].

Very little is yet known about the mechanism by which β-carotene and other pro-vitamins A are converted into the vitamin. It has been assumed that this process depends on a ferment, carotenase. It is very probable that the reaction occurs in the liver[21] or in the intestine[22]. In animals deficient of

* Cristalline vitamin A was not yet known at the time.

References p. 17–19.

vitamin A, the conversion of pro-vitamins into vitamin A takes place rapidly and fairly completely (up to 70 or 80 %). If the organism is saturated with vitamin A, however, or if high doses of pro-vitamins are given, then only a small proportion is converted into the vitamin[23]. For this reason, carotene and other carotenoids are always found in the faeces.

The form in which the pro-vitamins A are supplied to the organism is of decisive importance for their absorption and conversion into vitamin A. If the carotenoids are dissolved in animal or vegetable fats, they are easily taken up; if, on the other hand, solutions in paraffin oil or ethyl oleate are used, hardly any absorption takes place[24]. Ignorance of these facts is partly responsible for the contradictory results recorded in the early literature concerning the activity of carotene[24].

Very recently a number of attemps have been made to convert β-carotene into vitamin A *in vitro*. Although some of these attempts are claimed to have been successful, this problem cannot yet be regarded as finally solved, as it has not been possible to isolate the vitamin A formed in a pure state and to establish its identity with certainty. WILLSTAEDT[25] reports a transformation of this type, using liver preparations, while HUNTER and WILLIAMS[26] obtained traces of vitamin A by the action of hydrogen peroxide on β-carotene and subsequent reduction of the aldehyde produced.

It is an interesting fact that not all mammals have the same capacity for converting pro-vitamins A into vitamin A. The most suitable experimental animal appears to be the rat[27]. Guinea pigs[28], rabbits[29], pigs[30] and cattle[31] possess the capacity to a reduced extent, dogs only to a very small extent[32], whereas in cats it is completely absent[33]. Chicken also appear capable of transforming β-carotene into vitamin A[34]. The facts regarding fresh water and salt water fish are not yet completely known, but it appears that fish are capable of converting pro-vitamins A into vitamin A_1[35] (and vitamin A_2)[36].

After recognition of the fact that carotene consists of several isomers (cf. p. 125), and that α-carotene also possesses vitamin A activity, though to a reduced extent, a number of different investigations were begun with the view to elucidating the relationships between the structure of a compound and its vitamin A activity. In the course of these investigations several naturally occurring carotenoids were recognised as pro-vitamins A and a number of partly synthetic carotenoids were also shown to possess growth-promoting properties. Before dealing with the theoretical aspects of these results, a summary is given here of the compounds which possess vitamin A activity (see table 1, p. 14). As all these compounds (with the exception of vitamin A methyl ether and vitamin A acid) will be dealt with in detail in later sections, the reader is merely referred to the alphabetical index at this stage.

The relationships which exist between the vitamin A activity of a compound

TABLE 1

PRO-VITAMINS A

Naturally occurring	Partially synthetic	Totally synthetic
α-Carotene	β-Carotene mono-epoxide	Vitamin-A methyl ether*
β-Carotene	β-Carotene di-epoxide	Vitamin-A acid**
γ-Carotene	Dihydroxy-β-carotene	
α-Carotene epoxide	Semi-β-carotenone	
Citroxanthin = Mutatochrome	Semi-β-carotenone monoxime	
Cryptoxanthin	Anhydrosemi-β-carotenone	
Myxoxanthin	Luteochrome	
Aphanin	β-Apo-2-carotenal	
Echinenone	β-Apo-2-carotenal oxime	
Torularhodin	β-Apo-4-carotenal oxime	
	α-Carotene diiodide	
	β-Carotene diiodide	
	Product from zeaxanthin + PBr_3	
	Product from xanthophyll + PBr_3	
	β-Apo-2-carotenol	

* W. Oroshnik, *J. Am. Chem. Soc.* 67 (1945) 1627. — O. Isler, W. Huber, A. Ronco and M. Kofler, *Experientia 2* (1946) 31. — See also Festschrift 'Emil Barell', Basle 1946, p. 31.

** J. F. Arens and D. A. van Dorp, *Nature 157* (1946) 190. — P. Karrer, E. Jucker and E. Schick, *Helv. Chim. Acta 29* (1946) 704. — I. M. Heilbron, E. R. H. Jones, and D. G. O'Sullivan, *Nature 157* (1946) 485; *J. Chem. Soc.*, 1946, 866.

and its chemical constitution have now been clarified. In order to exhibit vitamin A activity a compound must contain an unsubstituted β-ionone ring and the unsaturated side-chain present in axerophtol (vitamin A). α-Semi-carotenone[37] possesses the unsaturated side chain but not the β-ionone ring and is biologically inactive. β-Euionone[38] contains an unsubstituted β-ionone ring but not the complete side chain and is incapable of replacing vitamin A in experiments with animals*.

Steric relationships also play a part in the biological activity of a pro-vitamin A. Most of the investigations in this field are due to Zechmeister and

* I. M. Heilbron, E. R. H. Jones and their collaborators (cf. I. M. Heilbron, Pedler Lecture, *J. Chem. Soc.*, 1948, 386; I. M. Heilbron, E. R. H. Jones and R. W. Richardson, *ibid.*, 1949, 287; I. M. Heilbron, E. R. H. Jones, D. G. Lewis and B. C. L. Weedon, *ibid.*, 1949, 2023) have recently synthesized a number of partly demethylated and acetylenic analogues of vitamin A acid and have shown that they exhibit some growth-promoting properties. This work is providing important new evidence concerning the relationships between chemical constitution and vitamin A activity.

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his collaborators[39]. These workers have shown that in general the greatest biological activity is exhibited by those pro-vitamins which possess a *trans*-configuration throughout*. The following table** demonstrates these relationships.

TABLE 2

RELATIONSHIP BETWEEN VITAMIN A ACTIVITY AND STERIC CONFIGURATION OF SOME CAROTENOIDS*

β-Carotene, *trans*-configuration throughout	100 %
Neo-β-carotene U (probably 1 *cis*-linkage)	38 %
Neo-β-carotene B (probably 2 *cis*-linkages)	53 %
α-Carotene, *trans*-configuration throughout	53 %
Neo-α-carotene U (probably 1 *cis*-linkage)	13 %
Neo-α-carotene B (probably 2 *cis*-linkages)	16 %
γ-Carotene, *trans*-configuration throughout**	28 %
Pro-γ-carotene (probably 5 *cis*-linkages)	44 %

* The potency of pure β-carotene is used as a standard (100 %).
** According to R. KUHN and H. BROCKMANN, *Klin. Wochschr. 12* (1933) 972, γ-carotene has the same vitamin A potency as α-carotene, instead of half the potency as shown here.

The fact that α-carotene mono-epoxide, β-carotene mono-epoxide, β-carotene di-epoxide and luteochrome all exhibit vitamin A activity although (with the exception of β-carotene mono-epoxide) they do not possess an unsubstituted β-ionone ring, deserves special mention[40]. It may be deduced that these compounds are partly de-oxygenated in the organism of the rat.

TABLE 3

COMPARISON OF THE BIOLOGICAL ACTIVITY OF SOME CAROTENOID EPOXIDES[41]

Carotenoid	Active daily dose, γ
β-Carotene	2.5
α-Carotene epoxide	10
β-Carotene di-epoxide	17
Luteochrome*	18

* P. KARRER and E. JUCKER, *Helv. Chim. Acta 28* (1945) 429, 430.

* γ-Carotene isolated from mimulus blossoms (p. 162) is an exception to this rule.
** Taken without alteration from L. ZECHMEISTER and co-workers, *Arch. Biochem.* 7 (1945) 247.

b. The Function of Carotenoids in the Visual Process

Some carotenoids appear to play a part in the process of vision[41]. Although there has been a good deal of theoretical speculation as well as experimental work in this connection, this field of research is still in an early stage of development and it is not yet possible to draw any definite conclusions. The following observations are merely meant to provide a brief summary.

The suggestion that visual purple is a carotenoid was first made by BOLL[42] about 70 years ago. For some time afterwards this question was not further investigated, presumably because science as a whole had not yet sufficiently progressed. In 1923, BLEGVAD[43] and in 1924, BLOCH[44] showed that vitamin A deficiency results in xerophthalmia, a sclerotic inflammation of the eyes. In 1925, FRIDERICIA and HOLM[45] found that night blindness (hemeralopia) is a direct consequence of vitamin A deficiency which must be related to the incapacity of forming visual purple in the retinal rods.

After the relationships between certain processes in the eye and vitamin A or the carotenoids had thus been demonstrated, several investigations were begun with the view to isolating the pigments concerned from the eye and to identifying them. However, this task proved to be a very difficult one. The eyes of animals only contain very small quantities of pigments and, furthermore, these substances are unstable and to some extent sensitive to light. The first success was achieved by VON EULER and ADLER[46], who isolated compounds of a carotenoid nature from the pigmented epithelial layer of bull and fish eyes. Shortly afterwards, WALD[47] proved the presence of vitamin A in the retina of bulls and frogs. Later, WALD[48] showed that the retina of frogs contained xanthophyll ester and recently WALD and ZUSSMAN[49] found strongly coloured oily discs in the pupils of many birds and reptiles. In the chicken these discs are red, golden and yellow-green and from them three carotenoids can be isolated, one of which appears to be identical with esterified astacene, while the other two are of as yet unknown constitution. HONIGMANN[50] has reported to have found a photolabile pigment in the retina of young chicken. This pigment appears to be of a carotenoid nature and to be similar to, but not identical with, rhodopsin and porphyropsin (cf. below).

The investigations just described show that carotenoids or very similar pigments occur in the eyes of numerous animals. Their function has not yet been clarified, but it seems possible that they act as light filters which ensure that only rays of certain wavelengths enter the inner part of the eye and reach the photolabile substance. The question then arises as to the nature of the photolabile substance. WALD[51] showed that after a brief illumination of the eyes of frogs or mammals, a new pigment with different spectral properties is formed from the rhodopsin (visual purple). He suggested the name of

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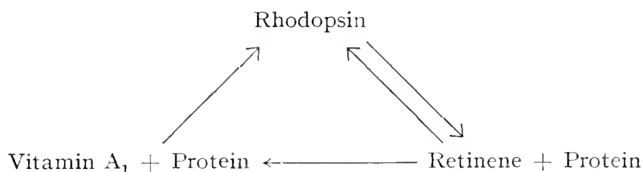
retinene for this yellow pigment. Retinene is subsequently converted into vitamin A and the latter is converted back into rhodopsin in the dark. To some extent retinene can also be directly transformed into rhodopsin. Both retinene and vitamin A always occur in the eye combined with protein[52]. The transformations described can be summarised schematically as follows:



According to WALD[51], retinene can also be obtained directly without illumination from eyes adapted to darkness by the extraction of visual purple with chloroform. This fact, as well as certain other considerations, led WALD to believe that rhodopsin is a carotenoid (retinene) -protein combination which can be destroyed by illumination, heat, or suitable solvents such as chloroform, thus liberating the protein-bound carotenoid (retinene). It should be mentioned that neither retinene nor rhodopsin have ever been obtained in the crystalline state or analysed. Nevertheless, it cannot be doubted that the chromophoric system of the carotenoids is utilised in the act of vision. Rhodopsin, retinene and vitamin A have also been found in the eyes of mammals and salt water fish[53] e.g. *Prionotus carolinus*, *Centropristes stiratus*, *Stenotomus chrysops*, and the cycle between these substances again appears to be the same.

Many fresh water fish contain a different light-sensitive pigment, porphyropsin[54], in place of rhodopsin. The reactions involved in the visual process of such fresh water fish, e.g. *Morone americana*, *Perca flavescens*, and *Esox reticulatus* were examined by WALD[55] who found that they were similar in character to those occurring with rhodopsin. During the illumination of porphyropsin, retinene$_2$ is formed, which is in turn converted into vitamin A_2.

MORTON and his collaborators have recently shown[56] that retinene$_1$ is identical with vitamin A_1 aldehyde and retinene$_2$ with vitamin A_2 aldehyde.

REFERENCES

1. P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. Chim. Acta* *13* (1930) 1084.
2. R. KUHN and CH. GRUNDMANN, *Ber. 65* (1932) 898, 1880. — R. KUHN and A. WINTERSTEIN, *Naturwissenschaften 21* (1933) 527; *Ber. 67* (1934) 344; *65* (1932) 646. — R. KUHN, *Forsch. u. Fortschr. 9* (1933) 426. — R. KUHN and A. DEUTSCH, *Ber. 66* (1933) 883.
   P. KARRER and T. TAKAHASHI, *Helv. Chim. Acta 16* (1933) 287.
3. A very detailed description of these processes will be found in L. ZECHMEISTER's Monograph *Die Carotinoide*, Berlin, 1934, Springer-Verlag.

4. Cf. R. Willstätter and A. Stoll, *Untersuchungen über die Assimilation der Kohlensäure*, Berlin, 1918.
5. K. Noack, *Z. Botan. 17* (1925) 481.
6. F. A. F. C. Went, *Rec. trav. botan. néerland. 1* (1904) 106.
7. O. Warburg and E. Negelein, *Z. physik. Chem. 106* (1923) 191; *Naturwissenschaften 10* (1923) 647.
8. A. Fodor and R. Schoenfeld, *Biochem. Z. 233* (1931) 243.
9. R. Emerson and D. Fox, *Proc. Roy. Soc. B 128* (1940) 275. — Cf. P. Karrer and coworkers, *Helv. Chim. Acta 26* (1943) 2121.
10. A. H. Blaauw, *Z. Botan. 6* (1914) 641; *7* (1915) 465. — H. Borris, *Planta 22* (1934) 644. — W. v. Buddenbrock, *Grundriss der vergleichenden Physiologie 1* (1937) 10. — S. Hecht, *Naturwissenschaften 13* (1925) 66.
11. E. Bünning, *Planta 26* (1937) 719; *27* (1937) 148, 583.
12. R. Kuhn, F. Moewus and D. Jerchel, *Ber. 71* (1938) 1541. For further references see *Jahrbücher für wissenschaftliche Botanik*.
13. P. Karrer, E. Jucker, J. Rutschmann and K. Steinlin, *Helv. Chim. Acta 28* (1945) 1149.
14. P. Karrer and H. Wehrli, *25 Jahre Vitamin-A Forschung*, Nova Acta Leopoldina, New series *1* (1933) 175. — U. Solmssen, *Dissertation*, Zürich, 1936. — G. R. Rosenberg, *Chemistry and Physiology of the Vitamins*, New York 1945, p. 38. — Cf. Otto Walker, *Dissertation*, Zürich, 1935.
15. H. Steenbock, M. T. Sell, E. M. Nelson and M. V. Buell, *J. Biol. Chem. 46* (1921) Proceed. XXXII. — Cf. H. Steenbock *et al.*, *Science 50* (1919)352; *J. Biol. Chem. 41* (1920) 163; *40* (1919) 501; *51* (1922) 63. For later, partly contradictory investigations see: J. C. Drummond and co-workers, *Biochem. J. 19* (1925) 1047.
16. B. v. Euler, H. v. Euler and P. Karrer, *Helv. Chim. Acta 12* (1929) 278.
17. P. Karrer, R. Morf and K. Schöpp, *Helv. Chim. Acta 14* (1931) 1036, 1431; *Helv. Chim. Acta 16* (1933) 557. — P. Karrer and R. Morf, *Helv. Chim. Acta 16* (1933) 625.
18. Cf. p. 133.
19. Th. Moore, *Biochem. J. 24* (1930) 692. — Cf. H. Brockmann and M.-L. Tecklenburg, *Z. physiol. Chem. 221* (1933) 117. — R. Kuhn and H. Brockmann, *Z. physiol. Chem. 200* (1932) 246. — R. Kuhn and H. Brockmann, A. Scheunert and M. Schieblich, *Z. physiol. Chem. 221* (1933) 129. — H. v. Euler, P. Karrer and A. Zubrys, *Helv. Chim. Acta 17* (1934) 24.
20. P. Karrer, H. v. Euler, H. Hellström and M. Rydbom, *Svensk. Kem. Tid. 43* (1931) 105.
21. H. S. Olcott and D. C. McCann, *J. Biol. Chem. 94* (1931) 185. — B. Ahmad, *Biochem. J. 25* (1931) 1195. — J. L. Rea and J. C. Drummond, *Z. Vitaminforsch. 1* (1932) 177. — J. G. Brazer, A. C. Curtis, *Arch. Internal. Med. 65* (1940) 90.
22. J. Glover, T. W. Goodwin and R. A. Morton, *Biochem. J. 41* (1947) Proceed. XLV; C. E. Wiese, J. W. Mehl and H. J. Devel, *Arch. Biochem. 15* (1947) 75; J. Glover, T. W. Goodwin and R. A. Morton, *Biochem. J. 43* (1948) 512; F. H. Mattson, *J. Biol. Chem. 176* (1948) 467.
23. Cf. L. Zechmeister, *Erg. physiol. Biol. Chem. exp. Parmakol. 39* (1937) 148. — C. A. Baumann, B. M. Riising and H. Steenbock, *J. Biol. Chem. 107* (1934) 705.
24. J. C. Drummond, B. Ahmad and R. Morton, *J. Soc. Chem. Ind.* (London) *49* (1929) Transact. 291. — Cf. W. Duliere, R. A. Morton and J. C. Drummond, *Chem. Centr.* 1930, I, 1639.
25. H. Willstaedt, *Enzymologia 3* (1937) 228. — Cf. also L. E. Baker, *Proc. Soc. Exp. Biol. Med. 33* (1935) 124. — H. S. Olcott and D. C. McCann, *J. Biol. Chem. 94* (1931) 185. — J. L. Rea and J. C. Drummond, *Z. Vitaminforsch. 1* (1932) 177. — H. v. Euler and E. Klussmann, *Arkiv. Kemi Mineral. Geol. B 11* (1932) 6. — B. Wolff and T. Moore, *Lancet 223* (1932) 13. — J. C. Drummond and R. J. McWalter, *Biochem. J. 27* (1933) 1342; *J. Physiol.* (London) 83 (1934) 236.
26. R. F. Hunter and N. E. Williams, *J. Chem. Soc.* 1945, 554.

27. B. Ahmad and K. Malik, *Indian J. Med. Research 20* (1933) 1033.
28. H. Brockmann and M.-L. Tecklenburg, *Z. physiol. Chem. 221* (1933) 117.
29. *Idem, ibid.*, see also H. Rosenberg, *Chemistry and Physiology of the Vitamins*, New York 1945, p. 55.
30. Th. Moore, *Biochem. J. 25* (1931) 2131.
31. Th. Moore, *Biochem. J. 26* (1932) 1.
32. A. J. Coombes, G. L. Ott and W. Wisnicky, *North. Am. Veterinarian 21* (1940) 601.
33. H. Rosenberg, cf. ref. 30.
34. N. S. Capper, JM. W. McKibbin, J. H. Prentice, *Biochem. J. 25* (1931) 265.
35. H. Rosenberg, *Chemistry and Physiology of the Vitamins*, p. 50, 73, New York 1945.
36. R. A. Morton and R. H. Creed, *Biochem. J. 33* (1939) 318.
37. P. Karrer, H. v. Euler and U. Solmssen, *Helv. Chim. Acta 17* (1934) 1169.
38. P. Karrer and co-workers, *Helv. Chim. Acta 15* (1932) 878; 17 (1934) 3.
49. L. Zechmeister and co-workers, *Arch. Biochem. 5* (1944) 107; 7 (1945) 247.
40. H. v. Euler, *Helv. Chim. Acta 28* (1945) 1150.
41. Cf. the review by P. Karrer, *Documenta Ophthalmologica*, 1938, p. 259.
42. F. Boll, *Arch. Anat. Physiol. Anat. Abt.* 1877, 4.
43. Blegvad, *Dissertation*, Kopenhagen, 1923.
44. Bloch, *J. Dairy Sci.* U.S.A. 7 (1924) 1.
45. Fridericia and Holm, *Am. J. Physiol. 73* (1925) 63.
46. H. v. Euler and E. Adler, *Arkiv. Kemi Mineral. Geol. B 11* (1933), Nr. 20, 21.
47. G. Wald, *Nature 132* (1933) 316; *134* (1934) 65; *J. Gen. Physiol. 18* (1934) 905.
48. G. Wald, *J. Gen. Physiol. 19* (1935) 351.
49. G. Wald and H. Zussman, *Nature 140* (1937) 197.
50. Honigmann, *Arch. Ges. Physiol. 189* (1921) 1. Cf. G. Wald, *Nature 140* (1937) 545.
51. G. Wald, *J. Gen. Physiol. 19* (1935) 351.
52. G. Wald, *J. Gen. Physiol. 21* (1937) 93.
53. G. Wald, *Nature 136* (1935) 913.
54. E. Köttgen and C. Abelsdorff, *Z. Psychol. Physiol. Sinnesorgane 12* (1896) 161.
55. G. Wald, *Nature 139* (1937) 1017.
56. S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J. 40* (1946) Procced. LIX. — R. A. Morton, *Nature*, London *153* (1944) 69. — R. A. Morton and T. W. Goodwin, *Nature*, London, *153* (1944) 405. — S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J. 42* (1948) 516.

CHAPTER III

The isolation of carotenoids

The isolation of carotenoids from vegetable or animal sources often presents difficulties, especially if extensive experience in this field is lacking. An attempt will therefore be made in the present section to describe some general methods of isolation. These have to be adapted in particular cases to take account of the mode of occurrence of the pigments and of the materials which accompany them.

The usual course of isolation consists of the following parts:

1. Preparation of the materials to be examined and extraction of the carotenoids.
2. Division of the carotenoids into hypophasic and epiphasic constituents.
3. Separation of the pigments in the two phases and preparation in a crystalline condition.

The individual steps are dealt with in more detail in the following section. No attempt has been made to summarise *all* the usual methods employed; instead, a few of the well-tried and common methods are given which are successful in most cases.

1. EXTRACTION OF CAROTENOIDS

Before the extraction of the carotenoids, the vegetable or animal material must be dried (dehydrated). With blossoms or fruit or other parts of plants, this is most easily accomplished by drying in the sun (preferably in a current of air), or in a well-aired room at 40–50° C. It is important that the material should be spread in thin layers and should be turned from time to time in order to achieve as uniform a drying as possible and in order to prevent fermentation which would destroy the carotenoids. If it is not possible to dry the material in this way, as is sometimes the case with algae, marine plants, or animals, the dehydration may be carried out by submersion in solvents such as acetone, methanol, ethanol, etc.

The extraction can be carried out by means of a wide variety of solvents. The following are most commonly used: benzene, petroleum ether, ether (free

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from peroxide), carbon disulphide, chloroform (free from hydrochloric acid), ethanol, methanol and acetone. Extraction at room temperature is carried out, by allowing the material to stand with the solvents in wide-necked bottles or percolators in a carbon dioxide or nitrogen atmosphere. If large amounts of material have to be extracted, large metal extractors can be used to advantage and also make it possible to work at higher temperatures. The extracts must be concentrated in vacuum as quickly as possible, and the concentrates thus obtained must be kept sealed up under vacuum in large ampoules until they are worked up.

2. SEPARATION INTO HYPOPHASIC AND EPIPHASIC CAROTENOIDS

As a result of the pioneering work of WILLSTÄTTER and STOLL[1], it is usual to divide the extracted carotenoids into two groups by partition between two immiscible solvents. Carotenoids with two or more hydroxyl groups are thus obtained as hypophasic pigments and those without hydroxyl groups as epiphasic pigments. Mono-hydroxy compounds such as cryptoxanthin and rubixanthin occupy an intermediate position and are found in the epiphase as well as in the hypophase. The following table provides a summary of the epiphasic and hypophasic carotenoids (table 4, p. 22).

As most of the hydroxylated carotenoids occur in nature in the form of esters as the so-called "pigment-waxes", it is necessary to saponify before partition between methanol and petroleum ether. This can be done by means of about 12 % methanolic potassium hydroxide at room temperature. The carotenoid mixture to be saponified is dissolved in petroleum ether and a requisite quantity of alkali is added. If the resulting mixture is homogenous it is simply allowed to stand for about 20 hours. If two phases are formed, the mixture must be shaken mechanically. In either case, the space above the solution should be filled with an inert gas (e.g. hydrogen or nitrogen) in order to prevent aerial oxidation.

In some cases a solution of sodium methoxide in methanol is preferred to methanolic potassium hydroxide. Saponification can also be carried out at more elevated temperatures (about 60–70° C.); in this case an alkali concentration of about 5% is usually sufficient.

After saponification is complete, petroleum ether is added, followed by sufficient water to result in a separation into two phases. The upper phase contains mainly the epiphasic carotenoids and the lower phase mainly the hypophasic carotenoids. The petroleum ether phase is then repeatedly extracted with methanol, and the methanol layer is repeatedly extracted with petroleum ether, and the appropriate extracts are combined. The solution of epiphasic pigments is washed with water, dried over sodium sulphate, concen-

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TABLE 4

DIVISION OF THE NATURAL CAROTENOIDS INTO HYPOPHASIC AND EPIPHASIC PIGMENTS ON THE BASIS OF PARTITION BETWEEN PETROLEUM ETHER AND 90 % METHANOL

Epiphasic	Almost equally distributed between epiphase and hypophase	Hypophasic
Actinioerythrin	Celaxanthin	Antheraxanthin
Aphanin	Gazaniaxanthin	Auroxanthin
Aphanicin	Cryptoxanthin	Aphanizophyll
α-Carotene	Lycoxanthin	Astacene
α-Carotene epoxide	Rhodoxanthin	Astaxanthin
β-Carotene	Rubichrome	Azafrin
γ-Carotene	Rubixanthin	Bixin
δ-Carotene (?)	Sarcinaxanthin	Capsanthin
Citroxanthin = Mutatochrome		Capsorubin
Echinenone		β-Citraurin
Flavorhodin		Chrysanthemaxanthin
Haematoxanthin		Crocetin
Leprotin		Cynthiaxanthin
Lycopene		Flavoxanthin
Mycoxanthin		Fucoxanthin
Pro-γ-carotene		Glycymerin
Pro-lycopene		Canary-xanthophyll
Rhodopsin		Lycophyll
Rhodopurpurin		Mytiloxanthin
Rhodovibrin		Myxoxanthophyll
Rhodoviolascin		Oscillaxanthin
Sarcinin		Pectenoxanthin
Torulin		Pentaxanthin
		Petaloxanthin
		Picofulvin
		Salmon acid
		Satinwood carotenoid
		Sulcatoxanthin
		Taraxanthin
		Torularhodin
		Trollixanthin
		Violaxanthin
		Violerythrin
		Xanthophyll
		Xanthophyll epoxide
		Zeaxanthin

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trated in vacuum, and is then subjected to chromatography on suitable adsorbents. The aqueous methanolic solution is diluted with water and extracted with ether. After washing and drying the ethereal solution, the solvent is removed by distillation in vacuum and the residue is dissolved in a suitable solvent and also subjected to chromatography.

3. SEPARATION OF THE CAROTENOID MIXTURES OF THE TWO PHASES

The method almost invariably employed at the present time for the separation of natural carotenoid mixtures is Tswett's adsorption analysis. It is probably no exaggeration to say that the extraordinary development of carotenoid research during the last 20 years is mainly due to the application of Tswett's chromatographic method and it therefore appears appropriate to describe it in some detail[2]. However, since a number of excellent monographs dealing with chromatography are already available, only the practical applications of the method to the separation of carotenoid pigments will be dealt with here.

Although TSWETT[3], a Russian botanist, published the first description of chromatographic adsorption analysis as early as 1906, and although he drew attention to the scope and manifold applications of his method, chromatography was but seldom made use of during the following 25 years[4]. It was not until 1931, when the older methods of separation had finally proved inadequate, that adsorption analysis was re-introduced by KUHN, by KARRER, and by ZECHMEISTER, although it had occasionally been used by DHÉRÉ[5] during the intervening period. The following short summary will give an indication of the progress subsequently achieved.

TABLE 5

NUMBER OF NATURAL CAROTENOIDS ISOLATED DURING THE PERIOD 1922–1946

Period	Number of carotenoids isolated
Up to 1922	7
,, ,, 1933	about 15
,, ,, 1937	,, 30
,, ,, 1948	,, 80

A decisive factor in this rapid development was the fact that the adsorptive capacity of the polyene pigments is strongly influenced by relatively small differences in molecular structure. It is therefore possible to separate even

References p. 28.

closely related pigments on the chromatographic column. A classical example of the efficiency of the method is the separation of γ-carotene from the α- and β-components of crude carotene, although the proportion of γ-carotene only corresponds to about one part in a thousand. Even differences in steric configuration of the pigments are sufficient for a quantitative separation, as was first shown by the investigations of WINTERSTEIN and STEIN[6] on *cis*- and *trans*-crocetin methyl esters.

The strengths of adsorption of different carotenoids on a given adsorbent bear a definite relationship to their chemical structures. Hydroxyl substituents exert the largest influence in this respect. (Carotenoids containing carboxyl groups are not considered here). Of two carotenoids otherwise possessing the same structure, that containing a larger number of

a) hydroxyl groups,
b) carbonyl groups,
c) esterified hydroxyl groups, or
d) double bonds,

is adsorbed more strongly.

The effectiveness of functional groups on the strength of adsorption decreases in the sequence: hydroxyl group, carbonyl group, esterified hydroxyl group, double bond.

The following table provides a summary of the positions of some carotenoids (those whose functional groups are known) on the chromatogram. At the top of the table are the pigments which are most strongly adsorbed, at the bottom those which are least strongly adsorbed.

In practice, adsorption analysis is carried out by percolating the solution of the carotenoids through a long column of suitable adsorbent. The amount of adsorbent used depends on the amount of pigments to be separated. The individual zones are then "developed" by further washing with the same (or a different) solvent. The solution of carotenoids is poured into a tube which is filled to the extent of about $^4/_5$ with the adsorbent and connected to an evacuated bottle. As soon as the solution has been almost completely adsorbed by the column, the latter is washed through with an organic solvent (usually the same as that employed to prepare the solutions) until an optimum separation of the zones has been achieved. It is of great importance that the chromatogram should never be allowed to dry, as this results in the destruction of the polyene pigments by aerial oxidation, and in a shrinking of the upper part of the column and a distortion of the zones. As soon as the individual pigments have been separated as shown by the formation of colourless zones between the individual coloured layers, the "development" is stopped. The adsorption column is extruded from the tube and the individual zones are mechanically divided. They are immediately placed in prepared vessels already filled with

References p. 28.

TABLE 6

THE POSITION OF CAROTENOIDS IN THE CHROMATOGRAM

Carotenoid	Hydroxyl groups	Carbonyl groups	Ether groups	Conjugated ethylenic bonds	Isolated ethylenic bonds
Myxoxanthophyll	6	1	0	10	0
Fucoxanthin	4–6		?	10?	?
Astaxanthin	2	2	0	11	0
Capsorubin	2	2	0	9	0
Capsanthin	2	1	0	10	0
Auroxanthin	2	0	2	7	2
Violaxanthin	2	0	2	9	0
Antheraxanthin	2	0	1	10	0
Lycophyll*	2	0	0	13	0
Eschscholtzxanthin	2	0	0	12**	0
Flavoxanthin	2	0	1	9	1
Chrysanthemaxanthin	2	0	1	9	1
Xanthophyll epoxide	2	0	1	10	0
Zeaxanthin	2	0	0	11	0
Xanthophyll	2	0	0	10	1
Rubichrome	1	0	1	10	1
Lycoxanthin	1	0	0	13	0
Rubixanthin	1	0	0	11	1
Cryptoxanthin	1	0	0	11	0
Rhodoxanthin	0	2	0	12	0
Myxoxanthin	0	1	0	11	1
Aphanin	0	1	0	11	0
Citroxanthin	0	0	1	9	1
Flavochrome	0	0	1	8	2
α-Carotene epoxide	0	0	1	9	1
Physalien		Di-ester		11	0
Helenien		Di-ester		10	1
Lycopene	0	0	0	11	2
Pro-lycopene	0	0	0	11	2
γ-Carotene	0	0	0	11	1
Pro-γ-carotene	0	0	0	11	1
δ-Carotene	0	0	0	11	0
α-Carotene	0	0	0	10	1

* The relative positions of antheraxanthin and lycophyll in the chromatogram are still uncertain.

** Possibly only 11 of the double bonds are conjugated.

an eluting solvent. When the whole chromatogram has thus been separated, the solutions of pigments from the different zones are filtered, the solvent is removed by distillation and the residues are crystallised. If the separation thus achieved

References p. 28.

is incomplete, the eluting solvent, usually methanol, can be removed by shaking with water and the remaining solution dried, concentrated and again subjected to chromatography.

Adsorbents. The following materials, in finely divided form, have been used for the chromatographic separation of carotenoids: alumina, FULLER's earth, calcium carbonate, calcium hydroxide, kaolin, kieselguhr, magnesium oxide, talcum, zinc carbonate, norite A, and others. The following 4 adsorbents are, however, adequate for most purposes: aluminium oxide*, calcium hydroxide, calcium carbonate, and zinc carbonate. Using these adsorbents it should be possible to carry out successfully all chromatographic separations of carotenoids.

Alumina Al_2O_3: Suitable for the separation of carotenoid hydrocarbons, but its use has recently decreased owing to its high cost.

Calcium hydroxide $Ca(OH)_2$: Calcium hydroxide was introduced for the separation of carotenoid hydrocarbons by KARRER and WALKER[7] in 1933 and has since become the adsorbent most widely used for this purpose. It is cheap and allows a complete separation of the epiphasic carotenoids.

Calcium carbonate $CaCO_3$: Calcium carbonate was first employed by TSWETT for the separation of carotenoids. Since 1931, it has frequently been used for the separation of phytoxanthins.

Zinc carbonate $ZnCO_3$: Zinc carbonate has frequently been employed, especially within recent years. It was introduced by KARRER and is very suitable for the separation of phytoxanthins. These are adsorbed somewhat more strongly on zinc carbonate than on calcium carbonate.

The following is the sequence of adsorbents of decreasing activity: Alumina, aluminium hydroxide, magnesium oxide, calcium oxide, calcium hydroxide, zinc carbonate, calcium carbonate, calcium sulphate, calcium phosphate, talcum, sucrose, inulin[7].

Solvents. The purity of the solvents used is of the greatest importance for the success of a chromatogram. The solvents must be dry and free from impurities, such as alcohol, pyridine, sulphur-containing compounds, etc. For the chromatography of epiphasic carotenoids, petroleum ether (b.p. 70–80°) or a mixture of petroleum ether with benzene or ether are commonly used. Recently petroleum ether-acetone mixtures have frequently been employed**

For hypophasic carotenoids it is usual to employ benzene or a mixture of benzene and ether or petroleum ether. Other solvents such as carbon disulphide, ethyl acetate, etc. are also used, although the first-named solvents are adequate in most cases. The following sequence of solvents, which is taken

* Different standardised grades of alumina are commercially available. For alumina according to H. BROCKMANN, cf. *Neuere Methoden der präparativen organischen Chemie*, 1943, p. 553.

** See p. 42, reference 10.

References p. 28.

from the excellent work of HESSE[7], is one of decreasing adsorptive capacity of the solutes: petroleum ether, carbon tetrachloride, trichloroethylene, benzene, methylene dichloride, chloroform, ether, ethyl acetate, acetone, *n*-propyl alcohol, ethanol, methanol, water, pyridine.

Experimental. A suitable adsorbent and solvent, and the size of the chromatogram column, are first selected by means of preliminary small-scale experiments. The chromatogram tube is then filled with the adsorbent. A variety of more or less complicated arrangements for large-scale chromatography have been described. Only a very simple apparatus which is readily assembled, inexpensive, and adequate for all purposes will be described here. It consists of a suction flask, a short glass tube *ca.* 10 mm in diameter and two good rubber stoppers. The stoppers are arranged back-to-back on the glass tube; one stopper is placed in the suction flask and the other in the chromatogram tube, as shown in the accompanying figure. The filling of the tube with the adsorbent can be carried out in various ways. The usual procedure is to add a small amount of the adsorbent at a time and to press it down with a half-bored cork attached to a glass rod. For larger tubes, ZECHMEISTER recommends a wooden stopper, the end of which has a diameter corresponding to two-thirds of that of the tube. After one layer has been well pressed down, more adsorbent is added and the operation is repeated until the tube is sufficiently full. It is important that the tube should be well and evenly filled, otherwise distorted colour-zones are obtained which are difficult to separate. When the tube has been filled, the vacuum is connected and the pressing and tapping (from the outside) is continued until the adsorbent no longer moves. If this is done, no shrinking should occur when solvent is added.

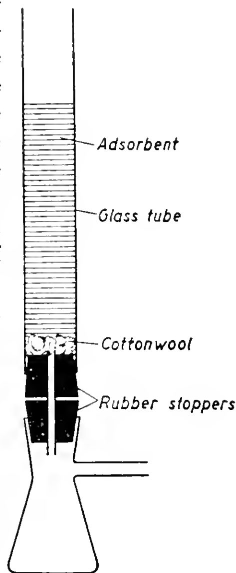

Fig. 1.
Arrangement for chromatographic analysis

WINTERSTEIN and STEIN[9] recommend that for filling large tubes the adsorbent should be moistened with the solvent and then poured into the tube.

When the column is ready, the solution of carotenoids is poured on and allowed to penetrate completely. More solvent is then added to "develop" the chromatogram. The development is best carried out by connecting the suction flask to the vacuum and then isolating the water pump by means of a screw tap. When the rate of flow of the solvent becomes too small, the flask is re-evacuated. It is important that the rate of flow should be neither too great nor too small. If the rate is too great, the zones tend to be blurred, while if it is too small, no sharp layers are formed because the rate of diffusion of the pigments then exceeds the rate of flow. After development is complete, the column is sucked dry until it has the appearance of a compact mass which can be extruded without falling to pieces. After mechanical separation of the coloured zones, the pigments of the individual layers are eluted with the solvent previously employed, but containing a little methanol (*ca.* 2–5 %). The eluates are evaporated to dryness in vacuum and the residue from each zone is either crystallised or, if the pigment is still non-homogeneous, again subjected to chromatography.

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4. CRYSTALLISATION

The crystallisation of carotenoids requires considerable practice, especially if small amounts of material are involved. It is not always possible to recrystallise a carotenoid from a single solvent. Solvent mixtures consisting of one component in which the pigment is easily soluble, and a second component in which the pigment is sparingly soluble, sometimes have to be employed. No attempt will be made here to give a complete list of all the solvents which have been used, since more information on this point will be found in later sections dealing with individual carotenoids. Epiphasic carotenoids can often be recrystallised from light petroleum or from ether-methanol, or benzene-methanol mixtures. For hypophasic pigments, benzene-methanol or ether-methanol mixtures are often used. Methanol alone is also employed. Certain carotenoids (e.g. violaxanthin, fucoxanthin, zeaxanthin) can be precipitated by adding water to a methanolic solution of the pigment covered with a layer of light petroleum. The water is added in very small portions and crystallisation can often be induced by scratching the sides of the vessel with a glass rod.

REFERENCES

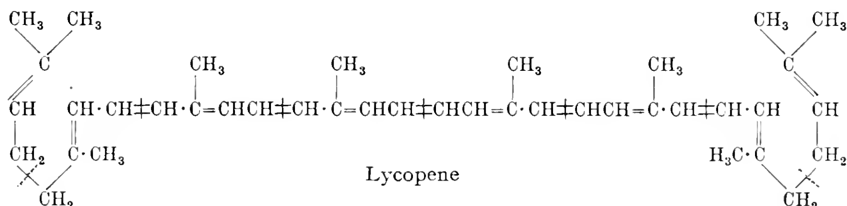
1. R. Willstätter and A. Stoll, *Untersuchungen über aas Chlorophyll*, Berlin 1913; *Untersuchungen über die Assimilation der Kohlensäure*, Berlin 1918. — Cf. also R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
2. An extensive literature dealing with chromatography is available. The following monographs should be consulted: L. Zechmeister and L. v. Cholnoky, *Die chromatographische Adsorptionsanalyse*, Berlin 1938. — H. Brockmann, *Die chromatographische Adsorption*, in: *Neuere Methoden der präparativen organischen Chemie*, Berlin 1943. — Gerhard Hesse, *Adsorptionsmethoden im chemischen Laboratorium*, Berlin 1943. — H. G. Cassidy *et al.*, "Chromatography" in: *Annals of the New York Academy of Sciences, 49* (1948) 141–326.
3. M. Tswett, *Ber. deut. botan. Ges. 24* (1906) 316, 384. — M. Tswett, *The chromophylls in the vegetable and animal kingdoms* (in Russian), Warsaw, 1910.
4. Cf. L. S. Palmer, *Carotinoids and related Pigments*, New York, 1922.
5. C. Dhéré, *Compt. rend. 158* (1914) 64–66. — Cf. also C. Dhéré, *Candollea 10* (1943) 60.
6. A. Winterstein and G. Stein, *Z. physiol. Chem. 220* (1934) 251.
7. Cf. G. Hesse, *Adsorptionsmethoden im chemischen Laboratorium*, Berlin, 1943, p. 31.
8. Cf. H. Brockmann, *Die chromatographische Adsorption*, in: *Neuere Methoden der präparativen organischen Chemie*, Berlin, 1943.
9. A. Winterstein and G. Stein, *Z. physiol. Chem. 220* (1933) 273. — Cf. D. C. Castle, A. E. Gillam, I. M. Heilbron and H. W. Thompson, *Biochem. J. 28* (1934) 1702.

CHAPTER IV

The chemical constitution of the carotenoids

Approximately 80 natural carotenoids are at present known. The constitutions of about 35 have been completely or largely elucidated and they are all closely related chemically. They all belong to the class of polyenes, their most characteristic structural feature being the large number of conjugated double bonds. Another characteristic feature is that of the 50 carotenoids the empirical formulae of which are known, 45 contain 40 carbon atoms, and only 5 have a different number of carbon atoms in the molecule.

The fact that there is some connection between the carotenoids and isoprene was first recognised by WILLSTÄTTER and MIEG[1]. To-day we know that isoprene is a constituent unit of the carotenoid pigments, which may be regarded as consisting of 8 isoprene molecules. It is characteristic of all carotenoids that the arrangement of the isoprene residues becomes reversed in the centre of the carotenoid molecule so that the central methyl groups occupy 1:6 instead of 1:5 positions[2]. The formula of lycopene is an example of this structural principle:



Lycopene

The important principle of the "reversal" of the central isoprene units has given rise to the hypothesis that a carotenoid molecule may be formed in the plant by the combination of two identical residues, e.g. two partially dehydrogenated phytyl groups, by a linking of the two terminal carbon atoms.

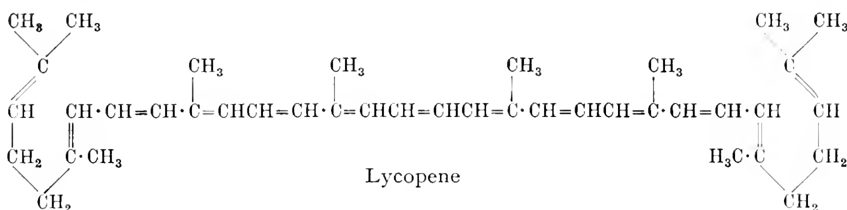
The following table, which contains the structural formulae of all naturally occurring carotenoids of known structure, shows the close structural relationship between these compounds. Formally, they can all be related to lycopene. γ-Carotene, β-carotene and α-carotene can be formed from lycopene by ring closure at one or both ends of the molecule, while bixin and crocetin can be

References p. 37.

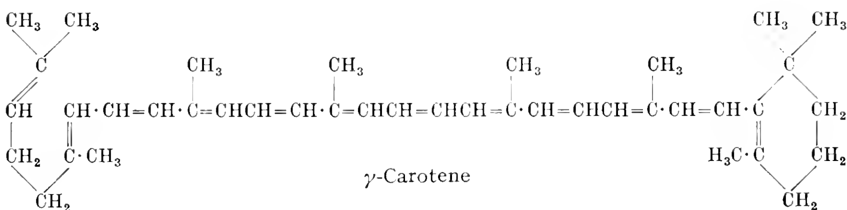
formed by oxydative degradation. From lycopene itself and from the three carotenes, numerous other pigments can then be derived by the introduction of oxygen, hydroxyl, methoxyl or carbonyl groups. Finally, the oxidation of certain of these compounds can give rise to the aldehydes and carboxylic acids of the carotene series such as β-citraurin and azafrin.

TABLE 7

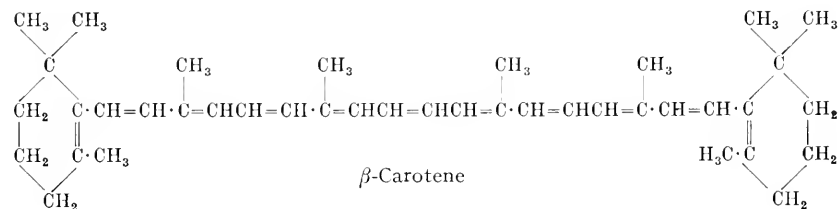
FORMULAE OF THE NATURALLY OCCURRING CAROTENOIDS OF KNOWN STRUCTURE



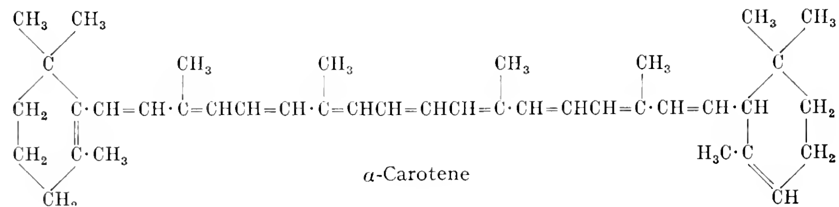
Lycopene



γ-Carotene



β-Carotene



α-Carotene

```
CH3  CH3
   C                                                                                   CH3  CH3
CH2  C═══CH CH3         CH3              CH3            CH3                             C
CH2  C    CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·C        CH2
  CH2   O                                                                        H3C·C       CH2
  CH3                                                                                  CH2
```

Mutatochrome=Citroxanthin

```
CH3  CH3                                                                              CH3  CH3
   C               CH3            CH3              CH3             CH3                    C
CH2  C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH    CH2
CH2  C  O                                                                        H3C·C      CH2
  CH2   CH3                                                                            CH
```

α-Carotene epoxide

```
CH3  CH3                                                                               CH3  CH3
   C               CH3            CH3              CH3             CH3                     C
CH    CH·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH    CH
CH2   C·CH3                                                                       H3C·C      CHOH
  CH2                                                                                   CH2
```

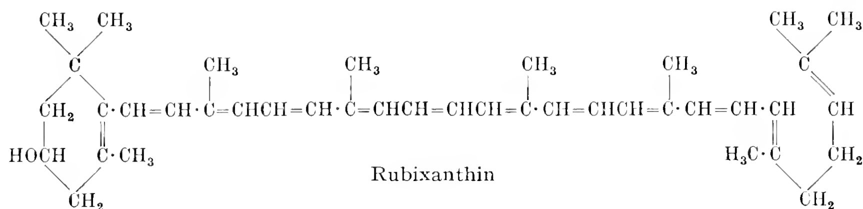
Lycoxanthin

```
CH3  CH3                                                                               CH3  CH3
   C               CH3            CH3              CH3             CH3                     C
CH      CH·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH    CH
HOCH    C·CH3                                                                     H3C·C      CHOH
  CH2                                                                                   CH2
```

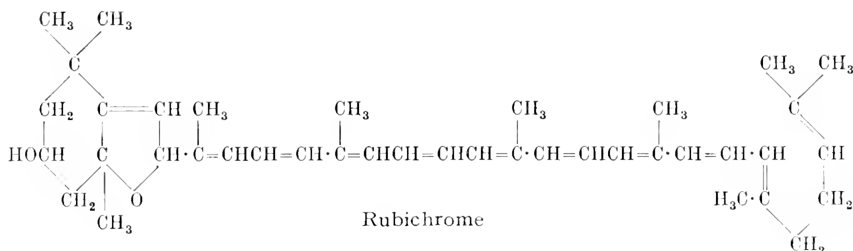
Lycophyll

```
CH3  CH3                                                                               CH3  CH3
   CH              CH3            CH3              CH3             CH3                    CH
CH2     CH·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH    CH2
H3COC   C·CH3                                                                     H3C·C      COCH3
  CH                                                                                     CH
```

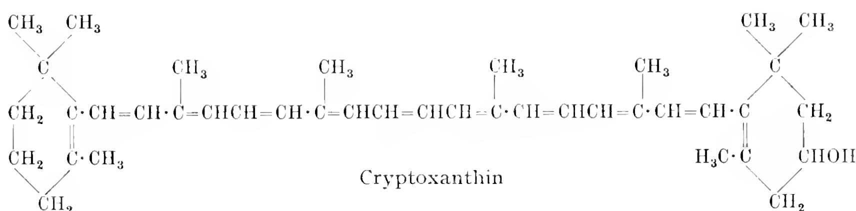
Rhodoviolascin(?)



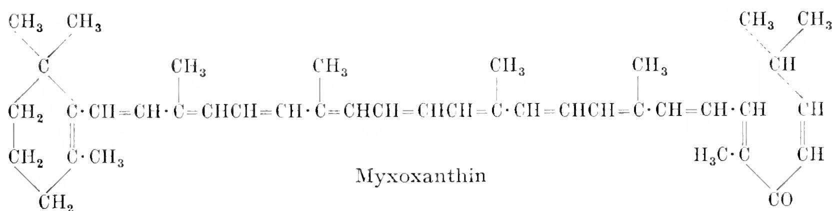
Rubixanthin



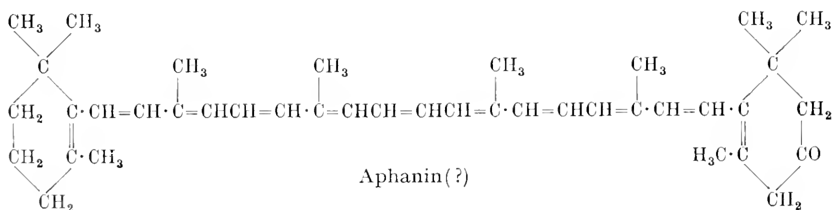
Rubichrome



Cryptoxanthin



Myxoxanthin



Aphanin(?)

```
 CH3  CH3                                                                           CH3  CH3
   \  /                                                                               \  /
    C           CH3          CH3            CH3          CH3                           C
   / \           |            |              |            |                           / \
CH2  C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·C      CH2
 |    ||                                                                        ||    |
HOCH  C·CH3                                                                H3C·C   CHOH
   \  /                                                                           \  /
   CH2                                                                             CH2
```

Zeaxanthin

```
 CH3  CH3                                                                           CH3  CH3
   \  /                                                                               \  /
    C           CH3          CH3            CH3          CH3                           C
   / \           |            |              |            |                           / \
CH2  C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH     CH2
 |    ||                                                                        |     |
HOCH  C·CH3                                                                H3C·C   CHOH
   \  /                                                                          \\  /
   CH2                                                                             CH
```

Xanthophyll

```
 CH3  CH3                                                                           CH3  CH3
   \  /                                                                               \  /
    C           CH3          CH3            CH3          CH3                           C
   / \           |            |              |            |                           / \
CH2  C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·C      CH2
 |    | \                                                                       ||    |
HOCH  C / O                                                                H3C·C   CHOH
   \  /  \                                                                        \  /
   CH2   CH3                                                                       CH2
```

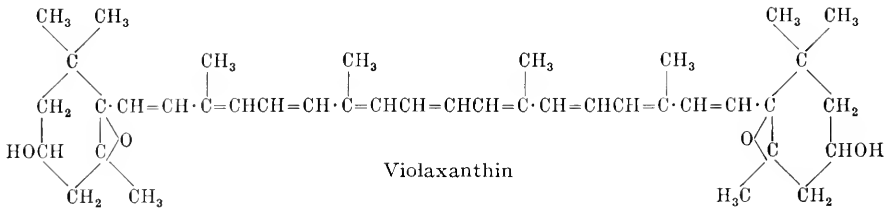
Antheraxanthin

```
 CH3  CH3                                                                           CH3  CH3
   \  /                                                                               \  /
    C           CH3          CH3            CH3          CH3                           C
   / \           |            |              |            |                           / \
CH2  C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH     CH2
 |    | \                                                                       |     |
HOCH  C / O                                                                H3C·C   CHOH
   \  /  \                                                                       \\  /
   CH2   CH3                                                                       CH
```

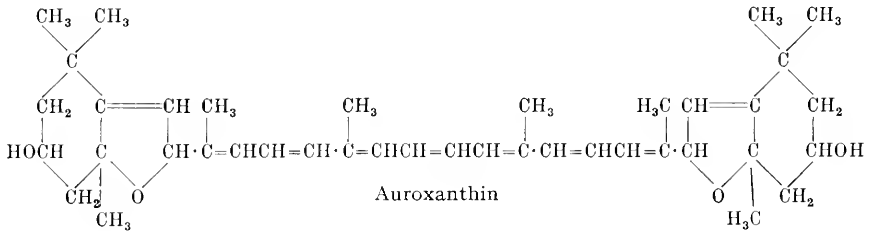
Xanthophyll epoxide

```
 CH3  CH3
   \  /                                                                             CH3  CH3
    C                                                                                 \  /
   / \                                                                                 C
CH2  C=====CH CH3          CH3            CH3          CH3                            / \
 |   |      |  |            |              |            |                   
HOCH C      CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH     CH2
   \ /|   \ /                                                                    |      |
   CH2|    O                                                                     C    CHOH
     CH3                                                                       /  \\  /
                                                                            H3C    CH
```

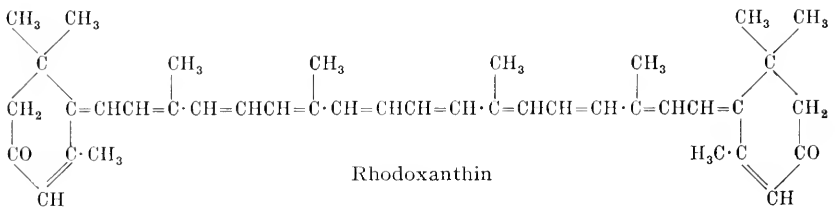
Flavoxanthin, Chrysanthemaxanthin



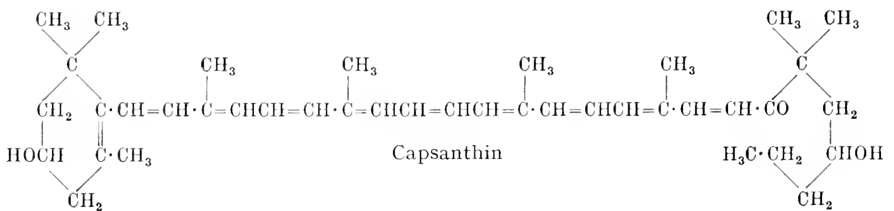
Violaxanthin



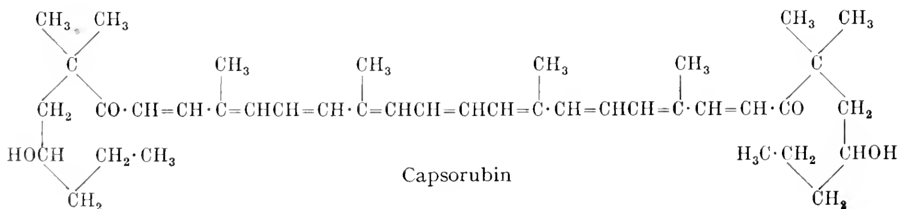
Auroxanthin



Rhodoxanthin



Capsanthin



Capsorubin

```
CH3  CH3                                                                          CH3  CH3
   \ /                                                                               \ /
    C           CH3          CH3            CH3           CH3                         C
   / \           |            |              |             |                         / \
CH2   C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·C   CH2
 |    ||                                                                       ||    |
HOCH  C·CH3                                                               H3C·C   CHOH
   \ /                                                                             \ /
    CO                                                                              CO
```

Astaxanthin

```
CH3  CH3                                                                          CH3  CH3
   \ /                                                                               \ /
    C           CH3          CH3            CH3           CH3                         C
   / \           |            |              |             |                         / \
CH2   C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·C   CH2
 |    ||                                                                       ||    |
CO    C·CH3                                                               H3C·C   CO
   \ /                                                                             \ /
    CO                                                                              CO
```

Astacene

```
CH3  CH3
   \ /
    C           CH3          CH3            CH3           CH3
   / \           |            |              |             |
CH2   C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH   COOH
 |    ||                                                                       ||    |
CH2   C·CH3                                                               H3C·C    CH
   \ /                                                                             \ //
    CH2                                                                             CH
```

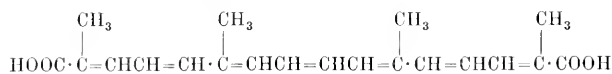
Torularhodin (?)

```
CH3  CH3
   \ /
    C     OH    CH3          CH3            CH3
   / \   /       |            |              |
CH2   C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CH·COOH
 |    |
CH2   C·CH3
   \ /    \
    CH2    OH
```

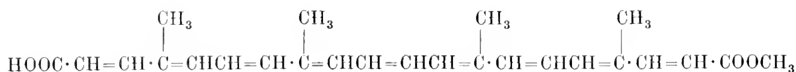
Azafrin

```
CH3  CH3
   \ /
    C           CH3          CH3            CH3           CH3
   / \           |            |              |             |
CH2   C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CHO
 |    ||
HOCH  C·CH3
   \ /
    CH2
```

β-Citraurin



Crocetin



Bixin

TABLE 8

CLASSIFICATION OF SOME CAROTENOIDS AS DERIVATIVES OF LYCOPENE AND THE CAROTENES

Lycopene	Lycoxanthin = 3-Hydroxylycopene Lycophyll = 3:3′-Dihydroxylycopene Rhodoviolascin (?)
γ-Carotene	Rubixanthin = 3-Hydroxy-γ-carotene Rubichrome = Furanoid oxide of rubixanthin Eschscholtzxanthin = Dihydroxy-γ-carotene(?)
β-Carotene	Cryptoxanthin = 3-Hydroxy-β-carotene Citroxanthin = Furanoid monoxide of β-carotene Zeaxanthin = 3:3′-Dihydroxy-β-carotene Antheraxanthin = Zeaxanthin mono-epoxide Violaxanthin = Zeaxanthin di-epoxide Auroxanthin = Furanoid zeaxanthin di-oxide Aphanin = 3-Keto-β-carotene(?) Rhodoxanthin = 3:3′-Diketo-β-carotene Astacene = 3:4:3′:4′-Tetraketo-β-carotene Astaxanthin = 3:3′-Dihydroxy-4:4′-diketo-β-carotene Capsanthin Capsorubin
α-Carotene	α-Carotene epoxide Xanthophyll = 3:3′-Dihydroxy-α-carotene Xanthophyll epoxide Flavoxanthin = Furanoid xanthophyll oxide Chrysanthemaxanthin = Furanoid xanthophyll oxide

It is not known with certainty to what extent carotenoids are interconvertible in nature. It seems very probable, however, that carotenoid epoxides, such as α-carotene epoxide and xanthophyll epoxide, are formed in the plant by oxidation of the corresponding carotenoids (α-carotene, xanthophyll), and

that they can be converted into the furanoid oxides (e.g. flavoxanthin, etc.[3]) under the influence of acids present in the plant.

REFERENCES

1. R. Willstätter and W. Mieg, *Ann.* *355* (1907) 1.
2. P. Karrer, A. Helfenstein, H. Wehrli and A. Wettstein, *Helv. Chim. Acta* *13* (1930) 1084.
3. P. Karrer and E. Jucker, *Helv. Chim. Acta* *28* (1945) 304.

CHAPTER V

Cis-trans isomerism of carotenoids

Cis-trans isomerism of the type exhibited by simple ethylenic compounds is also observed in the carotenoid series. Since a carotenoid contains several carbon-carbon double bonds, a considerable number of geometrically isomeric forms are possible. Thus a polyene of the formula $R(CH{=}CH)_9R'$ containing 9 double bonds can theoretically exist in 512 different *cis-trans* isomeric forms.

The fact that bixin occurs in two isomeric forms was discovered by HERZIG and FALTIS[1] in 1923. It was proved by KARRER and co-workers[2] in 1929 that these two forms were *cis-trans* isomers. Bixin is the more labile form. It is easily converted into *iso*-bixin which in view of its greater stability is regarded as the *trans*-form. Later, KUHN and WINTERSTEIN[3] found that crocetin, the chief pigment of saffron, is accompanied by a small quantity of a geometrical isomer, *iso*-crocetin. *Iso*-crocetin is a labile compound which can readily be converted into the more stable crocetin by means of iodine or other catalytic agents.

In 1935, GILLAM and EL RIDI[4] found that on repeated chromatographic adsorption of homogeneous β-carotene, two zones are obtained. The upper zone consists of β-carotene while the lower zone contains a new pigment, pseudo-α-carotene. It is possible that this isomer of β-carotene is not formed during adsorption, as assumed by GILLAM and EL RIDI, but spontaneously in the solution of the pigment[5].

In recent years, the reversible interconversion of carotenoids believed to be due to *cis-trans* isomerism has been investigated in detail, particularly by ZECHMEISTER and his collaborators. Although a considerable body of experimental material is already available, especially with regard to the spectroscopic and chromatographic properties of the different isomers, this field of work awaits further development, since the majority of interconversion products have not yet been isolated in crystalline form. Only a brief summary of these investigations will be given here. The properties of the different isomers are described in later chapters dealing with the parent carotenoids.

On the basis of recent studies employing x-ray analysis[6], spectral analysis and chromatographic analysis, it may be assumed that with a very few ex-

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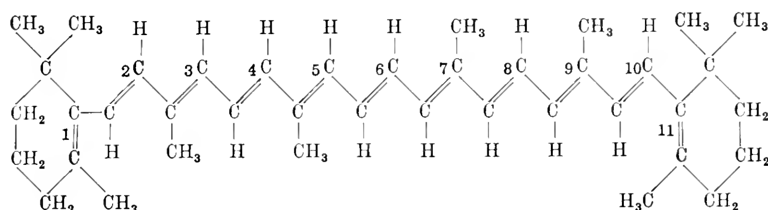
ceptions (e.g. bixin, pro-γ-carotene, pro-lycopene) the natural carotenoids all possess entirely *trans*-configurations. This is not surprising since the *trans*-configurations are those with the smallest energy content and the greatest stability. Thus natural β-carotene is believed to have the following structure.



On the basis of theoretical considerations, ZECHMEISTER, PAULING and collaborators[8] concluded that not all ethylenic groups of a carotenoid molecule are capable of taking part in *cis-trans* isomerisation, but only those of the type $-C(CH_3) = CH-$ and the double bond in the centre of the molecule. In the case of β-carotene only the 3-, 5-, 6-, 7-, and 9-double bonds could thus be involved in *cis-trans* isomerism. The remaining ethylenic groups are believed always to assume a *trans*-configuration owing to steric hindrance.

Isomerisation can be brought about in the following ways: a) refluxing of a solution of the carotenoid in an organic solvent, b) melting of the crystals, c) treatment with iodine[9], d) treatment with acids, and e) illumination. The separation of the isomerisation products is carried out by means of chromatographic analysis.

All the transformation products of natural *trans*-carotenoids so far obtained exhibit the following common features[10]:

1. The colour intensity of the pigment solution decreases as a result of isomerisation.

2. The isomerisation products are more soluble than the starting materials.

3. The melting points of *cis*-isomers are lower than those of the pigments with complete *trans*-configurations.

4. The isomerisation products often revert to the parent pigment with complete *trans*-configuration on crystallisation. Others crystallise inhomogeneously as shown by the fact that fresh solutions of the crystals give rise to several zones on chromatography. This is the case for instance, with pseudo-α-carotene, neo-carotene and neo-α-carotene[11].

5. If the molecule contains one or more asymmetric carbon atoms, isomerisation is often accompanied by large changes in optical rotation.

6. The strength of adsorption of the isomerised carotenoids on the chromatogram column differs considerably from that of the *trans*-pigments.

7. The isomerisation products always absorb at shorter wavelengths in the

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visible region of the spectrum than the parent carotenoids with complete *trans*-configuration. If the labile isomerisation products are treated with iodine, the absorption maxima are again displaced to longer wavelengths, but not as far as the location of the absorption maxima of the original *trans*-carotenoids. This is explained by ZECHMEISTER and his coworkers as due to the fact that in these isomerisations an equilibrium is always established so that the reverse change to the original *trans*-pigment is never complete.

8. The extinction coefficients of the isomerisation products are lower than those of the parent carotenoids with complete *trans*-configurations.

9. The isomerisation products are often characterised by the appearance of a new maximum in the ultra-violet spectrum. Following ZECHMEISTER and POLGÁR these new maxima are termed "cis-peaks". This phenomenon is illustrated in the schematic figure below:

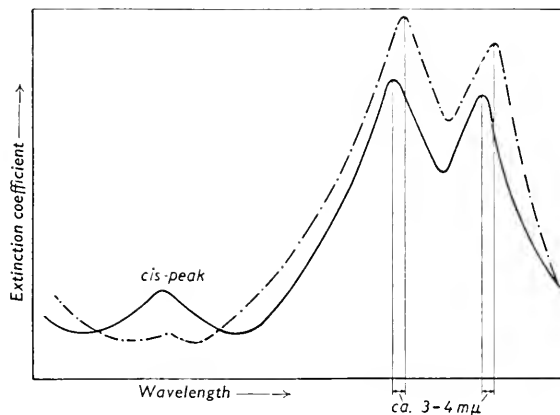

Fig. 2.
—·—·—· Carotenoid with complete *trans*-configuration
——— Isomerisation product

With all the isomerisation products so far examined, the centre of the *cis*-peak lies at a distance of 142 (±2) mμ from the long-wave maximum (in hexane solution).

For a theoretical interpretation of the "cis-peak", compare the review by ZECHMEISTER[10].

A more detailed discussion of this subject is beyond the scope of this monograph. The properties of the different isomers are briefly described in later sections dealing with the corresponding parent carotenoids. As a guide to the relevant literature all the carotenoids so far examined for *cis-trans* isomerisation are summarised in the following table.

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TABLE 9

CAROTENOIDS EXAMINED FOR CIS-TRANS ISOMERISM

Pigment	References
Capsanthin	L. ZECHMEISTER and co-workers, *Ann.* *530* (1937) 291; *543* (1940) 248; *J. Am. Chem. Soc.* *66* (1944) 186.
Capsorubin	L. ZECHMEISTER, L. v. CHOLNOKY, *Ann.* *543* (1940) 248; A. POLGÁR and L. ZECHMEISTER, *J. Am. Chem. Soc.* *66* (1944) 186.
α-Carotene	L. ZECHMEISTER and co-workers, *J. Am. Chem. Soc.* *65* (1943) 1522; *66* (1944) 137; *Arch. Biochem.* *6* (1945) 157. — A. E. GILLAM, M. S. EL RIDI and S. K. KON, *Biochem. J.* *31* (1937) 1605. — F. ZSCHEILE and co-workers, *Arch. Biochem.* *5* (1944) 77, 211.
β-Carotene	A. E. GILLAM and M. S. EL RIDI, *Nature* (London) *136* (1935) 914; *Biochem. J.* *30* (1936) 1735; *31* (1937) 251. — L. ZECHMEISTER and co-workers, *J. Am. Chem. Soc.* *64* (1942) 1856; *65* (1943) 1528; *Arch. Biochem.* *5* (1944) 107; *Arch. Biochem.* *7* (1945) 247; *Ber.* *72* (1939) 1340; *Nature* (London) *141* (1938) 249; *Biochem. J.* *32* (1938) 1305; *J. Am. Chem. Soc.* *66* (1944) 137.
γ-Carotene	L. ZECHMEISTER and A. POLGÁR, *J. Am. Chem. Soc.* *67* (1945) 108. — L. ZECHMEISTER and co-workers, *Arch. Biochem.* *5* (1944) 365. — Cf. also: R. F. HUNTER and A. D. SCOTT, *Biochem. J.* *35* (1941) 31. — L. ZECHMEISTER and co-workers, *Plant Physiol.* *17* (1942) 91, footnote 2. — L. ZECHMEISTER, L. PAULING and co-workers, *J. Am. Chem. Soc.* *65* (1943) 1940.
Celaxanthin	A. L. LE ROSEN and L. ZECHMEISTER, *Arch. Biochem.* *1* (1942) 17.
Fucoxanthin	H. H. STRAIN and W. M. MANNING, *J. Am. Chem. Soc.* *64* (1942) 1235.
Gazaniaxanthin	L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc.* *65* (1943) 1535.
Cryptoxanthin	L. ZECHMEISTER and co-workers, *Nature* (London) *141* (1938) 249; *Biochem. J.* *32* (1938) 1305; *Ber.* *72* (1939) 1340; *J. Am. Chem. Soc.* *66* (1944) 317.
Lycopene	L. ZECHMEISTER and co-workers, *Nature* (London) *141* (1938) 249; *Ber.* *72* (1939) 1340; *Biochem. J.* *32* (1938) 1305; *J. Am. Chem. Soc.* *65* (1943) 1942; *66* (1944) 137.
Physalien	L. ZECHMEISTER and co-workers, *Ber.* *72* (1939) 1340, 1678, 2039; *J. Am. Chem. Soc.* *66* (1944) 317.
Pro-γ-carotene	L. ZECHMEISTER and co-workers, *J. Am. Chem. Soc.* *64* (1942) 1173; *65* (1943) 1940.
Pro-lycopene	L. ZECHMEISTER and co-workers, *J. Am. Chem. Soc.* *65* (1943) 1940.
Spirilloxanthin	L. ZECHMEISTER and co-workers, *Arch. Biochem.* *5* (1944) 243.
Taraxanthin	L. ZECHMEISTER and P. TUZSON, *Ber.* *72* (1939) 1340.
Xanthophyll	H. H. STRAIN, *J. Biol. Chem.* *127* (1938) 191. — L. ZECHMEISTER and co-workers, *Ber.* *72* (1939) 1340; *J. Am. Chem. Soc.* *65* (1943) 1951; *66* (1944) 137.
Zeaxanthin	L. ZECHMEISTER and co-workers, *Ber.* *72* (1939) 1340, 1678, 2039; *J. Am. Chem. Soc.* *66* (1944) 317.

REFERENCES

1. J. Herzig and F. Faltis, *Ann. 431* (1923) 40.
2. P. Karrer and co-workers, *Helv. Chim. Acta 12* (1929) 741.
3. R. Kuhn and A. Winterstein, *Ber. 66* (1933) 209.
4. A. E. Gillam and M. S. El Ridi, *Nature 136* (1935) 914; *Biochem. J. 30* (1936) 1735; *31* (1937) 251.
5. A. E. Gillam and co-workers, *Ann. 530* (1937) 291; *Nature 141* (1938) 249; L. Zechmeister and co-workers, *Biochem. J. 32* (1938) 1305.
6. J. Hengstenberg and R. Kuhn, *Z. Krist. Mineral. 75* (1930) 301; *76* (1930) 174. — G. MacKinney, *J. Am. Chem. Soc. 56* (1934) 488.
7. R. S. Mulliken, *J. Chem. Phys. 7* (1939) 364; *Rev. Modern Phys. 14* (1942) 265.
8. L. Zechmeister, L. Pauling and co-workers, *J. Am. Chem. Soc. 65* (1943) 1940; L. Zechmeister, *Chem. Revs. 34* (1944) 267.
9. P. Karrer and co-workers, *Helv. Chim. Acta 12* (1929) 741.
10. L. Zechmeister, *Chem. Revs. 34* (1944) 267.
11. L. Zechmeister, *Chem. Revs. 34* (1944) 293. — Cf. A. E. Gillam, M. S. El Ridi and S. K. Kon, *Biochem. J. 31* (1937) 1605.

CHAPTER VI

Methods of elucidating the constitution of carotenoids

The elucidation of the constitution of the carotenoids is no easy task, and although carotene and some of its congeners have been known for a very long time, it is only within the last 20 years that it has been possible to obtain some insight into the chemical structure of these compounds. The following sections are meant to provide a short summary of the principal methods which have been used for this purpose. For detailed descriptions of the experimental methods the original literature should be consulted.

1. DETERMINATION OF THE NUMBER OF DOUBLE BONDS

The most characteristic structural feature of the polyene pigments is the large number of double bonds in the molecule. In determining the constitution of a carotenoid it is important to be able to establish the number of carbon-carbon double bonds with small amounts of material (about 5 mg). Useful information can be obtained by first determining the absorption spectrum of the carotenoid. The relationships between the number of double bonds and the absorption spectrum have been fully investigated and a knowledge of one of these properties enables one to make predictions about the other. More exact information is provided by quantitative measurements of the addition of hydrogen, halogen, or iodine chloride, or of oxygen. The most accurate values are obtained from catalytic hydrogenation, but the other methods have sometimes been used to confirm the results obtained.

Catalytic hydrogenation can be carried out on the macro- or microscale. In either case, *all* the double bonds in the molecule react including the carbonyl group. Epoxide groups are also reduced during catalytic hydrogenation with the formation of hydroxyl groups[1].

Colloidal platinum[2], platinum oxide[3], palladium oxide[3], or platinum adsorbed on Kieselguhr[4] can be used as catalysts.

The following are suitable solvents: acetic acid (free from higher homologues), ethyl acetate, acetic acid-ethanol mixtures, *cyclo*hexane, hexane, decalin, etc. Carotenoids are often so sparingly soluble that they have to be

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hydrogenated in suspension; under these conditions the reaction takes considerably longer than usual. Another characteristic feature of the hydrogenation of carotenoids is that a relatively large amount of catalyst is required for complete reduction.

The *microhydrogenation* of polyene pigments described by KUHN and MOELLER[4] is extremely valuable in view of the small amounts of material required. Using this method, numerous carotenoids which occur in only very small quantities in nature have been examined for the number of double bonds present in the molecule. Details regarding the apparatus and reagents required will be found in the memoir already cited[4].

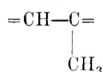
It was shown by ZECHMEISTER and TUZSON[5] that polyenes add bromine in chloroform solution. The titration method based on this reaction has the disadvantage, however, that not all double bonds participate. Thus, according to ZECHMEISTER, carotene and xanthophyll absorb only 8 molecules of bromine instead of 11. A more suitable reagent, which in most cases reacts with all the double bonds present, is iodine chloride as described by PUMMERER and REBMANN, and PUMMERER, REBMANN and REINDEL[6].

According to the last-named authors[6], the double bonds can also be saturated by the addition of oxygen. In practice, this method consists of reacting the carotenoid with perbenzoic acid in chloroform solution and back-titrating the excess perbenzoic acid after oxidation is complete. However, oxidation with perbenzoic acid again does not always result in the reaction of all double bonds present. Thus, the only completely reliable method of determining the number of double bonds in a carotenoid is catalytic hydrogenation.

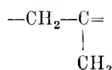
2. DETERMINATION OF SIDE-CHAIN METHYL GROUPS

The first method employed for the determination of side-chain methyl groups is due to KUHN, WINTERSTEIN and KARLOVITZ[7] and consists of oxidation by means of potassium permanganate in alkaline solution. Under these conditions a side-chain methyl group and the carbon atom to which it is attached give rise to one molecule of acetic acid. This method was later replaced by the oxidation with chromic acid, which was found to be more reliable[8].

KARRER, HELFENSTEIN, WEHRLI and WETTSTEIN[9] showed that oxidation by means of alkaline permanganate only degrades unsaturated groupings of the type



whereas more saturated groupings such as



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are only incompletely oxidised to acetic acid or not attacked. A micro-method for the determination of side-chain methyl groups has been described by KUHN and ROTH[10].

3. DETERMINATION OF *ISO*PROPYLIDENE GROUPS

For the determination of *iso*propylidene groups $(CH_3)_2C{=}C$ . . , a method introduced by KARRER, HELFENSTEIN, PIEPER and WETTSTEIN[11] and somewhat modified by KUHN and ROTH[12], is used.

The *iso*propylidene grouping is degraded by ozonisation to acetone which is determined iodometrically. The micro-determination of KUHN and ROTH depends on the same principle, but ozonisation is followed by oxidation with permanganate, to improve the yield of acetone.

4. DETERMINATION OF HYDROXYL GROUPS

By determination of active hydrogen by the method of ZEREWITINOFF, it was established by KARRER, HELFENSTEIN and WEHRLI[13] that the oxygen atoms present in xanthophyll are not present in the form of ether groupings as had previously been assumed, but as hydroxyl groups. An apparatus for the ZEREWITINOFF determination has been developed by FLASCHENTRÄGER[14] and a somewhat modified procedure has been described by ROTH[15]. The following empirical facts must be taken into account in interpreting the results of ZEREWITINOFF determinations: If several (4–6) hydroxyl groups are present in the polyene molecule, they may not all react. In the presence of two hydroxyl groups, on the other hand, somewhat high values are sometimes obtained[16]. Ketones which have a tendency to enolise also give rise to the production of methane, and thus simulate hydroxyl groups[17].

The determination of the position of the hydroxyl groups in the polyene molecule is more difficult than the determination of their number. In this connection, compare the investigations discussed on page 201. A clue to the position of the hydroxyl groups is often provided by the absence of certain oxidation products.

The question as to whether two hydroxyl groups are present in neighbouring positions can sometimes be decided by the method of CRIEGEE[18], e.g. in the case of azafrin (cf. page 282).

5. DETERMINATION OF METHOXYL GROUPS

Up to the present time, rhodoviolascin is the only known naturally occurring carotenoid containing methoxyl groups. These can be determined in the usual way by the method of ZEISEL[19].

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6. DETECTION AND ESTIMATION OF CARBONYL GROUPS

The detection of carbonyl groups in polyene pigments often presents difficulties as the usual carbonyl reagents (hydroxylamine, semicarbazide, etc.) do not always react. With some carotenoids, special methods of oximation have to be used*. Others (e.g. capsanthin) cannot be oximated at all by the methods at present available. In such cases the hydroxyl groups are first determined and the ZEREWITINOFF determination is repeated after complete reduction of the pigment. If the number of hydroxyl groups has increased, the presence of a carbonyl group which has been reduced to a secondary or primary alcohol grouping, is indicated.

If the carbonyl group is conjugated with the system of conjugated ethylenic bonds, it can be recognised by a strong red shift of the absorption maxima (cf. p. 56).

TABLE 10

NATURALLY OCCURRING CAROTENOIDS CONTAINING CARBONYL GROUPS*

Carotenoid	Number of carbonyl groups	Method of determining carbonyl groups
Aphanin	1	Formation of oxime
Astacene	4	Preparation of dioxime and bis-phenazine derivative
Astaxanthin	2	Analogy with astacene, but 2 hydroxyl groups present
Capsanthin	1	Absorption spectrum, Meerwein-Ponndorf reduction to capsanthol
Capsorubin	2	Analogy with capsanthin (not yet definitely proved)
β-Citraurin	1	Formation of oxime from aldehyde group
Myxoxanthin	1	Formation of oxime
Rhodoxanthin	2	Formation of dioxime

* Only those carotenoids the structure of which has been completely or largely determined, are mentioned in this table. Bixin, crocetin and azafrin which contain *carboxyl* groups have not been included.

7. DETERMINATION OF CARBOXYL GROUPS

Carboxyl groups are determined by titration with alkalis. The carotenoid is best hydrogenated before the titration which is then easier to carry out. Details will be found in the papers by KUHN and co-workers[20].

* The oximation of polyene pigments containing carbonyl groups is usually effected with hydroxylamine acetate. Sometimes it is necessary, however, to employ free hydroxylamine. Cf. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 828.

8. OXIDATION WITH PERMANGANATE AND OZONE

The introduction of oxidative degradation with potassium permanganate was of decisive importance for the elucidation of the structure of the carotenoids. By this means it was possible for the first time to obtain large fragments (dicarboxylic acids, etc.) which gave a clue to the constitution of these pigments. Details of these oxidations will be given in the description of individual carotenoids (cf. p. 132). The reader is also referred to the original literature[21]. The results of degradative oxidation are so reliable that it is possible to draw conclusions regarding the structure of a carotenoid from the *absence* of certain degradation products (cf. xanthophyll, p. 201).

Thus, potassium permanganate degradation of an unsubstituted β-ionone ring yields dimethylmalonic acid, $\alpha:\alpha$-dimethylsuccinic acid and $\alpha:\alpha$-dimethylglutaric acid. The same degradation products are formed from an α-ionone ring.

Degradation by means of ozone is also an important method for elucidating the constitution of carotenoids. By this means, it is possible for instance, to show that the end groups of lycopene are *iso*propylidene groups[22]. Similarly, an *iso*propylidene group can be shown to be present in γ-carotene. Furthermore, by the controlled ozonisation of β-carotene and β-ionone, PUMMERER, REBMANN and REINDEL[23] succeeded in isolating large degradation fragments identical with those obtained from permanganate oxidations.

An observation which was of considerable importance in the elucidation of the constitution of carotenoids was made by KARRER and co-workers[24]. They showed that, in addition to the degradation products obtained by means of permanganate, the ozonisation of β-carotene gives rise to geronic acid ($\alpha:\alpha$-dimethyl-δ-acetylvaleric acid), while α-carotene yields geronic as well as *iso*-geronic acid ($\gamma:\gamma$-dimethyl-δ-acetylvaleric acid)[25].

9. PARTIAL DEGRADATION OF CAROTENOIDS WITH PERMANGANATE AND CHROMIC ACID

Another important contribution to the elucidation of the constitution of carotenoids was the introduction of step-wise degradation with alkaline permanganate (KARRER and co-workers)[26] and of partial oxidation with chromic acid (KUHN and BROCKMANN[27]). Both methods allow the isolation of large degradation fragments from the structure of which it is possible to draw conclusions regarding the constitution of the parent pigments. Thus KARRER and co-workers[26] succeeded in preparing β-apo-2-carotenal, β-apo-3-carotenal and β-apo-4-carotenal by the stepwise degradation of β-carotene (cf. p. 144). KUHN and BROCKMANN obtained various ketonic products, e.g. β-carotenone and semi-β-carotenone by the mild chromic acid oxidation of β-carotene,

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depending on the quantity of oxidising agent employed. These partial oxidations are also of interest because they make it possible to interconvert different carotenoids and thus to prove the close relationships which exist between these compounds.

TABLE 11

CAROTENOIDS WHICH HAVE BEEN PARTIALLY OXIDISED*

Carotenoid	Oxidising agent	Degradation products
Azafrin	CrO_3	Azafrinone, "Azafrinal I" methyl ester
	$KMnO_4$	Apo-1-azafrinal, "Azafrinal II" methyl ester
Bixin (Stable form)	$KMnO_4$	Apo-1-norbixinal methyl ester (stable), apo-2-norbixinal methyl ester (stable), apo-3-norbixinal methyl ester (stable)
Bixin (labile form)	$KMnO_4$	Apo-1-norbixinal methyl ester (labile), apo-2-norbixinal methyl ester (labile) apo-3-norbixinal methyl ester (stable)
Capsanthin	CrO_3	Capsanthinone, capsanthylal, capsylaldehyde, 4-hydroxy-β-carotenone aldehyde
α-Carotene	CrO_3	Hydroxy-α-carotene, semi-α-carotenone, α-carotone
	$KMnO_4$	α-Apo-2-carotenal
β-Carotene	CrO_3	Hydroxy-β-carotene, semi-β-carotenone, hydroxy-semi-β-carotenone, β-carotenone, β-carotenone-aldehyde, hydroxy-β-neo-carotene
	$KMnO_4$	β-Apo-2-carotenal, β-apo-3-carotenal, β-apo-4-carotenal
Lycopene	$KMnO_4$	Apo-3-lycopenal
	CrO_3	Bixin dialdehyde, apo-2-lycopenal, apo-3:12-lycopenedial, apo-2:12-lycopenedial
Physalien	CrO_3	Physalienone
Rhodoviolascin	$KMnO_4$	Complex dialdehyde
Xanthophyll	$KMnO_4$	α-Citraurin
Zeaxanthin	$KMnO_4$	β-Citraurin

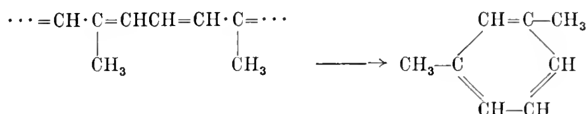
* Details concerning the various degradation products and literature references will be found in the relevant sections of the special part of this monograph.

10. THERMAL DEGRADATION

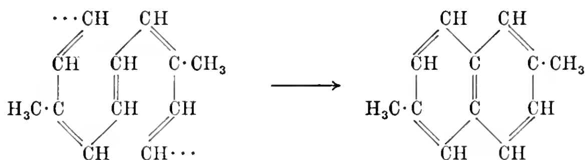
The thermal degradation of carotenoids is a method no longer employed to any extent. The yields of identifiable degradation products are only very small and, in any case, no certain conclusions can be drawn from them regarding

References p. 51–52.

the structure of the carotenoid. One advantage of the method of thermal degradation, however, is that the products formed give some indication of the relative position of the side-chain methyl groups. Thus VAN HASSELT[28] obtained *m*-xylene from bixin. According to KUHN and WINTERSTEIN[29], the xylene is derived from a part of the aliphatic chain.



A similar observation was made by ZECHMEISTER and VON CHOLNOKY[30] with capsanthin. Later, KUHN and WINTERSTEIN[31] re-investigated thermal decompositions and isolated 2:6-dimethylnaphthalene from different carotenoids. This must be derived from the central part of the polyene chain.



11. DETERMINATION OF OPTICAL ROTATION

Optical activity can be used to decide the question as to whether the carotenoid molecule has a symmetrical or unsymmetrical structure. The C-line of mercury (656.3 mμ) is often employed as light source as proposed by ZECHMEISTER and TUZSON[32]. KUHN, WINTERSTEIN and LEDERER, on the other hand, recommend a quartz cadmium lamp as a more powerful light source[33].

12. RELATIONSHIPS BETWEEN CHEMICAL CONSTITUTION AND BIOLOGICAL PROPERTIES

As was explained on p. 13, the relationship between the vitamin A potency of a carotenoid and its structure appears to be governed by the principle that high vitamin A activity depends on the presence of an unsubstituted β-ionone ring. In this way, conclusions can be drawn from the physiological activity of a polyene pigment regarding its content of β-ionone rings. It must be remembered, however, that some carotenoids (e.g. β-carotene di-epoxide), which do not contain an unsubstituted β-ionone ring, nevertheless possess vitamin A-activity because they undergo certain transformations in the animal organism (cf. p.148)[34] and that some growth-promoting properties are also exhibited by partly demethylated and acetylenic analogues of vitamin A (cf. p. 14, footnote).

References p. 51–52.

13. RELATIONSHIPS BETWEEN CHEMICAL CONSTITUTION AND COLOUR

It has been mentioned before that the colour of a carotenoid is one of its most important characteristics, and that it is often possible to make deductions regarding the structure of a polyene pigment from its absorption spectrum. A great deal of material is available in this field and many useful relationships have been established[35].

The shortest wavelength selective absorption in the visible region so far observed with a natural carotenoid is shown by auroxanthin (maxima at 454 and 423 mμ in carbon disulphide solution, cf. p. 196). The longest wavelength selective absorption in the visible region is shown by torularhodin (maxima at 582, 541, and 502 mμ in carbon disulphide solution*, cf. p. 330).

The light absorption properties are determined by the following factors:

a) Number and type of double bonds,
b) Number and type of carbonyl groups,
c) Number of epoxide groups,
d) Number and position of carboxyl groups,
e) Number of hydroxyl groups,
f) Steric configuration of the carotenoid.

In recent years the detailed relationships between the constitution and colour of the carotenoids have been investigated particularly by KUHN and co-workers[36], by HAUSSER and SMAKULA[37] and by KARRER and co-workers[38]. These investigations are discussed in more detail in the following chapter.

14. COMPARISON WITH PARTIALLY SYNTHETIC POLYENE PIGMENTS

Recently, comparison with partially synthetic carotenoids has often been used successfully for elucidating the structure of carotenoids of unknown constitution. An example is provided by the comparison of β-citraurin (p. 219) with β-apo-2-carotenal (p. 144) which, according to KARRER and SOLMSSEN[39] differ only by the presence of an extra hydroxyl group in β-citraurin. This comparison lead to the complete elucidation of the constitution of β-citraurin.

KARRER and JUCKER have recently succeeded in elucidating the structure of numerous carotenoids by comparison with partially synthetic pigments. Thus the following pairs of natural and partially synthetic pigments were proved to be identical: flavoxanthin and xanthophyll epoxide, antheraxanthin and zeaxanthin mono-epoxide, violaxanthin and zeaxanthin di-epoxide, citroxanthin and mutatochrome, etc.

* In this connection the partially synthetic dehydrolycopene is of interest. It exhibits a long wave absorption maximum in CS_2 at 601 mμ (p. 122).

15. DETERMINATION OF THE MOLECULAR WEIGHT

The molecular weight of carotenoids can be determined by RAST's method[10] as well as by other cryoscopic and ebullioscopic methods. X-ray analysis has also been employed[11].

REFERENCES

1. P. KARRER and E. JUCKER, *Helv. Chim. Acta 28* (1945) 300.
2. R. WILLSTÄTTER and E. WALDSCHMIDT-LEITZ, *Ber. 54* (1921) 113.
3. R. ADAMS and R. L. SHRINER, *J. Am. Chem. Soc. 45* (1923) 2171. — R. L. SHRINER and R. ADAMS, *J. Am. Chem. Soc. 46* (1924) 1683. — M. FRÄNKEL, *Abderhaldens Handbuch der biologischen Arbeitsmethoden*, part 12. — Cf. also H. GILMAN and A. H. BLATT, *Organic Syntheses, Coll. Vol.* I, 2nd ed., 1941, p. 463.
4. R. KUHN and E. F. MÖLLER, *Angew. Chem. 47* (1934) 145.
5. L. ZECHMEISTER and P. TUZSON, *Ber. 62* (1929) 2226.
6. R. PUMMERER and L. REBMANN, *Ber. 61* (1928) 1099; R. PUMMERER, L. REBMANN and W. REINDEL, *Ber. 62* (1929) 1411.
7. R. KUHN, A. WINTERSTEIN and L. KARLOVITZ, *Helv. Chim. Acta 12* (1929) 64.
8. R. KUHN and L. EHMANN, *Helv. Chim. Acta 12* (1929) 907; P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. Chim. Acta 13* (1930) 1084. — R. KUHN and F. L'ORSA, *Ber. 64* (1931) 1732; *Angew. Chem. 44* (1931) 847.
9. P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. Chim. Acta 13* (1930) 1084.
10. R. KUHN and H. ROTH, *Ber. 66* (1933) 1274.
11. P. KARRER, A. HELFENSTEIN, B. PIEPER and A. WETTSTEIN, *Helv. Chim. Acta 14* (1931) 435.
12. R. KUHN and H. ROTH, *Ber. 65* (1932) 1285.
13. P. KARRER, A. HELFENSTEIN and H. WEHRLI, *Helv. Chim. Acta 13* (1930) 87; *13* (1930) 268.
14. B. FLASCHENTRÄGER, *Z. Physiol. Chem. 146* (1925) 219.
15. H. ROTH, *Mikrochem. 11* (1932) 140. — Cf. also PREGL–ROTH, *Quantitative organische Mikro-analyse*, 5th edition, Springer-Verlag, Vienna, 1947.
16. P. KARRER and E. JUCKER, *Helv. Chim. Acta 28* (1945) 302.
17. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 828.
18. R. CRIEGEE, *Ber. 64* (1931) 260.
19. PREGL-ROTH, *Quantitative organische Mikroanalyse*, 5th edition, Springer-Verlag, Vienna, 1947.
20. R. KUHN and co-workers, *Helv. Chim. Acta 11* (1928) 716; *Ber. 64* (1931) 333.
21. P. KARRER and A. HELFENSTEIN, *Helv. Chim. Acta 12* (1929) 1142. — P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. Chim. Acta 13* (1930) 1084. — Cf. also R. KUHN and A. DEUTSCH, *Ber. 66* (1933) 883. — P. KARRER, *Helv. 12* (1929) 558.
22. P. KARRER and W. E. BACHMANN, *Helv. Chim. Acta 12* (1929) 285. — P. KARRER, A. HELFENSTEIN, B. PIEPER and A. WETTSTEIN, *Helv. Chim. Acta 14* (1931) 435.
23. R. PUMMERER, L. REBMANN and W. REINDEL, *Ber. 64* (1931) 492.
24. P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. Chim. Acta 13* (1930) 1084.
25. P. KARRER, R. MORF and O. WALKER, *Helv. Chim. Acta 16* (1933) 975.
26. P. KARRER and co-workers, *Helv. Chim. Acta 20* (1937) 682; *20* (1937) 1020, 1312; *21* (1938) 1171.
27. R. KUHN and H. BROCKMANN, cf. p. 139.

28. J. F. B. van Hasselt, *Rec. trav. Chim. Pays-Bas et Belg.* *30* (1911) 1; *33* (1914) 192. Cf. J. Herzig and F. Faltis, *Monatsh.* *35* (1914) 997.
29. R. Kuhn and A. Winterstein, *Helv. Chim. Acta* *11* (1928) 427.
30. L. Zechmeister and L. v. Cholnoky, *Ann.* *478* (1930) 95.
31. R. Kuhn and A. Winterstein, *Ber.* *65* (1932) 1873; *66* (1933) 429; *66* (1933) 1733.
32. L. Zechmeister and P. Tuzson, *Ber.* *62* (1929) 2226.
33. R. Kuhn, A. Winterstein and E. Lederer, *Z. Physiol. Chem.* *197* (1931) 141. — Cf. also P. Karrer and O. Walker, *Helv. Chim. Acta* *16* (1933) 641.
34. Cf. P. Karrer and J. Rutschmann, *Helv. Chim. Acta* *29* (1946) 355.
35. Cf. P. Karrer, *Die bisher bekannten natürlichen Carotinoide und ihre Absorptionsspektren*, Vierteljschr. Naturf. Ges., XV, Zürich 1945.
36. R. Kuhn and H. Brockmann, *Ber.* *65* (1932) 894; *66* (1933) 407; *66* (1933) 828; *66* (1933) 1319. — R. Kuhn and Ch. Grundmann, *Ber.* *65* (1932) 898; *65* (1932) 1880. — R. Kuhn and A. Deutsch, *Ber.* *66* (1933) 883.
37. K. W. Hausser and A. Smakula, *Angew. Chem.* *47* (1934) 663; *48* (1935) 152.
38. P. Karrer and E. Würgler, *Helv. Chim. Acta* *23* (1940) 955; *26* (1943) 116. — E. Würgler, *Dissertation*, Zürich 1943.
39. P. Karrer and U. Solmssen, *Helv. Chim. Acta* *20* (1937) 682.
40. Cf. Pregl–Roth, *Die quantitative Mikroanalyse*, Vienna, 1947.
41. Cf. e.g. G. Mackinney, *J. Am. Chem. Soc.* *56* (1934) 488. — H. Waldmann and E. Brandenberger, *Z. Krist.* *82* (1932) 77.

CHAPTER VII

Relationships between the colour and constitution of carotenoids

It has already been stressed that the yellow to violet colour of carotenoids is one of their most important characteristics. It is thus not surprising that repeated attempts have been made to elucidate the relationships between the colour and constitution of carotenoids and to employ absorption spectra for the characterisation and identification of polyene pigments. Many advances in this field have been made during the last 20 years and it is possible to-day to draw definite conclusions regarding the constitution of a carotenoid from its absorption spectrum. Conversely, certain changes in the spectrum can be predicted from a given change in structure[1].

Although carotenoids possess a relatively complex structure, the absorption spectra of these pigments are comparatively simple in character. In the visible region the spectrum usually consists of three, or occasionally four, absorption maxima and the position of the maxima is related in a relatively simple manner to the constitution of the pigments. In the ultra-violet region, the relationships between constitution and spectral properties are more complicated and it is

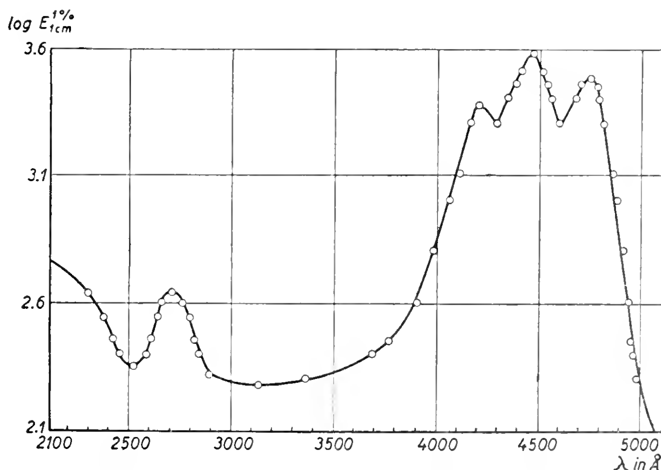


Fig. 3. Light absorption of xanthophyll in hexane solution

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not yet possible at the present time to predict the ultra-violet absorption of a carotenoid from its structure. As an example of a typical carotenoid spectrum the absorption curve of xanthophyll is shown above.

Most natural carotenoids, and also their degradation products such as β-apo-2-carotenal, β-apo-2-carotenol, β-citraurin, β-carotenone, etc., exhibit absorption curves similar to the one shown here.

Investigations by different workers have shown that the following empirical relationships exist between the constitution and absorption spectrum of polyenes:

1. The addition of a conjugated double bond without other changes in structure results in a displacement of the visible absorption bands towards longer wavelengths by 20–22 mμ (in carbon disulphide solution). The removal of a conjugated double bond has the opposite effect.

Example:

Crocetin, 7 conjugated ethylenic bonds, longest wavelength maximum in CS_2 at 482 mμ.

Bixin, 9 conjugated ethylenic bonds, longest wavelength maximum in CS_2 at 523.5 mμ.

2. If an ethylenic bond in a six-membered ring is moved from a conjugated to an isolated position the maxima are displaced by 9–11 mμ towards shorter wavelengths.

Examples:

β-Carotene, 11 conjugated ethylenic bonds, longest wavelength maximum in CS_2 at 520 mμ.

α-Carotene, 10 conjugated and 1 isolated ethylenic bond, longest wavelength maximum in CS_2 at 509 mμ.

Zeaxanthin, 11 conjugated ethylenic bonds, longest wavelength maximum in CS_2 at 517 mμ.

Xanthophyll, 10 conjugated and 1 isolated ethylenic bond, longest wavelength maximum in CS_2 at 508 mμ.

3. If a terminal conjugated double bond is replaced by an epoxide group. the maxima are displaced by 6–9 mμ towards the blue end of the spectrum,

Examples:

α-Carotene, 10 conjugated and 1 isolated ethylenic bond, longest wavelength maximum in CS_2 at 509 mμ.

α-Carotene epoxide, 9 conjugated and 1 isolated ethylenic bond and 1 epoxide group, longest wavelength maximum in CS_2 at 503 mμ.

Xanthophyll, 10 conjugated and 1 isolated ethylenic bond, longest wavelength maximum in CS_2 at 508 mμ.

Xanthophyll epoxide, 9 conjugated and 1 isolated double bond and 1 epoxide group, longest wavelength maximum in CS_2 at 501.5 mμ.

β-Carotene, 11 conjugated ethylenic bonds, longest wavelength maximum in CS_2 at 520 mμ.

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β-Carotene mono-epoxide, 10 conjugated double bonds and 1 epoxide group, longest wavelength maximum in CS_2 at 511 mμ.

Conversion of a mono-epoxide to a di-epoxide results in a further displacement of the absorption maxima by 6–9 mμ towards shorter wavelengths.

4. Conversion of a carotenoid mono-epoxide to the isomeric furanoid oxide results in a displacement of the absorption maxima towards the blue end of the spectrum. For the longest wavelength bands this displacement amounts to 19–22 mμ.

Examples:

β-Carotene mono-epoxide . . .	longest wavelength maximum in CS_2 at 511 mμ.
Mutatochrome	longest wavelength maximum in CS_2 at 489 mμ.
α-Carotene mono-epoxide . . .	longest wavelength maximum in CS_2 at 503 mμ.
Flavochrome	longest wavelength maximum in CS_2 at 482 mμ.
Capsanthin mono-epoxide . . .	longest wavelength maximum in CS_2 at 534 mμ.
Capsochrome	longest wavelength maximum in CS_2 at 515 mμ.

Conversion of a di-epoxide into the di-furanoid isomer results in a hypsochromic displacement approximately twice as great, i.e. ca 40 mμ.

5. Some carotenoids have a completely (e.g. lycopene) or partly (e.g. γ-carotene) open-chain structure. If such an open chain undergoes *one* ring-closure, the absorption maxima are displaced by 4–5 mμ towards the blue end of the spectrum. If both ends of the chain undergo ring closure, the longest wavelength maximum is displaced by ca. 10 mμ.

Examples:

γ-Carotene contains 11 conjugated and 1 isolated double bond. Taking β-carotene (11 conjugated double bonds), longest wavelength maximum in CS_2 at 520 mμ, as a basis, the position of the longest wavelength absorption maximum of γ-carotene is calculated to be 529–530 mμ. The observed longest wavelength maximum of γ-carotene is at 533.5 mμ.

Lycopene, contains 11 conjugated and 2 isolated double bonds. Taking β-carotene as a basis, the position of the longest wavelength maximum is calculated to be at 538–540 mμ. The observed longest wavelength maximum of lycopene lies at 548 mμ.

6. Introduction of a hydroxyl group only results in a small hypsochromic displacement (1–2 mμ).

Examples:

β-Carotene	longest wavelength maximum in CS_2 at 520 mμ.
α-Carotene	longest wavelength maximum in CS_2 at 509 mμ.
Lycopene	longest wavelength maximum in CS_2 at 548 mμ.
Zeaxanthin (Dihydroxy-β-carotene)	longest wavelength maximum in CS_2 at 517 mμ.
Xanthophyll (Dihydroxy-α-carotene)	longest wavelength maximum in CS_2 at 508 mμ.
Lycophyll (Dihydroxy-lycopene)	longest wavelength maximum in CS_2 at 546 mμ.

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7. Carbonyl groups (ketone, aldehyde or carboxyl groups) conjugated with the system of conjugated double bonds have a pronounced effect on the absorption spectrum, and produce bathochromic displacements, the magnitude of which varies from case to case. If the introduction of the carbonyl group, involves the opening of a ring, the effects of the two changes are, of course superimposed. Introduction of a second conjugated carbonyl group has a smaller effect on the position of the absorption maxima than the introduction of the first carbonyl group.

Examples:

β-Apo-2-carotenal. The system of 9 conjugated ethylenic bonds should exhibit an absorption maximum at about 480–485 mμ. The observed longest wavelength absorption maximum lies at 525 mμ and the difference of 40–45 mμ is to be ascribed to the carbonyl group.

Capsanthin. The system of 10 conjugated ethylenic bonds should exhibit an absorption maximum at 500–505 mμ. The longest wavelength absorption maximum actually observed lies at 542 mμ and the difference is to be ascribed to the carbonyl group.

8. *Cis-trans* configuration also has a definite, though small, influence on the position of the absorption maxima. Thus, the longest wavelength maximum of a compound containing one *cis*-ethylenic bond, is displaced by ca. 3–4 mμ towards shorter wavelengths as compared with the isomer with complete *trans*-configuration.

Examples:

Stable (*trans*-) bixin . . . . . longest wavelength maximum in CS_2 at 526.5 mμ.
Labile (*cis*-) bixin . . . . . . longest wavelength maximum in CS_2 at 523.5 mμ.
Stable (*trans*-) crocetin . . . . longest wavelength maximum in CS_2 at 463 mμ.
Labile (*cis*-) crocetin . . . . . longest wavelength maximum in CS_2 at 458 mμ.

After dealing with the effect of different atomic groups on the position of the absorption maxima, the influence of the solvent must be briefly discussed. Experiments have shown that the wavelength locations and fine structure of the absorption maxima are considerably dependent on the solvent. The solvent also has some influence on the absorption coefficients. The interaction between polar solvent such as alcohol and carotenoids with carboxyl groups, (e.g. capsanthin) is further described below.

The table below shows that the absorption maxima of most carotenoids are displaced by about 30–40 mμ towards shorter wavelengths in hexane and alcohol as compared with carbon disulphide. In chloroform, the hypsochromic displacement amounts to about 24 mμ. The differences in the positions of the maxima in hexane and carbon disulphide increase with the wavelengths at which the carotenoids absorb.

In carotenoids containing carbonyl groups (e.g. capsanthin, β-apo-2-

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TABLE 12

DISPLACEMENT OF THE POSITION OF MAXIMA BY DIFFERENT SOLVENTS*

Pigment	Maxima in carbon disulphide	Hexane	Ethanol	Chloroform
α-Carotene	509 mμ	478 mμ		485 mμ
Xanthophyll	508 mμ	476 mμ	479 mμ	487 mμ
β-Carotene	520 mμ	482 mμ		497 mμ
Cryptoxanthin	519 mμ	484 mμ	486 mμ	497 mμ
Zeaxanthin	517 mμ	482.5 mμ	483 mμ	495 mμ
γ-Carotene	533.5 mμ	494 mμ		508.5 mμ
Rubixanthin	533 mμ	494 mμ	496 mμ	509 mμ
Lycopene	548 mμ	506 mμ		517 mμ

* Only the longest wavelength maxima are given. Some of the data in the third column were obtained using petroleum ether instead of hexane as a solvent.

carotenal, etc.), relationships are more complicated, probably owing to the interaction which can occur between the pigment and certain solvents, e.g. alcohol. Thus, the spectrum of capsanthin in alcohol is completely blurred. The same behaviour is exhibited by β-apo-2-carotenal and other polyene ketones containing carbonyl groups conjugated with the system of ethylenic bonds. If the conjugation between the ethylenic bonds and the carbonyl group is broken, however, the absorption maxima are as sharp as usual[1].

The empirical relationships here described between the constitution of carotenoids and their light absorption properties have general validity. The exceptions which are occasionally encountered may often be ascribed to a lack of stability of the pigments involved and do not detract from the great value of spectroscopy in carotenoid research.

The theoretical interpretation of the visible and ultraviolet absorption spectra of natural and synthetic polyenes has also attracted considerable attention within recent years. The absorption of light in this region of the spectrum is thought to give rise to electronic oscillations along the axis of the polyene chain, and it can be predicted, on this basis, that the wavelengths and intensities of the maxima will increase with the number of conjugated ethylenic bonds[2].

TABLE 13

ABSORPTION SPECTRA OF NATURAL CAROTENOIDS

	Formula	Absorption maxima in CS_2			Number of conjugated ethylenic bonds	Number of hydroxyl groups	Number of carbonyl groups
		1st band	2nd band	3rd band			
(Violerythrin)*		625	576	540	?	?	?
Torularhodin	$C_{37}H_{48}O_2$	582	541	502	12	0	1
(Actinioerythrin)*		574	533	495	?	?	?
Rhodoviolascin	$C_{42}H_{60}O_2$	573.5	534	496	13	0	0
Bacterioruberin		571	532	498	?	?	?
Oscillaxanthin		568	528	494	?	?	?
Torulin		565	525	491	?	?	?
Rhodoxanthin	$C_{40}H_{50}O_2$	564	525	491	12	0	2
Celaxanthin	$C_{40}H_{56}O(-H_2?)$	562	521	487	13?	?	?
Rhodovibrin		556	517		?	?	?
Rhodopurpurin		550	511	479	?	?	?
Astacene	$C_{40}H_{48}O_4$	ca. 550–450, Maximum		510	11	0	4
Astaxanthin	$C_{40}H_{52}O_4$	?	?	?	11	2	2
Lycopene	$C_{40}H_{56}$	548	507.5	477	13	0	0
Rhodopin	$C_{40}H_{58}O(-H_2?)$	547	508	478	12	1	?
Lycoxanthin	$C_{40}H_{56}O$	547	507	473	13	1	0
Aphanizophyll**		547	506	474	?	?	?
Lycophyll	$C_{40}H_{56}O_2$	546	506	472	13	2	0
Myxoxanthophyll**	$C_{40}H_{56}O_7$	544	508	479	10	6	1
Capsanthin	$C_{40}H_{58}O_3$	542	503		10	2	1
Capsorubin	$C_{40}H_{60}O_4$	541	503	468	9	2	2
Eschscholtzxanthin	$C_{40}H_{54}O_2(\pm H_2)$	536	502	475	12	2	0
γ-Carotene	$C_{40}H_{56}$	533.5	496	463	12	0	0
Rubixanthin	$C_{40}H_{56}O$	533	494	461	12	1	0
Aphanin***	$C_{40}H_{54}O$	533	494		11	0	1
Aphanicin***		533	494		?	?	?
Gazaniaxanthin	$C_{40}H_{56}O(\pm H_2)$	531	494.5	461	11?	1	0
β-Citraurin	$C_{30}H_{40}O_2$	525	490	457	9	1	1
Bixin (labile)	$C_{25}H_{30}O_4$	523.5	489	457	9	0	2
β-Carotene	$C_{40}H_{56}$	520	485	450	11	0	0
Echinenone	$C_{40}H_{56}O(\pm H_2)$	(520)	488	(450)	?	0?	1?
Cryptoxanthin	$C_{40}H_{56}O$	519	483	452	11	1	0
Pectenoxanthin	$C_{40}H_{54}O_3(\pm H_2)$	518	486	452	11	2	?
Zeaxanthin	$C_{40}H_{56}O_2$	517	482	450	11	2	0
Cynthiaxanthin		517	483	451	?	?	?
Leprotin	$C_{40}H_{54}$	517	479	447	12	0	0

* It is not certain whether this compound belongs to the carotenoid series.

** The data given refer to the esterified pigment.

*** The pigments exhibit wide maxima (cf. p. 302 and 305).

TABLE 13 (CONTINUED)

	Formula	Absorption maxima in CS_2			Number of conjugated ethylenic bonds	Number of hydroxyl groups	Number of carbonyl groups
		1st band	2nd band	3rd band			
Sulcatoxanthin	$C_{40}H_{52}O_8$	516	482	450	?	?	?
Petaloxanthin	$C_{40}H_{56}O_3(+H_2?)$	514.5	481		?	2?	?
Haematoxanthin		One band, max. 513			?	?	?
Fucoxanthin	$C_{40}H_{56}O_6$	510	477	445	10?	?	?
Antheraxanthin	$C_{40}H_{56}O_3$	510	478	445	10	2	0
α-Carotene	$C_{40}H_{56}$	509	477		11	0	0
Xanthophyll	$C_{40}H_{56}O_2$	508	475	445	11	2	0
Pentaxanthin	$C_{40}H_{56}O_5(\pm H_2)$	506	474	444	?	3?	?
Rubichrome	$C_{40}H_{56}O_2$	506	476		11	1	0
α-Carotene epoxide	$C_{40}H_{56}O$	503	471		10	0	0
Flavorhodin		502	472		?	?	?
Xanthophyll epoxide	$C_{40}H_{56}O_3$	501.5	472		10	2	0
Taraxanthin	$C_{40}H_{56}O_4$	501	469	440	?	?	?
Violaxanthin	$C_{40}H_{56}O_4$	500.5	469	440	9	2	0
Trollixanthin	$C_{40}H_{56}O_4(?)$	501	473		?	3?	?
Prolycopene	$C_{40}H_{56}$	500.5	469.5		11	0	0
Mytiloxanthin		One band, max. 500			?	?	?
Sarcinaxanthin		499	466.5	436	?	?	?
Sarcinin *		?	?	?	?	?	?
Glycymerin		One band, max. 495			?	?	?
Pro-γ-carotene	$C_{40}H_{56}$	493.5	460.5		12	0	0
Flavacin		490	457	424	?	?	?
Mutatochrome (Citroxanthin)	$C_{40}H_{56}O$	489.5	459		10	0	0
Myxoxanthin	$C_{40}H_{54}O$	One band, max. 488			12	0	1
Azafrin	$C_{27}H_{38}O_4$	486	457		7	2	1
Crocetin (stable)	$C_{20}H_{24}O_4$	482	453		7	0	2
Chrysanthemaxanthin	$C_{40}H_{56}O_3$	480	451		10	2	0
Flavoxanthin	$C_{40}H_{56}O_3$	478	447.5	420	10	2	0
Auroxanthin	$C_{40}H_{56}O_4$	454	423		9	2	0

* In petroleum ether, the absorption maxima are located at 469 and 440 mμ.

REFERENCES

1. K. W. Hausser and A. Smakula, *Angew. Chem.* *47* (1934) 657. — R. A. Morton, *The Application of Absorption Spectra to the Study of Vitamins, Hormones and Co-enzymes*, London, 1942. — L. Zechmeister, *Chem. Reviews* *34* (1944) 267. — P. Karrer, *Die bisher bekannten natürlichen Carotinoide und ihre Absorptionsspektren*, Vjschr. Naturf. Ges., Zürich, 1945. — E. A. Braude, *Ann. Reports Chem. Soc. London* *42* (1946) 105.
2. G. N. Lewis and M. Calvin, *Chem. Reviews* *25* (1939) 73. — L. Pauling, *Proc. Nat. Acad. Sci.* *25* (1939) 577. — R. S. Mulliken, *J. Chem. Physics* *7* (1939) 121. — N. S. Bayliss, *ibid.* *16* (1948) 287. — W. Kuhn, *Helv. Chim. Acta* *31* (1948) 1780. — E. A. Braude, *Nature* *155* (1945) 753; *J. Chem. Soc.* 1950, 379.

CHAPTER VIII

The synthesis of carotenoids

In spite of many attempts, no total synthesis of a natural carotenoid has been achieved up to the present time. KUHN and his co-workers[1] have synthesized many carotenoid-like diphenylpolyenes and polyene dicarboxylic acids and thus provided valuable material for the comparison of structure and colour in polyenes. KARRER and his co-workers have synthesized the perhydro-derivatives of three natural carotenoids and thus established the constitution of the natural pigments. The compounds involved are perhydro-lycopene[2], perhydro-norbixin[3] and perhydro-crocetin[4].

The first conversion of one natural carotenoid into another was achieved by KARRER and SOLMSSEN[5] who reduced dihydrorhodoxanthin to zeaxanthin by means of aluminium *iso*propoxide and *iso*propyl alcohol (p. 182). Partial syntheses of natural carotenoids are also represented by the oxidative degradation of zeaxanthin and xanthophyll to β-citraurin* and by the conversion of lycopene into norbixin[6]. By the action of N-bromsuccinimide on lycopene, KARRER and RUTSCHMANN obtained dehydrolycopene, a carotenoid pigment with 15 conjugated double bonds (cf. p. 121).

Recently, KARRER and JUCKER[7] have succeeded in converting natural carotenoids containing isolated double bonds into other naturally occurring pigments. Thus, α-carotene can be converted into β-carotene by the action of sodium ethoxide at elevated temperatures, and similarly, xanthophyll can be converted into zeaxanthin. These conversions are of interest in showing that the isolated double bonds can be brought into conjugation.

Thus, until recently, only very few natural carotenoids had been partially synthesized. Within the last few years, however, KARRER and JUCKER[8] succeeded in preparing about 20 carotenoids by the introduction of oxygen into different carotenoid pigments by means of monoperphthalic acid. Some of these carotenoids were known to occur in nature, but their constitution had previously been unknown.

The addition of oxygen by means of perbenzoic acid was employed by

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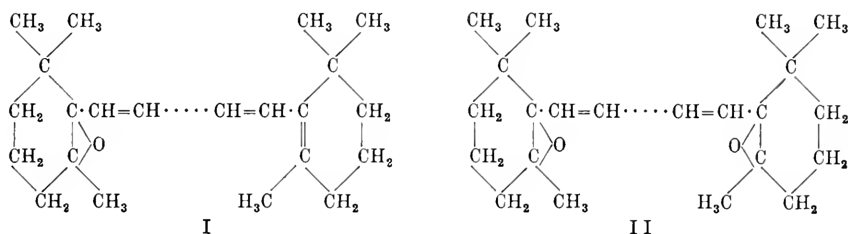
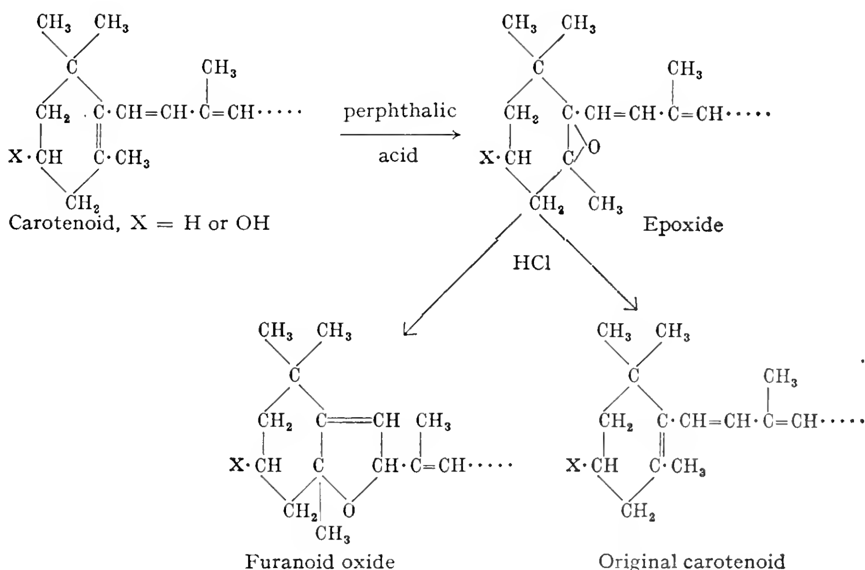
* Cf. p. 184. Later L. ZECHMEISTER and L. v. CHOLNOKY obtained β-citraurin by the hydrolytic fission of capsanthin, cf. p. 248.

References p. 64–65.

PUMMERER and co-workers[9] for the determination of the number of double bonds in polyene molecules (cf. p. 44). By the action of perbenzoic acid on β-carotene, KARRER and WALKER[10] prepared β-carotene oxide which has been shown by KARRER and JUCKER[11] to be identical with mutatochrome. Numerous investigations[12] by the two last-named authors have also shown that by using small quantities of monoperphthalic acid it is possible to oxidise individual double bonds in a carotenoid molecule. Well-defined crystalline compounds are obtained, which from their method of formation and their properties are regarded as 1:2-epoxides. Their properties show that only the double bonds in



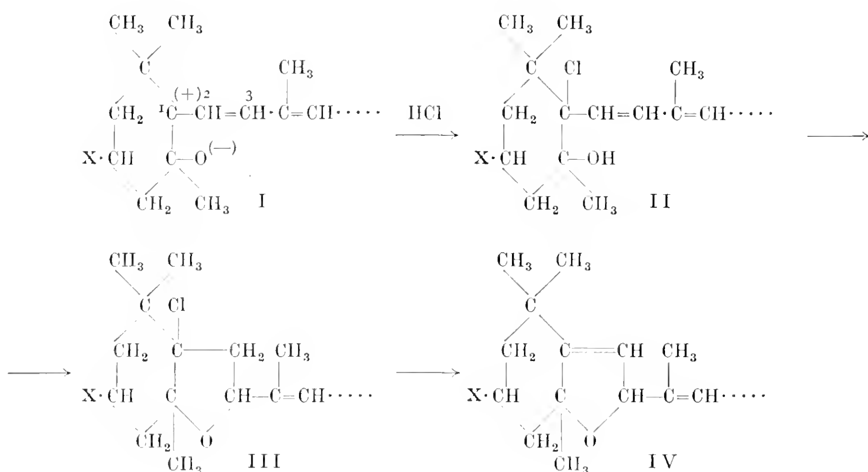
the β-ionone rings have been oxidised, and mono-epoxides or di-epoxides are obtained, depending on the number of β-ionone rings in the carotenoid molecule. No example of the oxidation of the isolated double bond in an α-ionone ring has so far been encountered. Thus, a mono-epoxide I and a di-epoxide II have been obtained from β-carotene, whereas α-carotene only yields a mono-epoxide.



References p. 64–65.

The most characteristic property of the epoxides is their extreme sensitivity to dilute mineral acids. Even traces of hydrogen chloride such as are present in chloroform after standing, are sufficient to rupture the epoxide ring[13]. The isomeric furanoid oxide is obtained, together with the original carotenoid which is formed as by-product by the loss of oxygen*.

The fact that the oxygen atom of these epoxides is readily lost, suggests that it is bound in an unusual way not well represented by the epoxide formulation. KARRER[13] has suggested a polar structure which explains the facile conversion into the furanoid oxide as well as the ready loss of oxygen.



According to this formulation, hydrogen chloride adds to the polar oxide I with the formation of II. This is transformed into III, converted in turn to the furanoid oxide IV by the loss of hydrogen chloride.

The conversion of the polar oxide I into the furanoid oxide IV can also be interpreted in the following way: an electron pair shared by carbon atoms 2 and 3 (formula I) is displaced towards carbon atom 1 under the influence of the positive charge, and simultaneously the oxygen atom and its electron pair adds to carbon atom 3.

The investigations of KARRER and JUCKER[14] showed that some natural carotenoids, the constitution of which had not previously been established, were the furanoid oxides of known carotenoids. Thus flavoxanthin proved to be identical with furanoid xanthophyll oxide, and auroxanthin with furanoid zeaxanthin dioxide.

The action of alkyl magnesium salts on carotenoid epoxides gives rise to the same products as the reaction with hydrogen chloride, namely the furanoid

* This applies to all partially synthetic epoxides so far prepared, cf. table 14.

References p. 64–65.

oxide, together with the parent carotenoid formed as a by-product by the loss of oxygen[15].

TABLE 14

PARTIALLY SYNTHETIC CAROTENOID EPOXIDES AND THEIR PROPERTIES

Epoxide	Absorption maximum in CS_2	M.P.	Colour reaction with concentrated HCl
α-Carotene mono-epoxide	503 471 mμ	175°	very faint, unstable
Xanthophyll mono-epoxide	501.5 472 mμ	192°	blue, fairly stable
β-Carotene mono-epoxide	511 479 mμ	160°	faint blue, unstable
Cryptoxanthin mono-epoxide	512 479 mμ	154°	blue, unstable
Zeaxanthin mono-epoxide*	510 478 mμ	205°	blue, unstable
Rubixanthin mono-epoxide	526 491 mμ	171°	blue, stable
Capsanthin mono-epoxide	534 499 mμ	189°	blue, unstable
β-Carotene di-epoxide	502 470 mμ	184°	deep blue, stable
Cryptoxanthin di-epoxide	503 473 mμ	194°	deep blue, stable
Zeaxanthin di-epoxide**	500 469 mμ	200°	deep blue, stable

* Identical with Antheraxanthin.
** Identical with Violaxanthin.

With regard to the elucidation of the constitution of the epoxides and the furanoid oxides, references should be made to the original literature[16].

TABLE 15

PARTIALLY SYNTHETIC FURANOID CAROTENOID OXIDES AND THEIR PROPERTIES

Oxide	Absorption maximum in CS_2	M.P.	Colour reaction with concentrated HCl
Flavochrome	482 451 mμ	189°	very faint, unstable
Flavoxanthin	479 449 mμ	180°	blue, fairly stable
Chrysanthemaxanthin	479 449 mμ	185°	blue, fairly stable
Mutatochrome	489 459 mμ	164°	faint blue, unstable
Cryptoflavin	490 459 mμ	171°	blue, unstable
Mutatoxanthin	488 459 mμ	177°	blue, unstable
Rubichrome	506 476 mμ	154°	blue, stable
Capsochrome	515 482 mμ	195°	blue, unstable
Aurochrome	457 426 mμ	185°	deep blue, stable
Cryptochrome	456 424 mμ	?	deep blue, stable
Auroxanthin	454 423 mμ	203°	deep blue, stable
Luteochrome	482 451 mμ	176°	deep blue, stable

References p. 64–65.

In the investigation of carotenoid epoxides and their isomeric furanoid oxides, the spectral properties of these compounds are of much importance. The following are some empirically recognised regularities:

Conversion of a carotenoid pigment into a mono-epoxide results in a displacement in the absorption bands towards the violet. The displacement of the longest wavelength band amounts, on the average, to 8 mμ in carbon disulphide. The formation of a di-epoxide results in a displacement of the bands by about 17 mμ. A rather larger displacement (about 21 mμ) of the absorption bands towards shorter wavelength accompanies the change of a mono-epoxide into the isomeric furanoid oxide. For a di-epoxide, the difference is about twice as great. These regularities allow a fairly certain prediction of the absorption spectra of carotenoid epoxides and their furanoid isomers.

Of the carotenoid epoxides and furanoid oxides so far prepared, the following have up to now been found in nature:

α-Carotene mono-epoxide
Flavochrome
(β-Carotene mono-epoxide)*
Citroxanthin = Mutatochrome
Zeaxanthin mono-epoxide = Antheraxanthin
Zeaxanthin di-epoxide = Violaxanthin
Xanthophyll mono-epoxide
Flavoxanthin
Chrysanthemaxanthin
Auroxanthin
Rubichrome

Trollixanthin[17], a pigment recently isolated from the blossoms of *trollius europaeus*, also possesses an epoxide structure.

The wide distribution of carotenoid epoxides in plants raises the question as to their physiological significance. No definite answer can be given, but the ready loss of oxygen suggests the possibility that these epoxides play a part in biological oxidation processes in the plant. Further investigations are required to elucidate this problem.

REFERENCES

1. Cf. R. Kuhn and co-workers, *Angew. Chem. 50* (1937) 703; *Ber. 69* (1936) 1757, 1979; *71* (1938) 1889.
2. P. Karrer, A. Helfenstein and R. Widmer, *Helv. Chim. Acta 11* (1928) 1201.
3. P. Karrer and co-workers, *Helv. Chim. Acta 15* (1932) 1218, 1399.

* β-Carotene mono-epoxide has not yet been found in nature, but as mutatochrome (= citroxanthin) is a natural pigment and almost certainly formed from β-carotene mono-epoxide, it is very probable that the latter also occurs in plants.

4. P. KARRER, F. BENZ and M. STOLL, *Helv. Chim. Acta 16* (1933) 297.
5. P. KARRER and U. SOLMSSEN, *Helv. Chim. Acta 18* (1935) 477.
6. R. KUHN and C. GRUNDMANN, *Ber. 65* (1932) 898, 1880.
7. P. KARRER and E. JUCKER, *Helv. Chim. Acta 30* (1947) 266.
8. P. KARRER and E. JUCKER and co-workers, *Helv. Chim. Acta 28* (1945) 300, 427, 471, 474, 717, 1143, 1146, 1156; *30* (1947) 531.
9. R. PUMMERER and co-workers, *Ber. 61* (1928) 1099; *62* (1929) 1411.
10. H. V. EULER, P. KARRER and O. WALKER, *Helv. Chim. Acta 15* (1932) 1507.
11. P. KARRER and E. JUCKER, *Helv. Chim. Acta 28* (1945) 427.
12. P. KARRER and E. JUCKER and co-workers, *Helv. Chim. Acta 28* (1945) 300, 427, 471, 474, 717, 1143, 1146, 1156; *30* (1947) 531.
13. P. KARRER, *Helv. Chim. Acta 28* (1945) 474.
14. P. KARRER and E. JUCKER, *Helv. Chim. Acta 28* (1945) 300.
15. P. KARRER, E. JUCKER and K. STEINLIN, *Helv. Chim. Acta 28* (1945) 233.
16. P. KARRER and E. JUCKER, *Helv. Chim. Acta 28* (1945) 300.
17. P. KARRER and E. JUCKER, *Helv. Chim. Acta 29* (1946) 1539.

CHAPTER IX

The distribution of carotenoids in nature

Since the discovery of carotene by WACKENRODER in 1831, the distribution of carotenoids in nature has been much investigated. Extensive studies have shown that polyene pigments are present in the whole of the vegetable and animal kingdoms. In the following sections the occurrence of polyene pigments is summarised in tabular form. The following arrangement is used:

A. Carotenoids in plants:
- 1. Phanerogams
  - (a) Unexposed parts of plants
  - (b) Exposed parts of plants
  - (c) Blossoms
  - (d) Fruit
- 2. Cryptogams

B. Carotenoids in amimals:
- 1. Invertebrates
  - (a) Arthropods
  - (b) Molluscs
  - (c) Echinoderms
  - (d) Worms
  - (e) Coelenterates and sponges
  - (f) Chordata
- 2. Vertebrates
  - (a) Mammals
  - (b) Birds
  - (c) Fish
  - (d) Amphibia
  - (e) Reptiles
  - (f) Miscellaneous

It should be mentioned that only limited significance can be attached to some of the older investigations which were carried out without the use of TSWETT's chromatography. In most cases, they merely indicate that the

presence of carotenoids may be assumed in the materials examined. Definite conclusions regarding the nature of the individual pigments can only be drawn after repeated chromatographic analysis. For this reason no attempt has been made to identify the pigments reported in these older investigations.

A. CAROTENOIDS IN PLANTS*, **

I. PHANEROGAMS

a) *Carotenoids in unexposed parts of plants*

TABLE 16 (References see p. 99–107)

CAROTENOIDS IN ROOTS

Beta vulgaris[1].
Brassica campestris[2].
Brassica Rapa[3].
Celastrus scandens[4]: β-Carotene (?).
Daucus Carota[5]: α-Carotene, β-carotene, γ-carotene, a hydrocarbon of unknown constitution (absorption maxima in CS_2: 482, 453 mμ), a second hydrocarbon of unknown constitution (absorption maxima in CS_2: 499, 469 mμ), xanthophyll.
Escobedia scabrifolia[6]: Azafrin.
Ipomoea Batatas[7]: α-Carotene, β-carotene.
Jaundiced potatoes[8]: Taraxanthin or violaxanthin, xanthophyll, α-carotene (?).
Sweet potatoes[9]: Carotene.
Pastinaca sativa[10].

b) *Carotenoids in exposed parts of plants*

Green parts of plants

It has long been known that besides chlorophyll, *all* green parts of plants contain carotenoids[1]. These are mainly β-carotene, xanthophyll and, according to the most recent investigations[2], xanthophyll epoxide. Small amounts of α-carotene are also usually present. The carotenoids are found together with chlorophyll in the chromatophores and are present in either an amorphous or crystalline state.

The ratio of carotene to xanthophyll in green leaves has been investigated by WILLSTÄTTER and STOLL[3] and by KARRER and co-workers[4]. Whereas the former found a ratio of 1.7, the latter obtained values tending to unity. In all the older investigations the fact that the so-called xanthophyll fraction consists

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* The following abreviations are employed in the tables in this chapter:
+ = isolated in the crystalline state;
++ = definitely present;
+++ = probably present.

** The literature references for the tables in this chapter will be found at the end of the general part (p. 99–107).

of *two* main pigments, xanthophyll itself and xanthophyll epoxide, was neglected.

Literature references regarding the occurrence of carotenoids in green plants, especially green leaves, are so numerous that no attempt will be made to summarise them. Only the most important investigations in this field can, therefore, be mentioned[1].

Non-green leaves

Numerous investigations have been carried out concerning the pigments which are responsible for the yellow colour of etiolated leaves[5]. However, most of these studies belong to the early period of carotenoid research. More recent investigations[2] show that besides water-soluble pigments, carotenoids, especially xanthophyll, are present in etiolated leaves.

Our knowledge concerning the pigments of *yellow* leaves (aurea varieties) is rather deficient. The last investigation of these pigments was made by WILLSTÄTTER and STOLL[6] and showed the presence of carotenoids and of water-soluble pigments. As the quantity of carotenoids was very small, however it appears doubtful whether they are, in fact, responsible for the yellow colour of the leaves.

As a result of further studies, our knowledge regarding the pigments of *yellow autumn leaves* is somewhat better, and it has been possible to recognise different stages in the rather complicated pigment metabolism. The carotenoids (possibly also the anthocyanins[7]) remain in the leaves after the degradation of the chlorophyll in the autumn and produce the well-known, striking colourations. Gradually the polyene pigments are also degraded and carotene seems to be decomposed more rapidly than xanthophyll. In the last phases of the necrobiosis, the well-known brown pigments are produced, to which fallen leaves owe their brown colouration. These brown pigments appear to be oxidation and decomposition products; they are soluble in water and produce dark yellow to brown colourations with alkalis.

An important question is whether only carotenoids already present in the green leaf are involved in the course of necrobiosis or whether new pigments are formed. According to WILLSTÄTTER and STOLL[8], the total quantity of pigments in autumnal leaves is about equal to that in green leaves. These findings are in agreement with the investigations of GOERRIG[9]. It is interesting that whereas the amount of carotene decreases in the course of necrobiosis, the quantity of xanthophyll is said to increase[10]. The yellow colouration of dead leaves is due, according to TSWETT[11] to epiphasic carotenoid pigments which, in contrast to carotene, can be adsorbed on calcium carbonate from petroleum ether solution. TSWETT named these carotenoids "autumn xanthophylls". According to KUHN

References p. 108.

and BROCKMANN[12], who confirmed the disappearance of carotene in the course of necrobiosis, these autumn xanthophylls are phytoxanthin esters, which are formed only in the autumn by the esterification of free phytoxanthins. The matter has not been completely elucidated, however, as with one exception no crystalline pigments could be obtained[13].

KARRER and WALKER isolated the carotenoids by precipitation in the form of sparingly soluble iodides and regeneration with sodium thiosulphate[14]. The main results of their investigations are as follows: as the leaves decay, the content of carotene and xanthophyll decreases, but the former decreases more rapidly. Xanthophyll can still be isolated in the crystalline state long after no carotene can be detected. Eventually the xanthophyll also disappears completely. The autumn xanthophylls observed by TSWETT were also found. These compounds first appear at the beginning of necrobiosis and their concentration steadily increases at the expense of the carotenoids, so that they become mainly responsible for the colouration of the leaves shortly before the postmortal phase. Nothing definite is yet known regarding the nature of these pigments, but they appear to consist of oxidation and degradation products of xanthophyll. Other pigments which absorb more strongly in the ultraviolet are also observed. Further investigations of these autumn xanthophylls would be of interest.

The pigments of *red winter leaves* have also been much studied. It has been found that some leaves owe their red colouration to anthocyanins, while others contain rhodoxanthin.

c) *Carotenoids in Blossoms*

Nature has been lavish in the distribution of carotenoids in blossoms. About 35 different carotenoids, i.e. about half of all the known polyene pigments, have been isolated from blossoms up to the present time. This variety is the more remarkable since nothing is at present known regarding the function of carotenoids in blossoms.

The following is a summary of the blossoms examined in this respect up to the end of 1948. Many of the older investigations, e.g. those of COURCHET, TAMMES and VAN WISSELINGH were carried out using inadequate techniques and only limited significance can be attached to them.

References p. 108.

TABLE 17 (References see p. 99–107)

CAROTENOIDS IN BLOSSOMS

(i). Monocotyledoneae

Gramineae
Hungarian wheat blossoms[427]: Xanthophyll, carotene.

Bromeliaceae
Tillandsia splendens[12].

Liliaceae
Allium siculum[13].
Aloe vera[14]: Rhodoxanthin+++.
Asphodelus cerasiferus[13].
Bulbina semibarbata[13].
Fritillaria imperialis[15, 16].
Hemerocallis Middendorffii[16].
Kniphofia aloides[12].
Lilium bulbiferum[17].
Lilium bulbiferum ssp. croceum[16].
Lilium candidum[19]: Antheraxanthin++(?), violaxanthin++, *cis*-antheraxanthin.
Lilium tigrinum (anther)[18]: Antheraxanthin+, capsanthin+.
Tulipa Gesneriana[16].
Tulipa hortensis[16].
Tulips, yellow variety[20]: Violaxanthin++.
Uvularia grandiflora[12].

Amaryllidaceae
Clivia miniata[16].
Narcissus poeticus[13, 16, 21].
Narcissus Pseudonarcissus[22]: Xanthophyll++.
Narcissus Tazetta[23].

Iridaceae
Crocus luteus[25]: Crocetin+.
Crocus neapolitanus[25]: Crocetin+.
Crocus reticulatus[25, 27]: Crocetin++.
Crocus sativus[25]: Crocetin+.
Crocus variegatus[25, 27]: Crocetin++.
Iris Pseudacorus[16, 24]: β-Carotene, xanthophyll, violaxanthin.
Saffron (Crocus sativus)[26]: α-Carotene++, β-carotene++, γ-carotene++, lycopene++, zeaxanthin++, crocetin+.
Tritonia aurea = *Ixia crocata*[25, 27]: Crocetin++.

Musaceae
Strelitzia Reginae[12, 15].

Orchidaceae
Cypripedium Argus[23].
Cypripedium Boxallii[23].
Cypripedium insigne[23].
Dendrobium thyrsiflorum[16].
Gongora galeata[16].
Lycaste aromatica[12].
Masdevallia Veitchiana[23].
Odontoglossum species[23].
Oncidium species[23].

(ii). Dicotyledoneae

Proteaceae
Grevillea robusta[69]: β-Carotene, cryptoxanthin+, xanthophyll+, carotenoid of unknown constitution+ (cf. p. 340).

Nymphaceae
Nuphar luteum[16].

Ranunculaceae
Adonis vernalis[12].
Caltha palustris[31, 434]: Xanthophyll+, xanthophyllepoxide++, trollixanthin++, β-carotene++, α-carotene++.
Eranthis hiemalis[12, 16].
Ranunculus acer (R. Steveni ?)[28, 29, 30]: Violaxanthin++, xanthophyll+, flavoxanthin+, chrysanthemaxanthin+, flavochrome+, xanthophyll epoxide++, α-carotene epoxide++, α-carotene++, β-carotene++, taraxanthin+.

Ranunculus arvensis[31]: Carotene^{++}, xanthophyll^{+}.
Ranunculus auricomus[12, 15].
Ranunculus Ficaria[12, 15].
Ranunculus gramineus[12].
Ranunculus repens[12, 15].
Ranunculus Steveni[32]: Xanthophyll^{+}.
Trollius asiaticus[12].
Trollius europaeus[31, 33]: β-Carotene^{++}, xanthophyll^{+}, xanthophyll epoxide^{++}, trollixanthin^{+}, epoxide of unknown constitution^{++}.

Berberidaceae
Epimedium macranthum[12].

Magnoliaceae
Liriodendron tulipifera[34].

Papaveraceae
Chelidonium majus[12, 16].
Corydalis lutea[16].
Eschscholtzia californica[13, 35, 36]: Eschscholtzxanthin^{+}.
Glaucium luteum[37].
Meconopsis cambrica[16].

Cruciferae
Alyssum saxatile[12].
Cheiranthus Cheiri[23, 28].
Cheiranthus Senoneri[22]: Xanthophyll^{++}.
Erysimum Perofskianum[16].
Isatis tinctoria[16].
Nasturtium species[23].
Raphanus Raphanistrum[28].
Sinapis officinalis[31]: Carotene^{++}, violaxanthin^{+}.
Sisymbrium Sophia[12].

Saxifragaceae
Ribes aureum[15].

Rosaceae
Geum coccineum[13].
Geum montanum[38].
Kerria japonica[15, 38, 12, 16, 39, 33]: Xanthophyll^{+}, xanthophyll epoxide^{++}, β-carotene^{++}.
Potentilla erecta (Tormentilla)[74]: β-Carotene, xanthophyll, zeaxanthin(?), flavoxanthin(?).
Rosa, yellow species[15, 17].
Waldsteinia geoides[12].

Leguminosae
Acacia decurrens var. mollis[10, 40]: Carotene^{++}, xanthophyll^{++}.
Acacia discolor[10]: Carotene^{+}, xanthophyll^{+}.
Acacia linifolia[10]: Carotene^{+}, xanthophyll^{+}.
Acacia longifolia[10]: Carotene^{+}, xanthophyll^{+}.
Colutea media[38].
Cytisus sagittalis[16].
Genista racemosa[13].
Genista tinctoria[13].
Genista tridentata[41]: α-Carotene^{+}, β-carotene^{+}, xanthophyll^{+}.
Laburnum anagyroides[31, 33]: Carotene^{++}, violaxanthin^{++}, β-carotene^{++}, xanthophyll^{++}, xanthophyll epoxide^{++}.
Lotus corniculatus[42]: α-Carotene^{++}, β-carotene^{++}, xanthophyll^{++}, xanthophyll epoxide^{++}, violaxanthin^{++}, carotenoid of unknown constitution^{++}.
Melilotus officinalis[38].
Sarothamnus scoparius[43, 137]: α-Carotene^{++}, β-carotene^{++}, xanthophyll^{+}, xanthophyll epoxide^{+}, chrysanthemaxanthin^{+}, flavoxanthin^{+}.
Spartium junceum[16].
Thermopsis lanceolata[16].
Ulex europaeus[28, 41]: α-Carotene^{+}, β-carotene^{+}, violaxanthin^{+}, taraxanthin^{+}, xanthophyll isomer^{+}(?) and a carotenoid of unknown constitution with spectral properties similar to those of flavoxanthin.
Ulex Gallii[41]: α-Carotene^{+}, β-carotene^{+}, violaxanthin^{+}, taraxanthin^{+}, xanthophyll isomer^{+}(?), flavoxanthin^{+++}(?).
Vicia, violet-blue varieties[31]: Lycopene.

Meliaceae
Cedrela Toona[44]: Crocetin^{+}.

Tropaeolaceae
Tropaeolum majus[15, 22]: Xanthophyll^{++}.

Malvaceae
Abutilon Darwini[12].
Abutilon megapotamicum[12].
Abutilon nervosum[15].

Balsaminaceae
Impatiens noli tangere[45]: Taraxanthin+.

Violaceae
Viola biflora[38].
Viola cornuta var. Daldowie[16].
Viola lutea[12, 38].
Viola odorata[15, 46].
Viola tricolor[47, 48]: Violaxanthin+, zeaxanthin++, flavoxanthin++, xanthophyll+, auroxanthin+, carotene++.

Loasaceae
Loasa (Cajophora) lateritia[15].

Oenotheraceae
Oenothera biennis[12, 15].

Umbelliferae
Ferula species[16].

Primulaceae
Primula acaulis[51].
Primula officinalis[12, 15].

Plumbaginaceae
Armeria vulgaris[13].

Oleaceae
Forsythia Fortunei[12].
Forsythia viridissima[12, 15, 38].
Jasminum Sambac[27]: Crocetin+++.
Nyctanthes Arbor-tristis[44, 49, 50]: Crocetin+.

Asclepiadaceae
Asclepias curassavica[16].

Boraginaceae
Nonnea lutea[12].

Labiatae
Ladanum hybridum[15].

Solanaceae
Atropa Belladonna[13].
Fabiana indica[25, 52]: Crocetin+.

Scrophulariaceae
Calceolaria species[28].
Calceolaria rugosa[16].
Calceolaria scabiosæfolia[14].
Mimulus longiflorus[53]: β-Carotene+, γ-carotene++, lycopene+, cryptoxanthin++, zeaxanthin+, pro-γ-carotene, prolycopene[54].
Mimulus moschatus[28].
Verbascum species[15].
Verbascum Thapsus[55]: Crocetin+.

Rubiaceae
Manettia bicolor[12].

Cucurbitaceae
Cucurbita foetidissima[15].
Cucurbita melanosperma[16].
Cucurbita Pepo[56]: Carotene+, cryptoxanthin+, xanthophyll+, zeaxanthin+, petaloxanthin+.
Momordica Balsamina[57].

Campanulaceae
Siphocampylus bicolor[12].

Compositae
Arnica montana[22, 42]: Xanthophyll+, xanthophyll epoxide++, zeaxanthin+.
Aster species[13].
Buphthalmum salicifolium[38].
Cacalia coccinea[38].
Calendula arvensis[16].
Calendula officinalis[58], dark variety: Carotene+, lycopene+, xanthophyll+, violaxanthin+, γ-carotene+++(?); light yellow variety: lycopene absent.
Chrysanthemum frutescens[28, 16].
Chrysanthemum segetum[59].
Chrysanthemum[60]: Xanthophyll+, xanthophyll epoxide+, chrysanthemaxanthin+ (carotene++).
Crepis species[138].
Crepis aurea[42]: α-Carotene++, β-Carotene++, xanthophyll++, violaxanthin++, pigment of unknown constitution, absorption maxima in CS_2 501, 470 mμ.

Dahlias (anther)[19]: Lycopene+.
Dimorphotheca aurantiaca[31]: Lycopene+.
Doronicum Columnae[15, 12, 38].
Doronicum Pardalianches[22]: Xantophyll++.
Doronicum plantagineum[16].
Doronicum excelsum[16].
Gaillardia splendens[38].
Gazania rigens, a) Portuguese origin[61]: Xanthophyll+, rubixanthin+, gazaniaxanthin+, carotenoid of unknown constitution, β-carotene+, γ-carotene.
b) Californian origin[62]: Xanthophyll+, gazaniaxanthin+, cryptoxanthin+, lycopene+, β-carotene+, γ-carotene+.
Gazania splendens[15, 16].
Helenium autumnale[40]: Xanthophyll.
Helenium autumnale var. grandicephalum[22]: Xanthophyll.
Helianthus annuus[40, 63]: Xanthophyll+, taraxanthin+, cryptoxanthin+, carotene+.
Heliopsis scabrae cinniaeflorae[22]: Xanthophyll.
Heliopsis scabra major[22]: Xanthophyll.
Hieracium aurantiacum[28].
Hieracium murorum[16].
Hieracium Pilosella[13].
Inula Helenium[16].
Kleinia Galpinii[16].
Leontodon autumnalis[45]: Xanthophyll+, taraxanthin+.
Rudbeckia Neumannii[40]: Xanthophyll.
Senecio Doronicum[31]: Zeaxanthin+.
Senecio vernalis[29]: Flavoxanthin+.
Silphium perfoliatum[26]: Xanthophyll.
Tagetes aurea[26]: Xanthophyll++.
Tagetes erecta[40]: Xanthophyll+.
Tagetes grandiflora[40]: Xanthophyll+, violaxanthin++.
Tagetes nana[40]: Xanthophyll+.
Tagetes patula[40, 64]: Xanthophyll+, xanthophyll epoxide+, rubixanthin++, rubichrome+, α-carotene++, β-carotene++.
Taraxacum officinale[65, 66, 67]: Xanthophyll+, flavoxanthin+, violaxanthin++, taraxanthin+(?).
Telekia speciosissima[38].
Tragopogon pratensis[30, 31]: α-Carotene++, β-carotene++, α-carotene epoxide++, xanthophyll+, xanthophyll epoxide++, violaxanthin+, flavoxanthin+.
Tussilago Farfara[68]: Taraxanthin+, violaxanthin+.

d) *Carotenoids in Fruit and Seeds*

TABLE 18 (References see p. 99–107)

CAROTENOIDS IN FRUIT AND SEEDS

(i). *Gymnospermae*

Taxaceae
Taxus baccata (Arillus)[70]: Rhodoxanthin.

(ii). *Angiospermae*

a) Monocotyledoneae

Pandanaceae
Pandanus polycephalus[6]: Lycopene+.

Gramineae
Avena sativa[71].
Barley germ[72].
Hordeum sativum[71].
Oryza sativa[71].
Rye-seed oil[80]: α-Carotene++, β-carotene++, γ-carotene+++, xanthophyll++, zeaxanthin++.
Triticum vulgare[71]: β-Carotene++, xanthophyll++.
Wheat germ[78, 79]: Xanthophyll, carotene(?).
Zea Mays[75, 77]: γ-Carotene++, crypto-

xanthin+, xanthophyll++, zeaxanthin+. [76] Hydroxy-α-carotene[434]. β-Carotene, α-carotene, K-carotene(?), neo-cryptoxanthin.

Palmae

Actinophloeus angustifolia[6]: Lycopene++.
Actinophloeus Macarthurii[6]: Lycopene+[81].
Archontophoenix Alexandrae[6]: Lycopene++.
Areca Alicae[6]: Lycopene++.
Attalea gomphococca[82]: Carotene.
Calyptrocalyx spicatus[6]: Lycopene.
Elaeis guineensis[83]: β-Carotene+++, lycopene+++[84].
Elaeis melanococca[83]: β-Carotene+++, lycopene+++.
Nenga Polycephalus[6]: Lycopene++.
Palm fruit[90]: Zeaxanthin, carotene.
Palm oil[85]: α-Carotene+, β-carotene+, γ-carotene++, lycopene+, neolycopene+, neo-γ-carotene(?), pigments of unknown constitution.
Palm oil from different species[87, 88, 89]: α-Carotene+, β-carotene, γ-carotene++, lycopene++.
Ptychandra elegans[6]: Lycopene++.
Ptychandra glauca[6]: Lycopene.
Sabal (serenaea) serrulatum[86]: β-Carotene+.
Synaspadix petrichiana[6]: Lycopene++.

Araceae

Aglaonema commutatum[12, 16].
Aglaonema nitidum[6]: Lycopene.
Aglaonema oblongifolium[6]: Lycopene++.
Aglaonema oblongifolium var. Curtisii[6]: Lycopene++.
Aglaonema simplex[6]: Lycopene++.
Arum italicum[6, 91, 92]: Lycopene++.
Arum maculatum[93]: Lycopene++.
Arum orientale[94]: Lycopene++, β-carotene++, xanthophyll.

Bromeliaceae

Ananas sativus[73]: Carotene, xanthophyll.

Liliaceae

Asparagus officinalis[96]: Zeaxanthin++.
Convallaria majalis[96, 97]: α-Carotene++, β-carotene+, γ-carotene+, lycopene+, xanthophyll+.

Dioscoraceae

Tamus communis[98, 99]: Lycopene+, lycoxanthin+, lycophyll+.

Musaceae

Musa paradisiaca[100]: Carotene+, xanthophyll+.

Amaryllidaceae

Clivia species[15].
Eriobotyra japonica Lindl.[439]: Cryptoxanthin, β-carotene, neo-β-carotene U and neo-β-carotene B.

β) Dicotyledoneae

Moraceae

Cannabis sativa[71].

Polygonaceae

Fagopyrum esculentum[95].

Berberidaceae

Berberis vulgaris[15].

Anonaceae

Polyalthia species[91].

Myristicaceae

Myristica fragrans[92, 15].

Cruciferae

Brassica campestris[101].
Brassica nigra[101].

Rosaceae

"*Apricot-peach*"[102]: Carotene++, lycopene++, xanthophyll++.
Cotoneaster species[15].
Cotoneaster occidentalis[103]: Violaxanthin++, xanthophyll.
Crataegus Crus galli[104].
Prunus armeniaca[105]: β-Carotene+, γ-carotene++, lycopene+.

Prunus persica[72]: β-Carotene, cryptoxanthin, xanthophyll, zeaxanthin, carotenoid of unknown constitution.

Rosa canina[32, 106, 107]: Lycopene^{+}, β-carotene^{+}, γ-carotene^{++}, rubixanthin^{+}, zeaxanthin^{++}, xanthophyll^{++}, taraxanthin^{++}.

Rosa damascena[107]: As for Rosa canina.

Rosa rubiginosa[107]: As for *Rosa canina*.

Rosa rugosa Thumb.[108]: α-Carotene^{+}, β-carotene^{+}, γ-carotene^{+}, lycopene^{+}, rubixanthin^{+}.

Rubus Chamaemorus[110]: α-Carotene, β-carotene, γ-carotene(?), lycopene, rubixanthin, zeaxanthin.

Sorbus Aria[12, 16].

Sorbus aucuparia[109]: α-Carotene^{+}, β-carotene^{+}.

Sorbus aucuparia dulcis[111]: Carotene.

Sorbus suecica[92].

Leguminosae

Afzelia cunazensis[34].

Soya beans[113, 428, 430]: α-Carotene, β-carotene.

Vigna sinensis[112]: β-Carotene^{+}, xanthophyll^{++}.

Linaceae

Linum usitatissimum[114].

Erythroxylaceae

Erythroxylon coca[94]: Lycopene^{++}.

Erythroxylon novogranatense[81, 94]: Lycopene^{+}.

Rutaceae

Citrus aurantium[115, 116, 117, 118]: β-Carotene^{+}, lycopene^{+++}, cryptoxanthin^{+}, xanthophyll^{+}, violaxanthin^{++}, zeaxanthin^{+}, β-citraurin^{+}, citroxanthin^{+} (= mutatochrome).

Citrus grandis[120]: β-Carotin, lycopene.

Citrus grandis Osbeck[119]: Lycopene.

Citrus Limonum[15, 38].

Citrus madurensis[121]: β-Carotene^{+}, xanthophyll^{+}, cryptoxanthin^{+}, violaxanthin^{+++}(?), zeaxanthin^{+++}(?).

Citrus poonensis hort.[122]: β-Carotene^{+}, cryptoxanthin^{+},violaxanthin^{+++}(?) cryptoxanthin^{+}, violaxanthin^{+++}(?). xanthophyll^{++}.

Anacardiaceae

Mangifera indica[123, 124]: α-Carotene^{+}, β-carotene^{+}, xanthophyll^{+}, carotenoid of unknown constitution.

Celastraceae

Celastrus scandens[125].

Evonymus europaeus[126]: Zeaxanthin^{++}.

Evonymus japonicus[13, 94]: Lycopene^{++}.

Evonymus latifolius[12, 16].

Icacinaceae

Gonocaryum obovatum[94, 97]: α-Carotene^{+}, β-carotene^{+}, γ-carotene^{++}, lycopene^{+}.

Gonocaryum pyriforme[94, 97]: α-Carotene^{+}, β-carotene^{+}, γ-carotene^{++}, δ-carotene^{++}(?), lycopene^{+}.

Vitaceae

Ampelopsis hederacea[17, 15].

Malvaceae

Gossypium hirsutum[127, 71].

Gossypium-species[128, 129]: Carotene^{++}, xanthophyll^{++}.

Bixaceae

Bixa orellana (cf. p. 256): Bixin^{+}.

Passifloraceae

Passiflora coerulea[13, 130]: Lycopene^{+}.

Caricaceae

Carica Papaya[131, 132]: cryptoxanthin^{+}, violaxanthin^{+}.

Myrtaceae

Eugenia uniflora[13].

Elaeagnaceae

Hippophae rhamnoides[93]: Zeaxanthin^{+}.

Ericaceae

Vaccinium Vitis idaea[161]: Lycopene^{++}, β-carotene^{++}(?), zeaxanthin^{++}, xanthophyll^{++}.

Arbutus Unedo[135]: α-Carotene++, β-carotene+, lycopene++, cryptoxanthin+, xanthophyll+, zeaxanthin+, violaxanthin+.

Ebenaceae

Capsicum frutecens jap.[142, 162]: Capsanthin+, carotene+.

Diospyros costata[134]: α-Carotene++, β-carotene+, lycopene+, cryptoxanthin+.

Diospyros Kaki[133]: Lycopene++, zeaxanthin++.

Apocynaceae

Tabernaemontana pentasticta[6]: Lycopene++.

Solanaceae

Lycium barbarum[96]: Zeaxanthin+.

Lycium carolinianum[92].

Lycium halimifolium[143]: Zeaxanthin+.

Lycium ovatum[92].

Lycopersicum cerasiforme[91].

Lycopersicum esculentum[144, 145]: Lycopene+.

Physalis Alkekengi[146]: Cryptoxanthin+, zeaxanthin+.

Physalis Franchetii[146]: Cryptoxanthin+, zeaxanthin+.

Solanum Balbisii[92].

Solanum corymbosum[13].

Solanum decasepalum[94]: Lycopene+.

Solanum Dulcamara[99]: Lycopene+, lycophyll+, lycoxanthin+.

Solanum Hendersonii[96]: Zeaxanthin+.

Solanum Lycopersicum[147]: Carotene, lycopene, xanthophyll.

Solanum Pseudocapsicum[92].

Pedaliaceae

Sesamum indicum[114].

Rubiaceae

Gardenia grandiflora[25]: Crocetin+.

Gardenia jasminoides[150, 151]: Crocetin+++.

Gardenia lucida[27]: Crocetin++.

Nertera depressa[94]: Lycopene++.

Caprifoliaceae

Lonicera tatarica[139].

Lonicera Xylosteum[130, 46, 15, 148].

Sambucus nigra[148].

Viburnum Opulus[16, 148, 149].

Viburnum Lantana[148, 16, 149].

Cucurbitaceae

Bryonia dioica[94, 96]: Lycopene+.

Citrullus vulgaris[152, 153]: Lycopene+, α-carotene+, β-carotene+, γ-carotene+.

Cucumis Melo[13].

Cucurbita maxima[154, 155]: α-Carotene+, β-carotene+, xanthophyll+, violaxanthin+.

Cucurbita Pepo[156].

Luffa species[157]: β-Carotene++, xanthophyll+.

Momordica Balsamina[158, 159]: Xanthophyll++, lycopene++.

Momordica Charantia[159]: β-Carotene++, lycopene++.

Trichosanthes species[160]: Lycopene++.

Compositae

Helianthus annuus[101].

Sunflower oil[163]: carotenoid of unknown constitution.

2. CRYPTOGAMS*

Much less is known about the carotenoids of cryptogams than about the carotenoids of phanerogams, no doubt partly due to the greater difficulty of collecting experimental material. Recent investigations, however, have yielded such interesting results that further studies in this field appear very desirable.

* The arrangement of the groups follows ENGLER's system. The species belonging to one group are given in alphabetical order.

Investigations on bacterial pigments are particularly difficult because of the shortage of materials. Recent observations show that bacteria produce carotenoids (e.g. rhodoviolascin, sarcinin, sarcinaxanthin, leprotin, rhodopin, etc.), not found in the higher plants. Further interesting results can be expected from a renewed study of bacterial carotenoids.

The carotenoids of fungi have been the subject of several investigations, some of recent date. In some mushrooms, certain other pigments (e.g. torulin, torularhodin) have been found besides carotene, and the question arises whether mushrooms produce these pigments by the degradation of other carotenoids or by independent synthesis.

A large number of data are to be found in the literature regarding carotenoids in algae. Many of these are of an early date and possess only relatively small significance, but others have been obtained more recently and provide some interesting results. In this connection the comprehensive studies of KYLIN[15] and HEILBRON[16] may be particularly mentioned.

We are least well informed regarding carotenoids in archegoniates, for which practically no data are available. It is probable, however, that the green organs of these plants also contain carotene, xanthophyll and xanthophyll epoxide besides chlorophyll. In the discussion of rhodoxanthin (p. 221) it will be mentioned that this pigment has been found in the internodes of *equisetum* and *selaginella*.

TABLE 19 (References see p. 99–107)

CAROTENOIDS IN CRYPTOGAMS

(i). Schizophyta

a) Bacteria

Bacillus Grasberger[261]: β-Carotene, γ-carotene, lycopene and a pigment resembling capsanthin.

Bacillus Lombardo Pellegrini[261]) ; β-Carotene, γ-carotene and a phytoxanthin of unknown constitution.

Bacterium chrysogloea[243].

Bacterium egregium[243].

Bacterium halobium[165, 244]: α-Bacterioruberin, β-bacterioruberin, hypophasic pigment, absorption-maxima in CS_2 : 571, 532 mμ (demethylated rhodoviolascin(?).

Corynebacterium[245]: β-Carotene.

Corynebacterium carotenum[255, 256, 257]: β-Carotene, yellow pigment with vitamin A activity.

Micrococcus erythromyxa[243]: Astacene(?).

Micrococcus rhodochrous[243]: Astacene(?).

Mycobacterium lacticola[265]: β-Carotene, two similar pigments, astacene.

Mycobacterium leprae[258, 259, 260]: yellow lipochromes, leprotin.

Mycobacterium phlei[240, 246, 247]: β-Carotene, γ-carotene, cryptoxanthin, xanthophyll, zeaxanthin, azafrin ester, leprotin, azafrin, α-carotene

Purple bacteria (sulphur-free)[264]: Hydrocarbon similar to lycopene and phytoxanthin of unknown constitution.

Rhodobacillus palustris[432].

Rhodovibrio Bacteria[252]: Rhodoviolascin, rhodopin, rhodovibrin, rhodopurpurin, flavorhodin, β-carotene(?).

Sarcina aurantiaca[238]: β-Carotene, lycopene(?), zeaxanthin[239].

Sarcina lutea[240, 239, 241, 242]: Sarcinin, new hypophasic pigment, yellow phytoxanthin ester, sarcinaxanthin.

Sphaerotilus roseus[175]: pigment of unknown constitution, absorption maxima in petroleum ether: 494, 449 mμ.

Spirillum rubrum Esmarch[262, 263]: Bacteriochlorophyll, rhodoviolascin =spirilloxanthin and two pigments of unknown constitution.

Staphylococcus pyrogenes aureus[243, 240]: Zeaxanthin.

Strepotothrix corallinus[238]: Coralin (absorption maxima in ether: 495, 457 mμ).

Thiocystis Bacteria[249, 250, 251]: Lycopene, α-carotene, β-carotene, flavorhodin, rhodoviolascin, rhodopin, rhodovibrin, rhodopurpurin, bacteriochlorophyll, bacteriopurpurin.

Timotheegras Bacteria[248]: β-Carotene, pigments of unknown constitution.

Torula rubra[253, 254]: β-Carotene, torulin, torularhodin.

β) Cyanophyceae

Anabaena flos-aquae[12, 16].

Aphanizomenon flos-aquae[231]: β-Carotene, aphanin, aphanicin, aphanizophyll, flavacin.

Calothrix species [232, 194].

Calothrix-scopulorum[199, 231]: Xanthophyll, myxoxanthin, myxoxanthophyll.

Microcystis flos aquae[221].

Nodularia species[16].

Nostoc species[232, 16].

Oscillatoria[184, 185, 233].

Oscillatoria limosa[232, 234].

Oscillatoria lapotricha[235].

Oscillatoria Froelichii[12, 15].

Oscillatoria rubescens[236, 237]: β-Carotene, myxoxanthin, myxoxanthophyll, oscillaxanthin, zeaxanthin, xanthophyll.

Phormidium vulgare[195, 15].

Rivularia species[15].

Rivularia nitida[198]: Carotene, myxoxanthin, xanthophyll.

Rivularia atra[198]: Carotene, myxoxanthin, myxoxanthophyll.

Tolypothrix species[15].

(ii). *Myxomycetes*

Lycogala epidendron[164]: Torulin(?), rhodoviolascin(?), β-carotene (β-carotene in spores[165]).

Lycogala flavofuscum[164].

Stemonitis ferruginea [164].

Stemonitis fusca[164].

(iii). *Flagellatae*

α) Chrysomonadales

Apistonema Carteri[198]: Carotene, xanthophyll, fucoxanthin.

Chromulina Rosanoffi[224, 225].

Chrysomonadina[226].

Glenochrysis maritima[198]: Carotene, xanthophyll, fucoxanthin.

Hydrurus penicillatus[195].

Thallochrysis litoralis[198]: Carotene, xanthophyll, fucoxanthin.

β) Euglenales

Euglena heliorubescens[228]: Astacene.
Euglena sanguinea[229, 230].
Euglena viridis[226, 227, 206].

γ) Dinoflagellatae

Ceratium tripos[223].
Ceratium fusus[223].
Ceratium furco[223]
Dinophysis acuta[223].
Dinophysis laevis[223].
Glenodinium species[223].
Gymnodinium helix[223].
Peridinium divergens[223].
Prorocentrum micans[223].

δ) Heterocontae

Botrydium granulatum[198]: Carotene, fucoxanthin.
Botrydium species[202].

(iv). Bacillariophyta

Diatomeae

Achnantidium lanceolata[212].
Cymatopleura solea[181].
Eunotia pectinalis[212].
Fragilaria species[12].
Gomphonema species[221, 15].
Melosira species[195].
Navicula species[15, 181]: Carotene, fucoxanthin, zeaxanthin.
Navicula torquatum[425]: β-Carotene, ε-carotene.
Nitzschia closterium[189, 205, 222, 435] β-Carotene, cryptoxanthin, xanthophyll, isoxanthophyll(?), fucoxanthin, pigment of unknown constitution.
Nitzschia Palea[181].
Nitzschia sigmoidea[181].
Nitzschia species[194, 436]: "Diatoxanthin", "diadinoxanthin", fucoxanthin, "neo-fucoxanthin A", "neo-fucoxanthin B".

(v). Conjugatae

Prasiola species[194].
Spirogyra crassa[203, 204, 12, 15, 194].
Spirogyra maxima[203, 16, 194].
Zygnema cruciatum[16].
Zygnema pectinatum[198]: Carotene, xanthophyll, fucoxanthin.

(vi). Chlorophyceae

α) Protococcales

Chlorella protothecoides[16].
Chlorella variegata[16].
Haematococcus pluvialis = *Sphaerella pluvialis*[213, 202, 214, 215, 15, 16, 216, 217, 218] (cf. *Z. physiol. Chem.* 267 (1941) 281): α-Carotene, β-carotene, xanthophyll, zeaxanthin, haematoxanthin, astacene. (In earlier investigations, J. TISCHLER reported a new pigment, euglenarhodon, later shown to be identical with astacene.)
Hydrodictyon utriculatum[12].
Palmellococcus miniatus[220].
Phyllobium dimorphum[219].
Phyllobium incertum[219].
Phyllobium Naegelii[298]: Carotene, xanthophyll.
Protococcus pluvialis = *Pleurococcus pluvialis*[213, 212].
Protococcus vulgaris[16].
Scotinosphaera paradoxa[219].
Volvox species[201].

β) Ulothrichales

Cephaleurus laevis[206, 207].
Cephaleurus solutus[206, 207].
Cephaleurus albidus[206, 207].
Cephaleurus parasiticus[206, 207].
Cephaleurus minimus[206, 207].
Phycopeltis epiphyton[206, 207].
Phycopeltis aurea[206, 207].
Phycopeltis amboinemis[206, 207].
Phycopeltis Treubii[206, 207].
Phycopeltis maritima[206, 207].
Trentepohlia aurea[198, 202, 172, 208, 165]: α-Carotene, β-carotene, xanthophyll, zeaxanthin.
Trentepohlia aureum tomentosum[209, 211].
Trentepohlia bisporangiata[206, 207].
Trentepohlia crassiaepta[206, 207].
Trentepohlia Cyania[206, 207].
Trentepohlia jolithus[206, 207, 15]: α-Carotene, β-carotene, xanthophyll, zeaxanthin.
Trentepohlia moniliformis[206, 207].
Trentepohlia umbrina[209, 210, 208, 172]: α-Carotene, β-carotene, xanthophyll, zeaxanthin.

γ) Ulvales

Enteromorpha compressa[198]: Carotene, xanthophyll.
Enteromorpha intestinalis[12, 194, 199]: α-Carotene, β-carotene, xanthophyll, violaxanthin(?).
Ulva lactuca[198, 188, 194]: Carotene xanthophyll.

δ) Oedogoniales

Bulbochaete setigera[15].
Bulbochaete species[200, 201].
Oedogonium species[12, 15, 16, 196, 198]: α-Carotene, β-carotene, xanthophyll, taraxanthin.

ε) Cladophorales

Cladophora glomerata[195, 12, 15, 16].
Cladophora rupestris[199]: β-Carotene, xanthophyll, violaxanthin.
Cladophora Sauteri[196, 198]: β-Carotene, xanthophyll, taraxanthin.
Spaeroplea species[201].

ζ) Siphonales

Vaucheria hamata[198]: Carotene, xanthophyll, violaxanthin.
Vaucheria species[195].

(vii). Charophyta

Chara ceratophylla Wallr.[197]: β-Carotene, γ-carotene, lycopene.
Chara fragilis[12, 15].
Nitella opaca[198]: Carotene, xanthophyll.
Nitella spores[16].
Nitella syncarpa (Thuill.)[197]: β-Carotene, γ-carotene, lycopene.

(viii). Phaeophyceae

Ascophyllum nodosum[12, 180]: Carotene+, [16, 198] fucoxanthin.
Chorda Filum[198]: Carotene, fucoxanthin.
Cladostephus spongiosus[198]: Carotene, fucoxanthin.
Cutleria multifida[183, 181].
Cystosira abrontanifolia[181].
Desmarestia aculeata[185].
Dictyota dichotoma[181, 183, 188, 198]: Carotene, fucoxanthin, xanthophyll.

Dictyota polypodioides[12, 181, 180].

Ectocarpaceae species[192].

Ectocarpus siliculosus[199]: β-Carotene, fucoxanthin, xanthophyll, violaxanthin, zeaxanthin.

Ectocarpus tomentosus[198]: Carotene, xanthophyll, fucoxanthin.

Elachistea species[183, 181].

Fucus ceranoides[198]: Carotene, fucoxanthin.

Fucus nodosus[183].

Fucus serratus[183, 184, 185, 12, 186, 181, 187, 180, 16, 188]: Fucoxanthin+, carotene++, xanthophyll.

Fucus species[182].

Fucus versoides[181].

Fucus vesiculosus[183, 189, 12, 190, 180, 16, 191, 198, 199]: β-Carotene, fucoxanthin, xanthophyll, violaxanthin, zeaxanthin.

Halydrys siliquosa[183, 185, 181, 180, 198]: Carotene, fucoxanthin.

Haliseris polypodioides[183, 181, 426]: β-Carotene, zeaxanthin.

Laminaria digitata[12, 181, 180, 16, 188, 198]: Carotene, fucoxanthin, xanthophyll.

Laminaria saccharina[183, 185, 12, 181, 180, 187, 16].

Leathesia marina[183, 181].

Padina Pavonia[181].

Pilayella littoralis[180, 198, 199]: Carotene, fucoxanthin, xanthophyll, violaxanthin.

Sphacellaria cirrhosa[198]: Carotene, fucoxanthin.

Stypocaulon scoparium[198]: Carotene, fucoxanthin.

(ix). *Rhodophyceae*

Ahnfeltia plicata[198]: Carotene, xanthophyll.

Bangia species[15, 195].

Batrachospermum moniliforme[185, 195, 15].

Callithamnion hiemale[193].

Ceramium diaphanum[193].

Ceramium rubrum[193, 194, 198, 199]: Carotene, xanthophyll, a little taraxanthin.

Chantransia species[15, 195].

Chondrus crispus[198, 16]: Carotene, xanthophyll.

Corallina officinalis[193, 198]: Carotene, xanthophyll.

Cystoclonium purpurascens[193].

Delesseria sanguinea[193].

Dilsea edulis[194, 199]: Carotene, xanthophyll[198].

Dumontia filiformis[193].

Furcellaria fastigiata[193, 194].

Gelidium corneum[198]: Carotene, xanthophyll.

Gigartina stellata[198]: Carotene, xanthophyll.

Laurencia pinnatifida[193].

Lemania fluviatilis[195, 15].

Lemania mamillosa[198]: Carotene, xanthophyll.

Nemalion multifidum[194].

Odonthalia dentata[194].

Phyllophora Brodiaci[180].

Phyllophora membranifolia[180].

Phyllophora membranifolia[198]: Carotene, xanthophyll.

Plocamium coccineum[198]: Carotene, xanthophyll.

Polyides rotundus[193, 194, 198]: Carotene, xanthophyll.

Polysiphonia fastigiata[198]: Carotene, xanthophyll.

Polysiphonia nigrescens[193, 194, 198]: Carotene, xanthophyll, fucoxanthin.

Polysiphonia species[12].

Porphyra hiemalis[193].

Porphyra laciniata[12, 194].

Porphyra umbilicalis[198]: Carotene, xanthophyll.

Porphyra vulgaris[184].

Rhodomela subfusca[193].

Rhodomela virgata[193].

Rhodymenia palmata[196, 198]: β-Carotene, xanthophyll, taraxanthin.

Spermothamnion roseolum[193].

(x). Fungi

a) Phycomycetes

Chytridium species[15].
Mucor flavus[15, 16].
Phycomyces[167].
Pilobolus crystallinus[15, 166]: Xanthophyll(?).
Pilobolus Kleinii[15, 166, 167]: Carotene, xanthophyll(?).
Pilobolus Oedipus[166]: Xanthophyll.
Pleotrachelus fulgens[166].

β) Eumycetes

1. Ascomycetes

Ascobolus species[171, 172].
Leotia lubrica[172, 173].
Nectria cinnabarina[174, 175, 15, 16].
Peziza aurantia[172].
Peziza (Lachnum) bicolor[174].
Peziza (Lachnea) scutellata[174].
Polystigma ochraceum = *Polystigma fulvum*[175].
Polystigma rubrum[167, 175]: Lycoxanthin and an acidic pigment.
Saccharomyces species[168]: Carotene.
Spathularia flavida[15, 172].
Sphaerostilbe coccaphili[16].
Sporobolomyces roseus[165]: Torulin, acidic pigments.
Sporobolomyces salmonicolor[165]: Torulin, acidic pigments.
Torula rubra[169, 170]: β-Carotene, torulin, torularhodin.

2. Basidiomycetes

Aecidio- and Basidio spores[15].
Aleuria aurantiaca[165]: α-Carotene, β-carotene, rubixanthin(?).
Allomyces[179]: β-Carotene, occasionally γ-carotene.
Calocera cornea[16].
Calocera palmata[16].
Calocera viscosa[16, 171].
Cantharellus cibarius[178]: α-Carotene, β-carotene, lycopene and two carotenoids of unknown constitution.
Cantharellus infundibuliformis[178]: Same pigments as in *Cantharellus lutescens* (below).
Cantharellus lutescens[178]: Lycopene, carotenoid of unknown constitution.
Coleosporium pulsatilla[178, 177].
Coleosporium senecionis[165]: α-Carotene, β-carotene, acidic pigment.
Dacryomyces stillatus[171].
Ditiola radicata[175].
Gymnosporangium juniperi-virginianae[136]: α-, β-, γ-Carotene.
Gymnosporangium juniperinum[174].
Melampsora aecidioides[176, 177].
Melampsora salicis capreae[174].
Neurospora crassa[440]: Neurospores.
Phragmidium violaceum[176].
Puccinia coronata[174].
Puccinia coronifera[165]: β-Carotene and acidic pigments.
Tremella mesenterica[165]: β-Carotene.
Triphragmium ulmariae[174].
Uredo (Coleosporium) euphrasie[177].
Uromyces alchemille[174].

B. CAROTENOIDS IN ANIMALS

I. INVERTEBRATES

Many investigations, some of which are now out of date, deal with the distribution of carotenoids in invertebrates. More recent studies in this field have shown that carotenoids are present in a variety of invertebrates, though it is not known with certainty whether the pigments are synthesized by the

animal or contained in the food. It would be of interest to examine the lower plants which serve as feeding stuffs for these classes of animals in more detail from this point of view.

a) *Arthropods*

TABLE 20 (References see p. 99–107)

CAROTENOIDS IN ARTHROPODS

(i) Insects

Bombyx mori[274, 275, 276, 266]: Carotene, xanthophyll.
Carausius morosus[278]: Protein-bound carotenoids.
Caterpillar of the cabbage butterfly[270]: α-Carotene, taraxanthin.
Coccinella septempunctata[269]: α-Carotene, β-carotene, lycopene.
Clythra quadripunctata[272]: Carotene.
Coleoptera coccinella[269]: α-Carotene, β-carotene, lycopene.
Locusta viridissima[278]: Protein-bound carotenoids.
Locustiden[278]: Protein-bound carotenoids.
Oedipoda coerulescens[269]: Traces of carotenoids.
Oedipoda miniata[269]: β-Carotene and an unknown pigment.
Perillus bioculatus[273]: Carotene.
Pieris brassicae[277]: Carotene, xanthophyll.
Pyrrhocoris apterus[268, 269]: Lycopene.
Rhynchota[267].
Schizoneura lanigera[270].
Sphinx ligustri[278]: Protein-bound carotenoids.
Tritogenaphis rudbeckiae[271].

(ii) Crustacea*, **

Ampelisca tenuicornis[282]: Carotene, xanthophyll.
Anapagurus chiroacanthus[281]: Astacene(?).
Astacus fluviatilis[287]: Astacene.
Astacus gammarus[288, 289]: Astacene, carotene.
Astacus[290]: Astacene.
Balanus balanus[281].
Balanus crenatus[282]: Carotene, xanthophyll.
Calanus finmarchianus[291]: Astacene, carotene.
Calocaris macandreae[282]: Carotene, xanthophyll.
Cancer pagurus[283, 284]: Astacene, Carotene.
Carcinus maenas[281]: Carotene.
Crangon allmani[282]: Carotene, xanthophyll.
Diaptomus bacillifer[279]: Carotene.
Ebalia tumefacta[282]: Carotene, xanthophyll.
Eupagurus prideauxii[291]: Astacene.
Eurynome aspera[281].
Galathea intermedia[282]: Astacene, carotene, xanthophyll.
Haploops tubicula[281].
Hyas araneus[281].
Idothea baltica[281].
Idothea emarginata[282]: Carotene, xanthophyll.
Idothea neglecta[282]: Carotene, xanthophyll.

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* This section is based on the summary given by O. Walker, *Dissertation*, Zürich, 1935, revised and completed up to 1946.

** Data concerning the occurrence of carotene and xanthophyll should not be regarded as conclusive as the pigments were only rarely isolated in the crystalline state.

Idya furcata[280].
Leander serratus[283]: Astacene.
Maja squinado[285, 286]: Astacene.
Munida banffia[282]: Astacene, carotene, xanthophyll.
Mysis flexuosa[282]: Carotene.
Neohela monstrosa[281].
Nephrops norvegicus[282, 283]: Astacene, carotene, xanthophyll.
Pagurus bernhardus[281]: Carotene, xanthophyll.
Pagurus rubescens[282]: Carotene.
Palaemon fabricii[281, 282]: Astacene, carotene, xanthophyll.
Palaemon serratus[283]: Astacene.
Palinurus vulgaris[283]: Astacene.
Pandalus brevirostris[282]: Astacene. carotene, xanthophyll.
Pandalus borealis[282]: Astacene(?).
Pandalus montagui[281]: Astacene, carotene.
Pontophilus spinosus[281].
Porcellana longicornis[282]: Carotene.
Portunus depurator[282]: Carotene.
Portunus longicornis[282]: Carotene, xanthophyll(?).
Portunus pusillus[282]: Carotene, xanthophyll(?).
Portunus puber[283]: Astacene.
Potamobius astacus = *Astacus fluviatilis*[283]: Astacene.
Scalpellum scalpellum[281].
Spirontocaris lilljeborgii[281].
Stenorhynchus species[281].

For further information regarding carotenoids in crustacea reference should be made to the investigations of LÖNNBERG (Ref. 17, p. 108).

b) *Molluscs**

The carotenoids of molluscs have been repeatedly studied. Recent investigations in this field are mainly due to LÖNNBERG and LEDERER.

TABLE 21 (References see p. 99–107)

CAROTENOIDS IN MOLLUSCS

(i) Amphineures

Lepidopleurus cancellatus[295]: Carotene, xanthophyll.
Tonicella marmorea[296]: Carotene(?), xanthophyll.
Chaetoderma nitidulum[296]: Carotene.

(ii) Lamellibranchia

Anomia ephippium[296]: Carotene.
Astarte banksi[295].
Astarte sulcata[296]: Carotene.
Axinus flexuosus[295].
Cardium echinatum[296]: Carotene. xanthophyll(?).
Cardium norvegicum[296].
Cardium tuberculatum[293]: Xanthophyll(?).
Cochleodesma praetenue[296]: Carotene.
Corbula gibba[295].
Cultellus pellucidus[296]: Carotene, xanthophyll.
Cyprina islandica[296, 297, 300]: Carotene.

* Crystalline pigments were obtained only in very few cases and most of the data are therefore not conclusive.

Dosina exolata[296, 297]: Xanthophyll(?).
Leda parvula[296]: Carotene, xanthophyll(?).
Lima loscombei[296]: Carotene, xanthophyll(?).
Lima hians[300].
Lucina borealis[296]: Carotene(?).
Lyonsia norvegica[295, 299].
Modiolaria marmorata[296]: Carotene(?). xanthophyll(?).
Mya truncata[296]: Carotene(?), xanthophyll(?).
Mytilus californianus[301]: Zeaxanthin, mytiloxanthin.
Mytilus edulis[297, 300]: Carotene (cryst.), xanthophyll(?).
Nucula sulcata[295, 296]: Carotene(?), xanthophyll.
Pecten jacobaeus[293]: Pectenoxanthin(?).
Pecten maximus[294]: Pectenoxanthin.
Pecten opercularis[296]: Carotene, xanthophyll(?).
Pecten septemradiatus[295].
Pecten striatus[296]: Carotene.
Pecten tigrinus[295].
Pectunculus glycimeris[297, 292]: Glycymerin, carotenoids.
Psammobia ferroensis[296]: Carotene, xanthophyll.
Saxicava rugosa[296]: Carotene, xanthophyll.
Solen ensis[296]: Carotene, xanthophyll.
Spisula solida[296]: Carotene(?), xanthophyll(?).
Spisula subtruncata[296]: Carotene, xanthophyll(?).
Syndosmia alba[295, 296]: Carotene(?).
Syndosmia nitida[295].
Tapes pullastra[296]: Carotene, xanthophyll.
Tellina crassa[296]: Carotene.
Thracia convexa[296]: Carotene.
Venus fasciata[296]: Carotene.
Venus gallina[296]: Carotene.
Venus ovata[296]: Carotene.
Vulsella barbata[296]: Carotene, xanthophyll(?).
Vulsella modiolus[296, 300]: Carotene, xanthophyll(?).

(iii) Scaphopoda

Dentalium entale[296]: Xanthophyll.

(iv) Gastropoda

α) *Opisthobranchia*:

Aeolis papillosa[295].
Acera bullata[296, 299]: Carotene.
Aplysia rosea[295].
Dendronotus frondosus[296]: Xanthophyll.
Doris repanda[295].
Doto coronata[296]: Carotene.
Philline aperta[296]: Carotene(?), xanthophyll.
Pleurobranchus species[293, 296]: Astacene(?).
Tritoma hombergi[296, 299].

β) *Prosobranchia*:

Acmaea virginea[295].
Aporrhais pes pelecani[296]: Carotene, xanthophyll.
Buccinum undatum[296]: Carotene, xanthophyll.
Calliostoma miliare[296]: Carotene, xanthophyll.
Capulus hungaricus[296]: Carotene, xanthophyll.
Emarginula crassa[295].
Emarginula fissura[295].
Gibbula cineraria[295]: Carotene, xanthophyll.
Gibbula tumida[296]: Carotene, xanthophyll(?).
Lacuna divaricata[295]: Carotene, xanthophyll.
Littorina littorea[296, 300]: Carotene, xanthophyll.
Nacella pellucida.

Nassa incrassata[296]: Carotene.
Nassa reticulata[296]: Carotene.
Natica nitida[296]: Carotene, xanthophyll(?).
Neptunea antiqua[295, 300].
Patella vulgaris[296]: Carotene, xanthophyll.
Purpurea lapillus[296]: Carotene, xanthophyll.
Rissoa species[296]: Carotene, xanthophyll.
Scalaria elatior[295, 299].
Stylifer stylifera[299].
Trivia europaea[296]: Carotene.
Trochus zizyphinus[296]: Carotene, xanthophyll(?).
Turritella communis[296]: Carotene, xanthophyll(?).
Velutina velutina[296]: Carotene, xanthophyll(?).

c) *Echinoderms*

Numerous species of echinoderms contain carotenoids. Here, too, specific polyene pigments are sometimes associated with particular animals. It is not known whether the carotenoids are derived from the food or whether they are synthesized by the animal. Most of the data regarding the distribution of carotenoids in echinoderms are of a qualitative nature, and cannot, therefore, be regarded as conclusive.

TABLE 22 (References see p. 99–107)

CAROTENOIDS IN ECHINODERMS

(i) Asteroidea

Asterias glacialis[304]: Carotene, xanthophyll(?), [296].
Asterias muelleri[295, 304].
Asterias rubens[296, 304, 305]: Asterinacid(?)[307].
Asterina gibbosa[306, 298]: Xanthophyll.
Astropecten irregularis[295, 304]: Xanthophyll(?), carotene.
Astropecten aurantiacus[306]: Xanthophyll.
Asteracanthion glacialis[306]: Xanthophyll.
Cribella oculata[298]: Carotene.
Crossaster papposus[296, 304]: Carotene, xanthophyll(?).
Goniaster equestris[298]: Carotene.
Henricia sanguinolenta[296]: Carotene, xanthophyll[304].
Hippasteria phrygiana[296]: Astacene, carotene, xanthophyll.
Luidia sarsii[296, 304]: Carotene.
Ophidiaster ophidianus[303]: Astacene.
Porania pulvillus[296]: Carotene, xanthophyll.
Solaster papposa[298]: Carotene.
Stichastrella endeca[305].
Stichastrella rosea[295].

(ii) Ophiuroidea

Amphiura chiajei[296, 304]: Carotene(?), xanthophyll.
Amphiura filiformis[304].
Ophiocoma nigra[296, 304]: Carotene(?), xanthophyll.
Ophiopholis aculeata[296, 304].
Ophiothrix fragilis[296, 304]: Astacene, carotene(?).
Ophiura affinis[304].
Ophiura texturata[296, 304]: Carotene(?), xanthophyll.

(iii) Crinoidea

Antedon petasus[295, 304]: Xanthophyll.

(iv) Echinoidea

Brissopsis lyrifera[296]: Xanthophyll.
Echinaster sepositus[293]: Astacene.
Echinus esculentus[296]: Xanthophyll(?)[305].
Psammechinus miliaris[296]: Xanthophyll.
Spatangus purpureus[296]: Carotene, xanthophyll.
Strongylocentrotus drobachiensis[296]: Carotene, xanthophyll.
Strongylocentrotus lividus[308]: α-Carotene, β-carotene, echinenone, pentaxanthin.

Holothurioidea

Cucumaria elongata[296]: Carotene, xanthophyll(?).
Cucumaria lactea[296, 304].
Holothuria brunnea[296]: Astacene(?).
Holothuria nigra [298]: Astacene(?).
Holothuria poli[306]: Astacene(?).
Holothuria tubulosa[302]: Astacene(?).
Mesothuria intestinalis[296, 304]: Xanthophyll.
Phyllophorus lucidus[296, 304]: Carotene.
Psolus phantapus[296, 304].
Thyone fusus[296, 304].

d) *Carotenoids in Worms*

Carotenoids in worms have recently been investigated, particularly by LÖNNBERG and by LÖNNBERG and HELLSTRÖM. Although these studies are mainly based on spectroscopic data, it appears very probable that carotene and xanthophyll occur in worms. The red carotenoids with the single absorption band previously reported by KRUKENBERG were not observed by the first-named authors. The following is a summary of carotenoids in worms which has been mostly taken from the dissertation of WALKER (Ref. 18, p. 108) and brought up to date.

TABLE 23 (References see p. 99–107)

CAROTENOIDS IN WORMS AND RELATED SPECIES

(i) Nemertini

Amphiporus pulcher[295].
Carinella annulata[295].
Cerebratulus fuscus[295].
Cerebratulus marginatus[295].
Malacobdella grossa[296]: Xanthophyll.

(ii) Polychaeta

Amphitrite affinis[295].
Aphrodite aculeata[295].
Arenicola marina[295].
Arenicola piscatorum[295].
Aricia norvegica[295].
Chaetopterus variopedatus[309]: Carotene.
Cirratulus cirratus[298]: Carotene.
Cirratulus tentaculatus[298]: Carotene.
Eumenia crassa[295].
Glycera goesii[296]: Carotene, xanthophyll(?).
Harmothoe sarsii[296]: Carotene.
Laetmonice filicornis[296]: Carotene.

Lepidonotus squamatus[295].
Lumbrinereis fragilis[296]: Carotene. xanthophyll(?).
Neoamphitrite figulus[296]: Carotene, xanthophyll.
Nephthys caeca[296]: Carotene.
Nephthys ciliata[295, 298]: Carotene.
Nereis virens[296, 298]: Carotene.
Nereis pelagica[295].
Pectinaria belgica[295].
Polymnia nebulosa[296]: Carotene(?), xanthophyll.
Polynoe spinifera[298].
Sabella penicillus[296]: Carotene, xanthophyll.
Siphonostoma diplochaitos[309]: Astacene(?).
Stylarioides plumosus[296]: Carotene, xanthophyll.
Terebella species[298].
Terebella stroemii[296]: Carotene, xanthophyll(?).
Thelepus cincinnatus[296]: Carotene, xanthophyll.

(iii) Gephyrea

Phascolosoma elongatum[296]: Carotene.
Priapulus caudatus[295].

(iv) Bryozoa

Alcyonidium gelatinosum[296]: Carotene, xanthophyll(?).
Bugula neritina[309]: Carotene.
Flustra foliacea[298]: Astacene.
Flustra securifrons[296]: Carotene, xanthophyll.
Lepralia foliacea[298]: Astacene.

(v) Brachiopoda

Crania anomala[296]: Carotene(?), xanthophyll.
Terebratulina caput serpentis[296]: Carotene.

e) *Coelenterates and Sponges*

Carotenoids in coelenterates and sponges have been repeatedly studied in recent years, especially by LÖNNBERG. In contrast to KRUKENBERG, and MACMUNN, this author did not observe any single-banded red carotenoids and describes only the presence of pigments of the carotene and xanthophyll type. Most studies in this field are again confined to spectroscopic observations, so that the data are not conclusive.

TABLE 24 (References see p. 99–107)

CAROTENOIDS IN COELENTERATES AND SPONGES

Actinia equina[297]: Actinioerythrin, [314, 293].
Alcyonium digitatum[296]: Carotene, xanthophyll.
Anemonia sulcata[314]: Sulcatoxanthin.
Aplysina aerophoba[311].
Axinea rugosa[295].
Axinella crista-galli[293]: Astacene.
Caryophyllia smithi[296]: Astacene(?), carotene.
Chondrosia reniformis[311].
Coccospongia species[311].
Dysidea fragilis[295].
Epiactis prolifera[315]: a carotenoid acid.
Esperia foliata[296]: Carotene.

Halcampa duodecirrhata[296]: Carotene. xanthophyll.
Halichondria albescens[312]: Astacene(?).
Halichondria caruncula[312]: Carotene.
Halichondria incrustans[312]: Carotene. xanthophyll.
Halichondria panicea[295]: Carotene.
Halichondria rosea[312]: Carotene.
Halichondria seriata[312]: Carotene, xanthophyll.
Halma Bucklandi[312]: Astacene(?).
Hircinia spinosula[311]: Carotene.
Hymeniacidon sanguineum[316]: Echinenone, α-carotene, γ-carotene.
Leuconia fossei[312]: Astacene(?).
Lucernaria quadricornis[296]: Carotene, xanthophyll(?).
Metridium dianthus[296]: Carotene, xanthophyll(?).
Metridium senile[313]: Astacene, carotene, phytoxanthins.
Papillina suberea[311].
Permatula phosphorea[296]: Carotene, xanthophyll.
Protanthea simplex[296]: Carotene(?), xanthophyll.
Radiella spinolaria[296]: Carotene.
Reniera aquaeductus[311].
Sagartia undata[296]: Xanthophyll.
Sagartia viduata[296]: Carotene, xanthophyll(?).
Stenogorgia rosea[296]: Carotene.
Suberites domuncula[293, 311]: Astacene(?).
Suberites ficus[296]: Carotene.
Suberites flavus[311]: Carotene, xanthophyll.
Suberites massa[296, 311]: Carotene.
Tedania muggiana[311]: Carotene.
Tentorium semisuberites[296]: Carotene.
Tealina felina[314]: Actinioerythrin.
Tethya lyncurium[311]: Carotene.
Tubularia indivisa[312]: Astacene(?).
Tubularia larynx[296]: Carotene.
Urticina felina[296]: Carotene, xanthophyll.

f) *Chordata*

TABLE 24a (References see p. 99–107)

CAROTENOIDS IN CHORDATA

(i) Hemichordata: Enteropneusta

Harrimania kupferi[295].

(ii) Tunicata

Ascidia virginea[295].
Botryllus schlosseri pallus[296, 310]. Xanthophyll, capsanthin, capsorubin, pectenoxanthin.
Ciona intestinalis[296]: Xanthophyll.
Clavellina lepadiformis[296]: Carotene, xanthophyll.
Corella parallelogramma[296]: Carotene(?), xanthophyll.
Cynthia papillosa[293, 291]: Astacene, cynthiaxanthin, α-carotene, β-carotene.
Dendrodoa grossularia[310]: α-Carotene, β-carotene, astacene.
Microcosmus sulcatus[291]: Phytoxanthins.
Molgula occulta[296]: Carotene(?), xanthophyll(?).
Muxilla mammillaris[296]: Carotene(?), xanthophyll(?).
Styela rustica[296]: Carotene, xanthophyll(?).
Synoicum pulmonaria[295].

2. VERTEBRATES

a) *Mammals*

A great deal of information is available in the literature concerning the occurrence of carotenoids in mammals. Carotenoids have been found in nearly every part of the mammalian organism. It is almost certain that these carotenoids are derived from the vegetable feeding stuffs. It is not known, however, whether the pigments undergo chemical changes in the animal body, or whether they are stored unchanged.

In the following sections the occurrence of polyene pigments in mammals is described. The treatment given is not complete; it is merely meant to illustrate the manifold distribution of carotenoids in mammalian organisms.

(i) *Faeces*

Recent investigations have shown that part of the carotenoids taken with the food leave the animal body unchanged[1], while the rest is absorbed in the intestine and then reaches the various organs.

Cow dung[316]*:* Carotene, xanthophyll.
Sheep dung[317]*:* Xanthophyll(?).

It is noteworthy that on feeding α-carotene or β-carotene to experimental animals, only the same pigment is found in the faeces[318].

(ii) *Blood serum*[319]

A variety of carotenoids have been found in the blood serum of human beings, horses, cows, calves, oxen, sheep, pigs, goats, cats and rats. The composition of the pigments depends on the diet fed to the animals. On the other hand, no polyene pigments have been found in the sera of dogs, guinea pigs and rabbits.

Blood of pregnant women[322]: Carotene.
Serum (cattle)[321]: Carotene, xanthophyll, cryptoxanthin.
Serum (human)[323]: Carotene, lycopene, xanthophyll.
Serum (human)[323]: β-Carotene, lycopene, β-hydroxycarotene(?), β-hydroxysemicarotenone(?) xanthophyll, occasionally zeaxanthin.

(iii) *Fat tissues*

Bone marrow (human)[328, 329]: Carotenoid.
Fat tissues (cats)[324]: Carotene, xanthophyll.
Fat tissues (cows)[327]: α-Carotene, β-carotene.
Fat tissues (horses)[325]: Carotene.
Fat tissues (human)[330]: Carotene, lycopene, xanthophyll, capsanthin.
Intestine and subcutaneous fat (horses and cattle)[326]: Xanthophyll.

References p. 99–107.

(iv) *Nerve tissues*

Nerves of humans and cows[331]: Carotenoids.

Peripheral nerves[332]: Carotenoids.

(v) *Inner Organs, Glands, Secretions*[333]

Corpora rubra of cows[356]: β-Carotene.
Corpus luteum of cows[349, 350, 351, 352, 353, 354, 355]: α-Carotene, β-carotene and traces of xanthophyll.
Gallstones (cattle)[346, 347]: Carotene, xanthophyll.
Heart tissues[348]: Carotenoids.
Hypophyses of cattle[358]: Carotenoids.
Kidneys (human beings, horses, guinea pigs, cats, dogs, pigs)[334, 335, 336, 337, 338, 326]: Carotene, xanthophyll.
Liver[335, 339, 326]: Carotenoids.
Liver (human)[323]: Carotene, lycopene, zeaxanthin, xanthophyll, violaxanthin(?), 2 pigments of unknown constitution (degradation products of β-carotene).
Liver (human)[343]: Carotene, xanthophyll, lycopene.
Liver (pigs)[344]: Carotene.
Milk fats (all mammals)[351, 360, 361, 362, 363, 364]: Carotene, a little xanthophyll, pseudo-α-carotene(?)[364].
Placenta (cow)[365, 322]: β-Carotene.
Placenta (human)[356, 357, 365, 322, 358]: Carotene and xanthophyll.
Skin[365]: Carotenoids[366].
Spleen[338]: Carotene.
Testes (various mammals)[359]: Carotenoids.
Yellow skin of diabetic subjects[369, 225, 370]: Carotenoids.
Yellow skin produced by special diet [367]: Carotenoid[368].

(vi) *Other parts*

Blood of umbilical cord[373]: Carotene.
Colostrum (women)[372]: Carotene.
Retina[371]: β-Carotene, retinene.

b) *Birds*

The numerous investigations on the pigments of the plumage of birds bear witness to the great interest shown by chemists and zoologists in these natural compounds. Other parts of birds, such as the skin, the inner organs, and the fat tissues, have also been repeatedly examined. The majority of these studies, have been carried out, however, with very small amounts of material, and have therefore yielded merely qualitative data. Crystalline pigments have only been obtained very rarely. Most of the available information consists of spectroscopic data or even colour reactions with concentrated sulphuric acid or antimony trichloride and cannot, therefore, be regarded as conclusive.

The following parts of birds have been examined:

(i) Feathers;
(ii) Fat tissues;
(iii) Foot skin, body skin, beaks;
(iv) Blood serum and inner organs;
(v) Egg yolk.

References p. 99–107.

As a result of these studies, certain regularities have emerged which are briefly summarised in the following paragraphs:

(i) *Carotenoids in Feathers*

The colours of the feathers of birds, particularly of sub-tropical and tropical birds, are often of extraordinary brilliance. Apart from basic blue and white pigments and melanin pigments, various red and yellow lipochromes are present, some of which possess carotenoid character. No example of the isolation of a crystalline carotenoid from feathers seems to have been recorded and the available information is wholly restricted to spectroscopic data. Nevertheless it appears certain that bird feathers contain *only* phytoxanthins (and their transformation products), especially those with two hydroxyl groups (xanthophyll, zeaxanthin, capsanthin[18]).

By suitable feeding experiments BROCKMANN and VÖLKER[19] showed that canaries which have white feathers as a result of a xanthophyll-free diet are unable to absorb β-carotene, lycopene or violaxanthin and to deposit these in the feathers, even though the birds were in an otherwise healthy condition. The feathers only assume their original yellow colour after feeding xanthophyll or zeaxanthin. It was shown by SAUERMANN[20] that paprica pigments are adsorbed in the same way as xanthophyll and zeaxanthin and are deposited in the feathers which, in this case, assume a red colouration. Examination of the feathers of xanthophyll-fed birds showed that, apart from xanthophyll, a transformation product of the latter was present, which has been termed canaryxanthophyll (p. 337). In some birds yet another carotenoid, picofulvin, has been found. The nature of canaryxanthophyll and picofulvin is not yet known. The former is *not* identical with xanthophyll epoxide (p. 206) as might be assumed in view of the similar absorption spectra (unpublished observation by KARRER and JUCKER).

Apart from well-defined pigments such as xanthophyll and zeaxanthin and pigments of unknown structure such as canaryxanthophyll and picofulvin, some birds also contain carotenoids which appear to be related to astacene. Even these more recent investigations are of a purely qualitative nature, while some of the older observations, e.g. those of KRUKENBERG, are now only of historical interest. Thus KRUKENBERG distinguished between 5 red and 5 yellow lipochrome-type pigments. Of the red pigments, zoonerythrin[21] and rhodophan[22] were most widely distributed. The behaviour of these two pigments is strongly reminiscent of polyene pigments and their spectral properties and possible combination with protein suggests a certain resemblance with astacene or astaxanthin. The latest investigations by LÖNNBERG[23] and by BROCKMANN and VÖLKER confirm the probable relation to astacene.

The nature of the yellow pigments mentioned by KRUKENBERG has been

References p. 108.

elucidated in one case: zoofulvin has been identified with xanthophyll. The structure of picofulvin, on the other hand, is still unknown.

(ii) *Carotenoids from fat tissues, foot skin, body skin and beaks*

It was mentioned above that no carotenoid, or other polyene pigment of hydrocarbon nature, has been found in bird feathers. By contrast, the beaks and skin of various animals contain mixtures of carotene and xanthophyll. At an early date, KRUKENBERG[24] established the presence of lipochromes in the fat tissues and foot skin of many animals. In 1930, LÖNNBERG[25] showed that chloriosulfurin consisted of a mixture of xanthophyll and carotene, and zoofulvin of almost pure xanthophyll. Further investigations in this field have been carried out by KUHN and BROCKMANN[26] and by CAPPER, MCKIBBIN and PRENTICE[27]. With two exceptions (*phasianus colchicus*, "Rosen" and *Anser domesticus*), all these investigations are of a purely qualitative nature.

(iii) *Carotenoids from blood serum and inner organs*

It has long been known that a lipochrome pigment is present in the blood serum of pigeons and chicken. It was later identified by spectroscopic means as xanthophyll by SCHUNCK[28]. The same pigment has been found in the livers of chicken[29]. It appears that the carotene and cryptoxanthin contained in the diet (maize) are quickly degraded, while xanthophyll, which has no vitamin A activity, is stored.

(iv) *Carotenoids from egg yolk*

The pigments from egg yolk have long aroused the interest of chemists. STÄDELER[30] was the first to isolate a crystalline pigment with well-defined properties from chicken egg yolk. THUDICHUM[31] classified this pigment as one of the "luteins" (lipochromes) and it was related to xanthophyll by SCHUNCK[32]. In 1912, WILLSTÄTTER and ESCHER[33] proved the xanthophyll nature of the pigment isolated by STÄDELER and in 1931 KUHN and co-workers showed it to be a mixture of xanthophyll and zeaxanthin[34]. The composition of the egg yolk pigments can be altered by varying the diet[35]. Carotene has also been shown to be present in chicken egg yolk[36].

TABLE 25 (References see p. 99–107)

CAROTENOIDS IN BIRDS*

Acanthis flammea (red forehead)[374]: Astacene(?), carotenoids.
Ampelis garrula[374]: Astacene(?), carotenoids.
Anser domesticus (retina)[375]: Astacene, astaxanthin[376].
Anas penelope (foot skin)[374].
Anas platyrhyncha (red foot skin, beak

* The above table is taken from O. WALKER, *Dissertation*, Zürich, 1935.

skin)[374]: Carotene, xanthophyll, and decomposition products*.

Anas platyrhyncha domestica[374] (yellow foot skin and beak skin): Carotene, xanthophyll and decomposition products.

Anser domesticus (beak skin)[356]: Carotene, xanthophyll and decomposition products.

Aprosmictus melanurus (yellow feathers)[377]: Astacene(?) carotenoids of unknown constitution.

Astur gentilis (yellow feathers)[374]: Carotene, xanthophyll.

Cacatua roseicapilla (red feathers)[377]: Carotenoids with single absorption bands.

Calurus auriceps (red feathers): Carotenoids with single absorption bands.

Campethera nubica (feathers): Carotenoids of unknown constitution[377].

Cardinalis virginianus (feathers)[377]: Carotenoids with single absorption bands.

Carduelis spinus (feathers)[378]: Xanthophyll and transformation products.

Carduelis carduelis (feathers)[378]: Xanthophyll and transformation products.

Certhiola mexicana (feathers): Carotenoids of unknown constitution[377].

Chloris chloris (feathers)[378]: Xanthophyll and transformation products.

Chloronerpes aurulentus (feathers)[377]: unknown pigments.

Chloronerpes kirki (feathers): Carotenoids of unknown constitution[377].

Chloronerpes yucatensis (feathers)[378]: Picofulvin (violaxanthin and taraxanthin(?).

Chlorophanes atricapilla (feathers)[377]: unknown pigments.

Chrysoptilus punctigula (feathers)[377]: Carotenoids of unknown constitution.

Colaptes auratus (feathers)[377].

Colaptes olivaceus (feathers)[377].

Cotinga coerulea (feathers)[377]: Carotenoids with single absorption bands.

Cymbirhynchus macrorhynchus[377]: Carotenoids with single absorption bands.

Dendropicos cardinalis (feathers)[377].

Diphyllodes magnifica (yellow neck feathers)[377].

Dryocopus auratus (feathers)[377].

Dryobates major (black feathers)[378]: Picofulvin.

Eclectus polychlorus (feathers)[377]: Carotenoids with single absorption bands and carotenoids of unknown constitution with two or three absorption bands.

Emberiza citrinella (feathers): Xanthophyll and decomposition products[374, 378].

Emberiza icterica (feathers)[378]: Xanthophyll and decomposition products.

Euphone nigricollis (feathers)[377]: Carotenoids of unknown constitution.

Fringilla canaria (feathers)[377, 378].

Gallus bankiva domesticus[377, 378] (yellow footskin): Carotenoids.

Hypoxanthus rivolii (feathers)[378]: Picofulvin.

Ithaginis cruentatus (feathers)[377]: Carotenoids with single absorption bands.

Loxia curvirostra (feathers)[374, 378]: Carotenoids with single absorption bands, xanthophyll and decomposition products.

Lyrurus tetrix[377, 374]: Carotenoids with single absorption bands.

Megaloprepia magnifica (feathers)[377]: Carotenoids with single absorption bands.

Milvus (foot skin)[377]: Carotenoids with single absorption bands.

Motacilla cinerea (feathers)[378]: Xanthophyll and decomposition products.

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* Canaryxanthophyll predominates amongst the transformation products of xanthophyll.

Oriolus galbula (feathers)[377].

Oriolus oriolus (feathers)[378]: Xanthophyll and transformation products.

Oriolus xanthonotus (feathers)[374]: Xanthophyll and transformation products.

Paradisea papuana (feathers)[377]: Carotenoids with single absorption bands.

Paradisea rubra (feathers)[377]: Carotenoids with single absorption bands.

Paroaria cucullata (feathers)[377]: Carotenoids with single absorption bands.

Parus coeruleus (feathers)[378]: Xanthophyll and transformation products.

Parus major (feathers)[378]: Xanthophyll and transformation products.

Phasianus colchicus x torquatus (feathers)[374]: Astacin (?).

Phasianus colchicus ("Rosen")[378, 375]: Astacin (crystallised).

Phlogoena cruenta (feathers)[377]: Carotenoids with single absorption bands.

Phoenicopterus antiquorum (feathers)[377]: Carotenoids with single absorption bands.

Phylloscopus sibilatrix[378]: Xanthophyll and transformation products.

Picidae species (feathers)[377]: Carotenoids with single absorption bands and picofulvin.

Picus canus (feathers)[378]: Picofulvin.

Picus major (feathers)[377]: Carotenoids with single absorption bands.

Picus viridis (feathers)[378]: Picofulvin.

Pinicola species (feathers)[374]: unknown pigments.

Ploceus cucullatus (feathers)[378]: Xanthophyll and transformation products.

Pyrocephalus rubineus (feathers)[377]: Carotenoids with single absorption bands.

Pyromelana franciscana (feathers)[378]: Carotenoids with single absorption bands.

Pyrrhula pyrrhula[374, 378]: Carotenoids with single absorption bands, xanthophyll and transformation products.

Pyrrhula vulgaris (feathers)[377]: Carotenoids with single absorption bands.

Regulus regulus (feathers)[377]: Carotenoids with single absorption bands.

Seleucides alba (feathers)[377].

Serinus canaria (feathers)[378]: Xanthophyll and transformation products.

Serinus canaria serinus (feathers)[378]: Xanthophyll and transformation products.

Sittace macao (feathers)[377]: Carotenoids with single absorption bands and other unknown pigments.

Somateria mollissima (yellow foot skin)[374]: Carotenoids of unknown constitution.

Tetrao tetrix ("Rosen")[374]: Carotenoids with single absorption bands.

Tiga tridactyla (feathers)[377]: Carotenoids of unknown constitution.

Trogon massena (feathers)[377]: Carotenoids with single absorption bands.

Turdus merula (Beak skin and throat skin)[374]: unknown pigments.

c) *Fish*

The carotenoids of fish have been repeatedly investigated, but the isolation of the crystalline pigments has only rarely been achieved. It can be regarded as certain, however, that fish (skin, flesh, inner organs, eyes) almost invariably contain carotenoids. Xanthophyll, carotene, astacin, and according to LÖNN-

BERG*, taraxanthin, appear to predominate. It should be mentioned, however, that most of the available data merely show that the occurrence of carotenoids is probable. The designation of individual pigments appears unjustified in the majority of cases since the pigments were neither isolated nor chromatographically separated. It is probable that a carotenoid mixture was generally obtained in which one component predominated and that only the absorption bands of this component were observed in the spectroscopic determination. A careful chromatographic analysis would probably have shown the presence of other pigments as well. The carotenoids present in the eyes of fish mostly include xanthophyll and taraxanthin(?) (cf. LÖNNBERG, Ref. 37, p. 109).

TABLE 26 (References see p. 99–107)

CAROTENOIDS IN FISH**

Abramis brama[380].
Agonus cataphractus[380].
Ammodytes lanceolatus[380].
Anguilla anguilla[370]: Xanthophyll[382], carotene.
Antherina presbyter[381]: Xanthophyll.
Aphiga minuta[379]: Taraxanthin.
Arnoglossus megastoma[381]: Xanthophyll.
Barbus fluviatilis[383].
Belone belone[379].
Belone rostrata[383].
Beryx decadactylus[384]: Astacene[385].
Bothus maximus[379]: Taraxanthin.
Bothus rhombus[379]: Xanthophyll, taraxanthin.
Callionymus lyra[382, 379]: Xanthophyll, carotene.
Carassius auratus[381, 386]: Lycopene(?)[385], astacene, carotene.
Caranx trachurus[379]: Xanthophyll.
Centrolabrus exoletus[380]: Astacene[379], xanthophyll, taraxanthin.
Clupea harengus[379, 381]: Xanthophyll, taraxanthin.
Coregonus albula[387] (eggs): Asterin acid.
Cottus bubalis[379, 381, 382]: Carotene, xanthophyll, taraxanthin.
Cottus scorpius (skin and muscles)[397, 380]: Xanthophyll, taraxanthin.
Crenilabrus melops[379, 380]: Xanthophyll or taraxanthin.
Crenilabrus suillus[379, 380]: Taraxanthin or xanthophyll.
Cyclopterus lumpus (fins)[380]: Taraxanthin or xanthophyll.
Cyclopterus lumpus[379]: Taraxanthin.
Cyclopterus lumpus (liver)[388]: Astacene.
Cyprinus auratus[389].
Cyprinus carpio[389].
Eleginus navaga (Ovaries)[165]: β-Carotene, 3 phytoxanthins.
Embiotocidae[390]: Carotene, a carotenoid acid and a phytoxanthin.
Esox anguilla[380].
Esox lucius[380].
Esox lucius (fins)[393]: 2 Phytoxanthins, similar to taraxanthin or eloxanthin = xanthophyll epoxide.
Esox lucius (liver)[339]: Xanthophyll.
Esox lucius (roe)[338]: Carotene, xanthophyll.
Esox lucius (spermatozoa)[338]: Carotene, xanthophyll.
Fundulus parvipinnis[391]: Taraxanthin(?), phytoxanthins.
Gadus aeglefinus[379]: Taraxanthin.
Gadus callarias[379, 381, 382]: Carotene, xanthophyll, taraxanthin.

* It is doubtful whether the pigment found by E. LÖNNBERG, *Arkiv. Zool. 31* A (1938) No. 1 is really taraxanthin as the latter is hypophasic and not epiphasic as described by LÖNNBERG.

** Unless otherwise stated, the data refer to skin.

Gadus callarias (roe): Carotene, xanthophyll[338].
Gadus esmarkii[380].
Gadus merlangus[380, 379]: Taraxanthin.
Gadus minutus[379, 382]: Carotene, xanthophyll, taraxanthin.
Gadus pollachius[379, 380]: Taraxanthin.
Gadus virens[379, 380]: Taraxanthin.
Gaidropsarus cimbrius[379]: Xanthophyll.
Gaidropsarus mustela[382]: Xanthophyll, carotene.
Gasterosteus aculeatus[379]: Taraxanthin.
Gasterosteus spinachia[381]: Xanthophyll.
Gobius niger[382]: Carotene, xanthophyll.
Hippoglossus hippoglossus[379, 338] (roe): Xanthophyll, carotene, zeaxanthin.
Hippoglossus platessoides[379, 380].
Labrus bergsnyltrus (scales, fins)[382]: Carotene, xanthophyll, taraxanthin.
Labrus bergsnyltrus[379]: Xanthophyll.
Labrus melops[382]: Carotene, xanthophyll
Labrus ossifagus[380, 379]: Carotene, xanthophyll.
Leuciscus rutilus (liver)[339, 379]: Xanthophyll, taraxanthin.
Lophius piscatorius[402] (liver): Astacene, taraxanthin[165, 379] and a carotenoid similar to eloxanthin.
Lota vulgaris (roe)[338]: Carotene, xanthophyll.
Molva molva[379, 380].
Muraena helena[394].
Mullus barbatus[394].
Nerophis aequoreus[382]: Carotene, xanthophyll.
Nerophis ophidion[379]: Taraxanthin.
Orthagoriscus mola[381]: Carotene.
Osmerus eperlanus[381]: Carotene.
Perca fluviatilis (fins)[165]: Astacene, taraxanthin.
Perca fluviatilis[380] (fins): Astacene.
Pholis gunellus[379, 380]: Carotene, xanthophyll, taraxanthin.
Pleuronectes flesus[382, 381]: Carotene, xanthophyll.
Pleuronectes kitt[379, 380, 382]: Carotene, xanthophyll, taraxanthin.
Pleuronectes limanda[379, 381]: Carotene, xanthophyll, taraxanthin.
Pleuronectes microcephalus[381]: Carotene.
Pleuronectes platessa[381]: Carotene.
Raja clavata[379]: Xanthophyll.
Raja batis[379]: Xanthophyll.
Raniceps raninus[379, 380]: Xanthophyll.
Regalecus glesne (liver)[396]: Astacene.
Salmo salar (meat)[397]: Carotene, salmon acid (astacene?)[398].
Salmo salar (liver)[339]: Xanthophyll.
Salmo trutta[379]: Taraxanthin.
Salmo irideus[438]: β-Carotene, astaxanthin.
Scomber scombrus[382, 381, 379]: Carotene, xanthophyll, taraxanthin.
Scophthalmus norvegicus[379, 382]: Carotene, xanthophyll, taraxanthin.
Scorpaena scrofa[394]: Astacene.
Sebastes marinus[399]: Astacene.
Shark (embryo)[392]: Carotene, xanthophyll, zeaxanthin.
Siphostoma typhle[379, 381]: Xanthophyll, taraxanthin.
Solea solea[379, 380].
Solea variegata[381]: Carotene.
Solea vulgaris (roe)[338]: Carotene.
Spinachia spinachia[380].
Syngnatus acus[379, 381]: Xanthophyll, taraxanthin.
Trachinus draco[379]: Taraxanthin.
Trigla gurnardus[379, 381]: Taraxanthin.
Trigla hirundo[381]: Xanthophyll.
Zeus faber[381]: Xanthophyll.
Zoarces viviparus[379, 382]: Carotene, xanthophyll, taraxanthin.

d) *Amphibia*

TABLE 27 (References see p. 99–107)

CAROTENOIDS IN AMPHIBIA

Batrachier species[405].
Bombinator igneus[404]: Lacertofulvin (xanthophyll?).
Bufo calamita[403, 411]: Xanthophyll.
Bufo calamita (ovaries): Xanthophyll(?)[403].
Bufo viridis[403].
Bufo vulgaris[403].
Frogs (retina)[400]: Carotenoids of unknown constitution.
Frogs (retina, fat tissues, skin)[401]: xanthophyll(?).
Hyla arborea and Rana esculenta[403].
Rana esculenta (skin, ovaries, fat tissues)[406]: Carotene, xanthophyll, zeaxanthin.
Rana esculenta (liver): α-Carotene, β-carotene, xanthophyll, zeaxanthin[406].
Rana temporaria, rana esculenta, rana bufo (skin, liver, ovaries, ovarian ducts, eggs, fat tissues, kidneys, testes)[407, 408]: Carotene, xanthophyll.
Salamandra maculosa[403]: Xanthophyll(?).
Triton cristatus[403].
Triton cristatus (fat tissues)[403]: Xanthophyll(?).

e) *Reptiles*

Our knowledge of reptile pigments is even more deficient than that of amphibia pigments. The investigations of KÜHNE and KRUKENBERG show that the pigments of snakes are not carotenoids. Salamanders and tortoises, on the other hand, appear to contain carotenoids (Ref. 38 and 39, p. 109).

TABLE 28 (References see p. 99–107)

CAROTENOIDS IN REPTILES

Chamaeleon vulgaris[404]: Lacertofulvin (xanthophyll?).
Chrysemis scripta elegans (eye)[165]: γ-Carotene; (back): α-carotene(?); (intestine): β-carotene and a phytoxanthin.
Clemmys insculpata (retina)[410]: Astacene.
Lacerta agilis[404].
Lacerta muralis[404].
Tortoise (blood serum, fat tissues)[409]: Carotenoids.

f) *Miscellaneous*

Alfalfa (after acid treatment)[424]: five new carotenoids.
Bees wax[417, 429]: Xanthophyll derivative, xanthophyll, carotene.
Carrot leaves[431]: α-Carotene, β-carotene, γ-carotene.
Egg yolk[415]: β-Carotene, cryptoxanthin, xanthophyll.
Elodea canadensis[412, 413]: Carotene, eloxanthin = xanthophyll epoxide, xanthophyll.
Marine soil[419]: α-Carotene, β-carotene, xanthophyll.
Oil from acacia acuminata[420]: β-Carotene and pigments of unknown constitution.

Plankton[422]: Carotenoids.

Pyracantha coccinia[414]: α-Carotene, β-carotene, γ-carotene, lycopene, xanthophyll epoxide, xanthophyll (flavoxanthin).

Sardine oil[421]: Carotene, xanthophyll and (sometimes) fucoxanthin.

Seaweed[423]: Carotene, xanthophyll.

Seatang[418]: Carotene.

Swamp[416]: Carotene, xanthophyll.

LITERATURE REFERENCES

1. WACKENRODER, cf. p. 128. — P. KARRER, O. WALKER, *Helv. Chim. Acta 16* (1933) 641. — P. KARRER, K. SCHÖPP and R. MORF, *Helv. Chim. Acta 15* (1932) 1158. — D. VAN STOLK, J. GUILBERG and H. PENAU, *Chimie & Industrie 27* (1932) 550. — R. KUHN and E. LEDERER, *Naturwissenschaften 19* (1931) 306; *Ber. 64* (1931) 1349. — R. KUHN and H. BROCKMANN, *Naturwissenschaften 21* (1933) 44; *Ber. 66* (1933) 407. — H. v. EULER and E. NORDENSON, *Z. physiol. Chem. 56* (1908) 223, etc.
2. WAKEMANN, *Am. J. Pharm. 23* (1935) 873.
3. R. KUHN, A. WINTERSTEIN and H. ROTH, *Ber. 64* (1931) 333. — C. LIEBERMANN, *Ber. 44* (1911) 850.
4. J. FORMANEK, *J. prakt. Chem. 62* (1900) 310.
5. L. S. PALMER, *Carotenoids and related Pigments*, New York 1922.
6. M. W. LUBIMENKO, *Rev. gén. botan. 25* (1914) 475. — H. KYLIN, *Z. physiol. Chem. 163* (1927) 229.
7. L. S. PALMER, *Carotenoids and related Pigments*, New York 1922.
8. L. S. PALMER, cf. footnote 7. — J. C. LANZING and A. G. VAN VEEN, *Chem. Centr. 1938*, I, 2081.
9. L. SCHMID and R. LANG, *Monatsh. 72* (1939) 322.
10. J. M. PETRIE, *Biochem. J. 18* (1924) 957.
11. J. C. MILLER and H. M. CORINGTON, *Proc. Am. Soc. Hort. Sci. 40* (1942) 519.
12. T. TAMMES, *Flora 87* (1900) 205.
13. COURCHET, *Ann. sci. nat. Botan.* Ser. VII, 7 (1888) 263.
14. H. KYLIN, *Z. physiol. Chem. 163* (1927) 229.
15. F. G. KOHL, *Untersuchungen über das Carotin und seine physiologische Bedeutung in den Pflanzen*, 1–165, Berlin 1902.
16. C. VAN WISSELINGH, *Flora 107* (1914) 371.
17. A. HANSEN, *Dissertation Würzburg, Botan. Centr. 20* (1884) 36.
18. P. KARRER and A. OSWALD, *Helv. Chim. Acta 18* (1935) 1303.
19. W. GUGELMANN, *Dissertation Zürich* 1938; G. TAPPI and P. KARRER, *Helv. Chim. Acta 32* (1949) 50.
20. BIDGOOD, *J. Roy. Hort. Soc. 29* (1905) 463. — C. A. SCHUNCK, *Proc. Roy. Soc.* (London) *72* (1903) 165.
21. BIDGOOD, *J. Roy. Hort. Soc. 29* (1905) 463.
22. R. KUHN and A. WINTERSTEIN, *Naturwissenschaften 18* (1930) 754.
23. BIDGOOD, *J. Roy. Hort. Soc. 29* (1905) 463.
24. W. F. O'CONNOR, P. J. DRUMM, *Nature* (London) *147* (1941) 58.
25. R. KUHN, A. WINTERSTEIN and W. WIEGAND, *Helv. Chim. Acta 11* (1928) 716.
26. R. KUHN and A. WINTERSTEIN, *Naturwissenschaften 18* (1930) 754.
27. WEHMER, *Pflanzenstoffe* (1931) 2nd Ed., Vol. II, p. 957.
28. C. A. SCHUNCK, *Proc. Roy. Soc.* (London), *72* (1903) 165.
29. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 213* (1932) 192.
30. P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. Chim. Acta 28* (1945) 1146.
31. P. KARRER and A. NOTTHAFFT, *Helv. Chim. Acta 15* (1932) 1195.
32. H. H. ESCHER, *Helv. Chim. Acta 11* (1928) 752.
33. P. KARRER and E. JUCKER, *Helv. Chim. Acta 29* (1946) 1539.
34. H. SCHRÖTTER–KRISTELLI, *Botan. Centr. 61* (1895) 33.
35. L. DIPPEL, *Flora 61* (1878) 17.
36. H. H. STRAIN, *J. Biol. Chem. 123* (1938) 425.
37. H. SCHMALFUSS, *Z. physiol. Chem. 131* (1923) 166. — H. SCHMALFUSS and K.

Keitel, *Z. physiol. Chem. 138* (1924) 156.
38. A. Tschirch, *Ber. deut. botan. Ges. 22* (1904) 414.
39. T. Ito, H. Suginome, K. Ueno and Sh. Watanabe, *Bull. Soc. Chem. Japan 11* (1936) 770.
40. R. Kuhn, A. Winterstein and E. Lederer, *Z. physiol. Chem. 197* (1931) 141.
41. K. Schön, *Biochem. J. 30* (1936) 1960.
42. P. Karrer, E. Jucker and E. Krause-Voith, *Helv. Chim. Acta 30* (1947) 538.
43. P. Karrer and E. Jucker, *Helv. Chim. Acta 27* (1944) 1585.
44. A. G. Perkin, *J. Chem. Soc. 101* (1912) 1538.
45. R. Kuhn and E. Lederer, *Z. physiol. Chem. 213* (1932) 188.
46. H. Molisch, *Ber. deut. botan. Ges. 14* (1896) 18.
47. R. Kuhn and A. Winterstein, *Ber. 64* (1931) 326.
48. P. Karrer and J. Rutschmann, *Helv. Chim. Acta 27* (1944) 1684; *25* (1942) 1624.
49. E. G. Hill and A. P. Sikkar, *J. Chem. Soc. 91* (1907) 1501.
50. R. Kuhn and A. Winterstein, *Helv. Chim. Acta 12* (1929) 899.
51. Keegan, *Chem. News 113* (1916) 85,114.
52. M. E. Filhol, *Compt. rend. 39* (1854) 194.
53. L. Zechmeister and W. A. Schroeder, *Arch. Biochem. 1* (1942) 231.
54. W. A. Schroeder, *J. Am. Chem. Soc. 64* (1942) 2510.
55. L. Schmid and E. Kotter, *Monatsh. 59* (1932) 341.
56. L. Zechmeister, T. Béres and E. Ujhelyi, *Ber. 68* (1935) 1321; *69* (1936) 573.
57. G. and F. Tobler, *Ber. deut. botan. Ges. 28* (1910) 365.
58. L. Zechmeister and L. v. Cholnoky, *Z. physiol. Chem. 208* (1932) 27.
59. H. C. Sorby, *Proc. Roy. Soc.* (London) *21* (1873) 442.
60. P. Karrer and E. Jucker, *Helv. Chim. Acta 26* (1943) 626. — Cf. P. Karrer and co-workers, *Helv. Chim. Acta 28* (1935) 1155.
61. K. Schön, *Biochem. J. 32* (1938) 1566.
62. L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc. 65* (1943) 1535.
63. L. Zechmeister and P. Tuzson, *Ber. 63* (1930) 3203; *67* (1934) 170.
64. P. Karrer, E. Jucker and K. Steinlin, *Helv. Chim. Acta 30* (1947) 531.
65. P. Karrer and H. Salomon, *Helv. Chim. Acta 13* (1930) 1063.
66. R. Kuhn and E. Lederer, *Z. physiol. Chem. 200* (1931) 108.
67. P. Karrer and J. Rutschmann, *Helv. Chim. Acta 25* (1942) 1144.
68. P. Karrer and R. Morf, *Helv. Chim. Acta 15* (1932) 863.
69. L. Zechmeister and A. Polgár, *J. Biol. Chem. 140* (1941) 1.
70. R. Kuhn and H. Brockmann, *Ber. 66* (1933) 828. Who also give references to the earlier literature.
71. L. S. Palmer and H. L. Kempster, *J. Biol. Chem. 39* (1919) 299, 331.
72. G. Mackinney, *Plant Physiol. 10* (1935) 365.
73. O. C. Magistad, *Plant Physiol. 10* (1935) 187.
74. L. Schmid and A. Polaczek–Wittek, *Mikrochem. 27* (1939) 42.
75. P. Karrer, H. Salomon and H. Wehrli, *Helv. Chim. Acta 12* (1929) 790.
76. J. W. White, F. P. Zscheile and A. M. Brunson, *J. Am. Chem. Soc. 64* (1942) 2603.
77. R. Kuhn and Ch. Grundmann, *Ber. 67* (1934) 593.
78. B. Sullivan, C. H. Bailey, *J. Am. Chem. Soc. 58* (1936) 383.
79. H. v. Euler and M. Malmberg, *Chem. Centr. 1936*, II, 117.
80. H. A. Schuette and R. C. Palmer, *Oil and Soap 14* (1937) 295; *Chem. Centr. 1938*, I, 1898.
81. J. Zimmermann, *Rec. trav. Chim. 51* (1932) 1001.
82. W. J. Blackie and G. R. Cowgill, *Chem. Centr. 1939*, II, 1309.
83. A. H. Gill, *J. Ind. Eng. Chem. 9* (1917) 136; *10* (1918) 612.
84. W. Brash, *J. Soc. Chem. Ind.* (London) *45* (1926) 1483; *Chem. Centr. 1927*, I, 821.
85. R. F. Hunter and A. D. Scott, *Biochem. J. 35* (1941) 31.
86. C. Griebel and E. Bames, *Chem. Centr. 1916*, II, 102.
87. P. Karrer, H. v. Euler and H. Hellström, *Chem. Centr. 1932*, I, 1800.

88. R. KUHN and H. BROCKMANN, *Z. physiol. Chem.* *200* (1931) 255.
89. P. KARRER and O. WALKER, *Helv. Chim. Acta* *16* (1933) 641.
90. C. MANUNTA, *Helv. Chim. Acta* *22* (1934) 1153.
91. V. N. LUBIMENKO, *Chem. Abstracts* *14* (1920) 1697.
92. H. KYLIN, *Z. physiol. Chem.* *163* (1927) 229.
93. P. KARRER and H. WEHRLI, *Helv. Chim. Acta* *13* (1930) 1104.
94. M. W. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 475.
95. K. FESSLER, *Z. physiol. Chem.* *85* (1913) 148.
96. A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem.* *207* (1932) 25.
97. A. WINTERSTEIN, *Z. physiol. Chem.* *215* (1933) 51; *219* (1933) 249.
98. L. ZECHMEISTER and L. v. CHOLNOKY, *Ber.* *63* (1930) 423.
99. L. ZECHMEISTER and L. v. CHOLNOKY, *Ber.* *69* (1936) 422.
100. H. v. LOESECKE, *J. Am. Chem. Soc.* *51* (1929) 2439.
101. A. H. GILL, *J. Ind. Eng. Chem.* *10* (1918) 612.
102. H. THALER and K. E. SCHULTE, *Biochem. Z.* *306* (1940) 1.
103. P. KARRER and J. RUTSCHMANN, *Helv. Chim. Acta* *28* (1945) 1528.
104. J. L. W. THUDICHUM, *Proc. Roy. Soc.* (London) *17* (1869) 253.
105. H. BROCKMANN, *Z. physiol. Chem.* *216* (1933) 45.
106. P. KARRER and R. WIDMER, *Helv. Chim. Acta* *11* (1928) 751.
107. R. KUHN and CH. GRUNDMANN, *Ber.* *67* (1934) 341.
108. H. WILLSTAEDT, *Chem. Centr.* *1935*, II, 707.
109. R. KUHN and E. LEDERER, *Ber.* *64* (1931) 1349.
110. H. WILLSTAEDT, *Skand. Arch. physiol.* *75* (1936) 155.
111. H. v. EULER, *Arkiv. Kemi Mineral Geol.* Ser. B, *11* (1933) No. 18.
112. G. E. HILBERT and E. F. JANSEN, *J. biol. Chem.* *106* (1934) 97.
113. W. C. SHERMAN, *Chem. Centr.* *1941*, I, 2673.
114. A. H. GILL, *J. Ind. Eng. Chem.* *10* (1918) 612.
115. L. ZECHMEISTER and P. TUZSON, *Naturwissenschaften* *19* (1931) 307.
116. P. G. F. VERMAST, *Naturwissenschaften* *19* (1931) 442.
117. L. ZECHMEISTER and P. TUZSON, *Ber.* *69* (1936) 1878.
118. P. KARRER and E. JUCKER, *Helv. Chim. Acta* *27* (1944) 1695; *30* (1947) 536.
119. M. B. MATLACK and J. WASHINGTON, *Acad. Sci.* *24* (1934) 385.
120. M. B. MATLACK, *J. Biol. Chem.* *110* (1935) 249.
121. L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem.* *221* (1933) 278; *240* (1936) 191.
122. R. YAMAMOTO, S. TIN, *Chem. Centr.* *1934*, I, 1660.
123. R. YAMAMOTO, Y. OSIMA and T. GOMA, *Chem. Centr.* *1933*, I, 441.
124. G. B. RAMASARMA, S. D. RAO and D. N. HAKIM, *Biochem. J.* *40* (1946) 657.
125. KELLER, *Am. J. Pharm.* *68* (1896) No. 64.
126. L. ZECHMEISTER and K. SZILÁRD, *Z. physiol. Chem.* *190* (1930) 67. — L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem.* *196* (1931) 199.
127. L. S. PALMER, *J. Biol. Chem.* *23* (1915) 261.
128. A. H. GILL, GREENUP, *J. Oil Fat Ind.* *5* (1929) 288.
129. G. S. JAMIESON and W. F. BAUGHMAN, *Chem. Centr.* *1924*, I, 2752.
130. P. KARRER, F. RÜBEL and F. M. STRONG, *Helv. Chim. Acta* *19* (1935) 28.
131. R. YAMAMOTO and S. TIN, *Chem. Centr.* *1933*, I, 3090.
132. P. KARRER and W. SCHLIENTZ, *Helv. Chim. Acta* *17* (1934) 7.
133. P. KARRER and co-workers, *Helv. Chim. Acta* *15* (1932) 490.
134. K. SCHÖN, *Biochem. J.* *29* (1935) 1779.
135. K. SCHÖN, *Biochem. J.* *29* (1935) 1782.
136. B. L. SMITS, W. J. PETERSON, *Science* *96* (1942) 210.
137. P. KARRER and E. KRAUSE–VOITH, *Helv. Chim. Acta* *30* (1947) 1158.
138. J. L. COLLINS, *Science* *63* (1926) 52.
139. A. F. W. SCHIMPER, *Jb. wiss. Bot.* *16* (1885) 1.
140. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *509* (1934) 269.
141. R. KUHN and CH. GRUNDMANN, *Ber.* *67* (1934) 593.

142. L. Zechmeister and L. v. Cholnoky, *Ann. 489* (1931) 1.
143. L. Zechmeister and L. v. Cholnoky, *Ann. 481* (1930) 42.
144. R. Willstätter and H. H. Escher, *Z. physiol. Chem. 64* (1910) 47.
145. M. B. Matlack and Ch. E. Sando, *J. biol. Chem. 104* (1934) 407.
146. R. Kuhn and Ch. Grundmann, *Ber. 66* (1933) 1746.
147. K. Brass, A. Beyrodt and J. Mattausch, *Naturwissenschaften 25* (1937) 60.
148. G. Nowak and J. Zellner, *Monatsh. 42* (1921) 293.
149. F. Kryz, *Chem. Centr. 1920*, I, 533.
150. J. Stenhouse, *Ann. 98* (1856) 316; *Chem. Gaz. 1856*, 40.
151. J. Stenhouse and Ch. E. Groves, *J. Chem. Soc. 31* (1877) 551; *35* (1879) 688.
152. L. Zechmeister and P. Tuzson, *Ber. 63* (1930) 2881.
153. L. Zechmeister and A. Polgár, *J. biol. Chem. 139* (1941) 193.
154. H. Suginome and K. Ueno, *Chem. Centr. 1931*, II, 2892.
155. L. Zechmeister and P. Tuzson, *Ber. 67* (1934) 824.
156. H. Schrötter–Kristelli, *Botan. Centr. 61* (1895) 33. — G. Michaud and J. F. Tristan, *Arch. Soc. Phys. Nat.*, Genève, *37* (1913) 47.
157. T. N. Godnew and S. K. Korschenewsky, *Chem. Centr. 1931*, I, 1299.
158. G. and F. Tobler, *Ber. deut. botan. Ges. 28* (1910) 365, 496.
159. B. M. Duggar, *Washington Univ. Stud. 1* (1913) 22.
160. N. A. Monteverde and V. N. Lubimenko, *Bull. Acad. Sci. Petrograd* p. 6, 7, 11 (1913) 1105.
161. H. Willstaedt, *Chem. Centr. 1937*, I, 3658.
162. W. M. Brown, *J. biol. Chem. 110* (1935) 91.
163. R. Řetovský and A. Urban, *Chem. Centr. 1935*, I, 3068. — H. Dubljanskaja, *Chem. Centr. 1941*, I, 717.
164. W. Zopf, *Flora* (N.S.) *47* (1889) 353.
165. E. Lederer, *Bull. Soc. Chim. Biol. 20* (1938) 554.
166. W. Zopf, *Beitr. Phys. Morph. nied. Org. 2* (1892) 3.
167. E. Bünning, *Planta 26* (1937) 719; *27* (1937) 148.
168. H. S. Olcovich and H. A. Mattill, *Proc. Soc. Exp. Biol. Med. 28* (1930) 240.
169. H. Fink and E. Zenger, *Wschr. Brauerei 51* (1934) 89.
170. P. Karrer and J. Rutschmann, *Helv. Chim. Acta 26* (1943) 2109.
171. W. Zopf, *Z. wiss. Mikrosk. 6* (1889) 172.
172. W. Zopf, *Beitr. Phys. Morph. nied. Org. 2* (1892) 3.
173. W. Zopf, *"Die Pilze"*, Breslau 1890, p. 1–500.
174. E. Bachmann, *Ber. deut. botan. Ges. 4* (1886) 68.
175. W. Zopf, *Beitr. Phys. Morph. nied. Org. 3* (1893) 26.
176. Müller, *Landw. Jahrb. v. Thiel* (1886) 719.
177. G. Bertrand and G. Poirault, *Compt. Rend. 115* (1892) 828.
178. H. Willstaedt, *Chem. Centr. 1938*, II, 2272.
179. R. Emerson and D. L. Fox, *Proc. Roy. Soc.* (B) London, *128* (1940) 275.
180. H. Kylin, *Z. physiol. Chem. 82* (1912) 221.
181. H. Molisch, *Botan. Z. 63* (I) (1905) 131.
182. S. Rosanoff, *Mém. Soc. Sci. nat. Cherbourg 13* (1867) 195.
183. A. Millardet, *Ann. sci. nat. Botan.* Ser. 5, *10* (1869) 59.
184. H. C. Sorby, *Proc. Roy. Soc.* (London) *21* (1873) 442.
185. J. Reinke, *Jb. wiss. Bot. 10* (1876) 399.
186. N. Gaidukov, *Ber. deut. botan. Ges. 21* (1903) 535.
187. M. Tswett, *Botan. Z. 63* (II) (1905) 273; *24* (1906) 235.
188. R. Willtätter and H. J. Page, *Ann. 404* (1914) 237.
189. A. Hansen, *Botan. Z. 42* (1884) 649.
190. M. Tswett, *Ber. deut. botan. Ges. 24* (1906) 235.
191. I. M. Heilbron and R. F. Phipers, *Biochem. J. 29* (1935) 1373.
192. E. Askenasy, *Botan. Z. 27* (1869) 785.
193. H. Kylin, *Z. Physiol. Chem. 74* (1911) 105.
194. H. Kylin, *Z. physiol. Chem. 166* (1927) 39.
195. H. Nebelung, *Botan. Z. 36* (1878) 369, 385, 401.

196. I. M. HEILBRON and R. F. PHIPERS, *Biochem. J. 29* (1935) 1369.
197. P. KARRER, W. FATZER, M. FAVARGER and E. JUCKER, *Helv. Chim. Acta 26* (1943) 2121.
198. I. M. HEILBRON, *J. Chem. Soc.*, London 1942, 79.
199. H. KYLIN, *Sällskap. Lund Förh. 9* (1939) 213; *Chem. Abstracts 1939*. 5115³.
200. DE BARY, *B. Naturf. Ges. Freib. 13* (1856) 222.
201. F. COHN, *Arch. Mikrosk. Anat. 3* (1867) 1.
202. J. ROSTAFINSKY, *Botan. Z. 39* (1881) 461.
203. BORODIN, *Bull. l'Acad. imper. Sci. St-Petersbourg 28* (1883) 328; *Botan. Z. 41* (1883) 577.
204. H. MOLISCH, *Ber. deut. botan. Ges. 14* (1896) 18.
205. BASHIR AHMAD, *Biochem. J. 24* (1930) 860.
206. G. KARSTEN, *Botan. Z. 47* (1891) 300.
207. W. ZOPF, *Beitr. Phys. Morph. nied. Org. 1* (1892) 30.
208. J. TISCHER, *Z. physiol. Chem. 243* (1936) 103.
209. R. CASPARY, *Flora* (N.S.) *16* (1858) 579.
210. A. B. FRANK, *Cohn's Beitr. Biol. Pfl. 2* (1877) 123.
211. HILDEBRAND, *Botan. Z. 19* (1861) 81.
212. F. G. KOHL, *Ber. deut. botan. Ges. 24* (1906) 124, 222.
213. F. COHN, *Nova Acta Leopoldina Carolin. Acad. 22*, II (1850) 649.
214. G. KLEBS, *Untersuchungen bot. Inst. Tübingen, 1* (1883) 261.
215. W. ZOPF, *Biol. Z. 15* (1895) 417.
216. JACOBSEN, *Botan. Z. 72* (1912) 38.
217. W. MEVIUS, *Ber. deut. botan. Ges. 41* (1923) 237.
218. J. TISCHER, *Z. physiol. Chem. 250* (1937) 147.
219. G. KLEBS, *Botan. Z. 39* (1881) 249, 265, 281, 297, 313, 329.
220. K. BORESCH, *Ber. deut. botan. Ges. 40* (1923) 288.
221. W. ZOPF, *Ber. deut. botan. Ges. 18* (1900) 461.
222. NELLO PACE, *J. biol. Chem. 140* (1941) 483.
223. F. SCHÜTT, *Ber. deut. botan. Ges. 8* (1890) 9.
224. N. WILLE, *Jb. wiss. Bot. 18* (1887) 473.
225. N. GAIDUKOV, *Ber. deut. botan. Ges. 18* (1900) 331.
226. G. KLEBS, *Untersuchungen bot. Inst. Tübingen 1* (1883) 261.
227. V. WITTICH, *Virchows Arch. path. Anat. 27* (1863) 573.
228. J. TISCHER, *Z. physiol. Chem. 239* (1936) 257; *267* (1941) 281; cf. R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber. 72* (1939) 1688.
229. A. GARCIN, *J. bot. 3* (1889) 189; *Z. wiss. Mikrosk. 6* (1889) 529.
230. F. KUTSCHER, *Z. physiol. Chem. 24* (1898) 360.
231. J. TISCHER, *Z. physiol. Chem. 251* (1938) 109; *260* (1939) 257.
232. G. KRAUS and A. MILLARDET, *Compt. rend. 66* (1868) 505.
233. N. A. MONTEVERDE, *Acta Horti Petropolitani 13* (1893) 201.
234. G. KRAUS, *Zur Kenntnis der Chlorophyllfarbstoffe und ihrer Verwandten, spektroskopische Untersuchungen*, Stuttgart 1872.
235. H. MOLISCH, *Ber. deut. botan. Ges. 14* (1896) 18.
236. I. M. HEILBRON and B. LYTHGOE, *J. Chem. Soc. 1936*, 1376.
237. P. KARRER and J. RUTSCHMANN, *Helv. Chim. Acta 27* (1944) 1691.
238. V. READER, *Biochem. J. 19* (1925) 1039.
239. E. CHARGAFF, *Compt. rend. 197* (1933) 946.
240. E. CHARGAFF and J. DIERYCK, *Naturwissenschaften 20* (1932) 872.
241. T. NAKAMURA, *Bull. chem. Soc. Japan 11* (1936) 176.
242. Y. TAKEDA, T. OHTA, *Z. physiol. Chem. 268* (1941) I–II.
243. W. ZOPF, *Ber. deut. botan. Ges. 9* (1891) 22.
244. H. F. M. PETTER, *Amsterdam Acad. Sci. 34* (1931) No. 10.
245. C. A. BAUMANN, H. STEENBOCK, M. A. INGRAHAM and E. B. FRED, *J. biol. Chem. 103* (1933) 339.
246. M. A. INGRAHAM and H. STEENBOCK, *Biochem. J. 29* (1935) 2553.
247. Y. TAKEDA and T. OHTA, *Z. physiol. Chem. 262* (1939) 168; *265* (1940) 233.
248. E. CHARGAFF, *C. Bakt. Parasitenkunde* 1. Abt., *119* (1930) 121.
249. P. KARRER and U. SOLMSSEN, *Helv. Chim. Acta 18* (1935) 25.

250. H. Gaffron, *Biochem. Z. 279* (1935) 33.
251. P. Karrer and U. Solmssen, *Helv. Chim. Acta 18* (1935) 1306; *19* (1936) 3; cf. H. Koenig, *Dissertation* Zürich 1940.
252. P. Karrer and U. Solmssen, *Helv. Chim. Acta 18* (1935) 25, 1306; *19* (1936) 3, 1019. — P. Karrer, U. Solmssen and H. Koenig, *Helv. Chim. Acta 21* (1938) 454. — P. Karrer and H. Koenig, *Helv. Chim. Acta 23* (1940) 460.
253. E. Lederer, *Compt. rend. 197* (1933) 1694.
254. P. Karrer and J. Rutschmann, *Helv. Chim. Acta 26* (1943) 2109.
255. C. E. Skinner and M. F. Gunderson, *J. Biol. Chem. 97* (1932) 53.
256. M. A. Ingraham and C. A. Baumann, *J. Bact. 28* (1934) 31.
257. E. Chargaff, *Z. physiol. Chem. 218* (1933) 223.
258. F. B. Gurd and W. Denis, *J. exper. Med. 14* (1911) 606.
259. Ch. Grundmann and Y. Takeda, *Naturwissenschaften 25* (1937) 27.
260. Y. Takeda and T. Ohta, *Z. physiol. Chem. 258* (1939) 7.
261. E. Chargaff and E. Lederer, *Ann. Inst. Pasteur 54* (1935) 383.
262. C. B. van Niel and J. H. C. Smith, *Arch. Microbiol. 6* (1935) 219; cf. *Arch. Biochem. 5* (1944) 243.
263. L. Zechmeister and co-workers, *Arch. Biochem. 5* (1944) 243.
264. E. Schneider, *Chem. Centr. 1936*, I, 3525.
265. H. F. Haas and L. D. Bushnell, *J. Bact. 48* (1944) 219.
266. M. Oku, *Chem. Centr. 1930*, II, 2538; *Chem. Centr. 1932*, II, 2325.
267. H. C. Sorby, *Quart. J. Sci.* (N.S.) *29* (1871) 64.
268. C. Phisalix, *Compt. rend. 118* (1894) 1282.
269. E. Lederer, *Compt. rend. Soc. biol. 117* (1934) 413.
270. Fr. N. Schulz, *Biochem. Ztschr. 127* (1922) 112.
271. L. S. Palmer and H. H. Knight, *J. biol. Chem. 59* (1924) 451.
272. W. Zopf, *Beitr. Phys. Morph. nied. Org. 1* (1892) 30.
273. L. S. Palmer and H. H. Knight, *J. biol. Chem. 59* (1924) 443.
274. F. G. Dietel, *Klin. Wschr. 12* (1933) 601.
275. C. Rand, *Biochem. Z. 281* (1935) 200.
276. Barbera, *Ann. Chim. applic. 23* (1933) 501.
277. C. Manunta, *Chem. Centr. 1935*, I, 3559.
278. H. Junge, *Z. physiol. Chem. 268* (1941) 179.
279. W. Zopf, *Beitr. Phys. Morph. nied. Org. 3* (1893) 26.
280. A. Lwoff, *Compt. rend. Soc. Biol. 93* (1925) 1602.
281. E. Lönnberg, *Arkiv. Zool. 22*, A (1931) No. 14, 1.
282. E. Lönnberg and H. Hellström, *Arkiv. Zool. 23* A (1931) No. 15, 1.
283. R. Fabre and E. Lederer, *Compt. rend. Soc. Biol. 113* (1933) 344; *Bull. Soc. Chim. Biol. 16* (1934) 105.
284. C. A. MacMunn, *Proc. Roy. Soc.* London *35* (1883) 370.
285. R. Maly, *Monatshefte 2* (1881) 351.
286. R. Kuhn, E. Lederer and A. Deutsch, *Z. physiol. Chem. 220* (1933) 229.
287. W. D. Halliburton, *J. Physiol. 6* (1885) 300.
288. R. Kuhn and E. Lederer, *B. 66* (1933) 488.
289. J. Verne, *Compt. rend. Soc. Biol. 83* (1920) 963, 988.
290. H. v. Euler, H. Hellström and E. Klussmann, *Z. physiol. Chem. 288* (1934) 77.
291. E. Lederer, *Bull. Soc. chim. Biol. 20* (1938) 567.
292. E. Lederer, *Bull. Soc. Chim. Biol. 20* (1938) 567. — E. Lederer and R. Fabre, *Chem. Centr. 1934*, I, 3757.
293. P. Karrer and U. Solmssen, *Helv. Chim. Acta 18* (1935) 915.
294. E. Lederer, *Compt. rend. Soc. Biol. 116* (1934) 150.
295. E. Lönnberg, *Arkiv. Zool. 22*, A (1931) No. 14, 1.
296. E. Lönnberg and H. Hellström, *Arkiv. Zool. 23*, A (1931) No. 15, 1.
297. R. Fabre and E. Lederer, *Compt. rend. Soc. Biol. 113* (1933) 344; *Bull. Soc. Chim. Biol. 16* (1934) 105.
298. Ch. A. MacMunn, *Quart. J. Microscop. Sci.* (N.S.) *30* (1890) 51.
299. E. Lönnberg, *Arkiv. Zool. 25*, A (1932) No. 1.

300. E. LÖNNBERG, *Arkiv. Zool. 26* (1933) No. 7.
301. B. T. SCHEER, *J. Biol. Chem. 136* (1940) 275.
302. C. DE MEREKOWSKY, *Compt. rend. 93* (1881) 1029.
303. P. KARRER and F. BENZ, *Helv. chim. Acta 17* (1934) 412.
304. E. LÖNNBERG, *Arkiv. Zool. 26*, A (1933) No. 7.
305. E. LÖNNBERG, *Arkiv. Zool. 25*, A (1932) No. 1.
306. C. F. W. KRUKENBERG, cf. *Physiol. Stud.* Ser. II, part *3* (1882) 41, 92.
307. H. v. EULER, and H. HELLSTRÖM, *Z. physiol. Chem. 223* (1934) 89.
308. E. LEDERER, *Compt. rend. 201* (1935) 300.
309. C. F. W. KRUKENBERG, cf. *Physiol. Stud.* Ser. II, part *3* (1882) 6, 22.
310. E. LEDERER, *Compt. rend. Soc. Biol. 117* (1934) 1086.
311. C. F. W. KRUKENBERG, cf. *Physiol. Stud.* Ser. I, part 2 (1880) 65; Ser. II, part 3 (1882) 108.
312. C. A. MACMUNN, *J. Physiol. 9* (1888) 1.
313. D. L. FOX and C. F. A. PANTIN, *Trans. Roy. Soc.* London, B, *230* (1941) 415.
314. I. M. HEILBRON, H. JACKSON and R. N. JONES, *Biochem. J. 29* (1935) 1384.
315. D. L. FOX and C. R. MOE, *Chem. Centr. 1938*, II, 2272.
315. P. KARRER and A. HELFENSTEIN, *Helv. Chim. Acta 13* (1930) 86.
317. H. FISCHER, *Z. physiol. Chem. 96* (1915) 292.
318. R. KUHN and H. BROCKMANN, *Ber. 64* (1931) 1859.
319. E.g. J. L. W. THUDICHUM, *Proc. Roy. Soc.* (London) *17* (1869) 253. — KRUKENBERG, *Sitz.-Ber. Jenaer. Ges. Med. Naturwissenschaften 19* (1885) 1886. — A. A. HYMANS, H. VAN DEN BERGH and J. SNAPPER, *Dtsch. Arch. F. klin. Med. 110* (1913) 540. — L. S. PALMER and C. H. ECKLES, *J. biol. Chem. 17* (1914) 223. — B. v. EULER, H. v. EULER and H. HELLSTRÖM, *Biochem. Z. 203* (1920) 370. — C. L. CONNOR, *J. biol. Chem. 77* (1928) 619. — H. v. EULER and E. VIRGIN, *Biochem. Z. 245* (1932) 252. — H. v. EULER and E. KLUSSMANN, *Biochem. Z. 250* (1932) 1.
320. E. DÁNIEL and G. J. SCHEFF, *Proc. Soc. exper. Biol. Med. 33* (1935) 26. — E. DÁNIEL and T. BÉRES, *Z. physiol. Chem. 238* (1936) 160. — H. SÜLLMANN and co-workers, *Biochem. Z. 283* (1936) 263. — H. WILLSTAEDT and T. LINDQVIST, *Z. physiol. Chem. 240* (1936) 10.
321. A. E. GILLAM and M. S. EL RIDI, *Biochem. J. 29* (1935) 2465.
322. G. GAEHTGENS, *Klin. Wochschr. 16* (1937) 894, 1073.
323. H. WILLSTAEDT and T. LINDQVIST, *Z. physiol. Chem. 240* (1936) 10.
324. L. S. PALMER and C. H. ECKLES, *J. biol. Chem. 17* (1914) 211.
325. H. VAN DEN BERGH, P. MULLER and J. BROEKMEYER, *Biochem. Z. 108* (1920) 279.
326. H. v. EULER and E. KLUSSMANN, *Biochem. Z. 256* (1932) 11.
327. L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 154.
328. C. F. W. KRUKENBERG and H. WAGNER, *Z. Biol. 21* (1885) 25.
329. B. v. EULER, H. v. EULER and H. HELLSTRÖM, *Biochem. Z. 202* (1932) 370.
330. L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 231* (1935) 259.
331. D. H. DOLLEY and F. V. GUTHRIE, *J. med. research 40* (1919) 295.
332. H. v. EULER and E. KLUSSMANN, *Chem. Centr. 1932*, II, 2201.
333. H. VAN DEN BERGH and co-workers, *Biochem. Z. 108* (1920) 279; A. A. HYMANS, and co-workers, *Dtsch. Arch. klin. Med. 110* (1913) 540.
334. C. F. W. KRUKENBERG, *Virchows Arch. path. Anat. 101* (1885) 542.
335. H. VAN DEN BERGH, P. MULLER and J. BROEKMEYER, *Biochem. Z. 108* (1920) 279.
336. G. M. FINDLAY, *J. Path. Bact. 23* (1920) 482.
337. O. BAILLY and R. NETTER, *Compt. rend. 193* (1931) 961.
338. H. v. EULER, U. GARD and H. HELLSTRÖM, *Chem. Centr. 1932*, II, 2201.
339. H. v. EULER and E. VIRGIN, *Biochem. Z. 245* (1932) 252.
340. L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 234* (1935) 235.
341. L. RANDOIN and M. R. NETTER, *Bull. Soc. Chim. Biol. 15* (1933) 944.

342. O. Bailly, *Bull. Acad. Med. 107* (1935) 932.
343. L. Zechmeister and P. Tuzson, *Z. physiol. Chem. 234* (1935) 241.
344. L. Zechmeister and P. Tuzson, *Z. physiol. Chem. 239* (1936) 147.
345. G. Gaehtgens, *Klin. Wochschr. 16* (1937) 894, 1073.
346. H. Fischer and H. Röse, *Z. physiol. Chem. 88* (1913) 331.
347. H. Fischer and R. Hess, *Z. physiol. Chem. 187* (1930) 133.
348. D. H. Dolley and F. V. Guthrie, *J. Med. research 42* (1921) 289.
349. G. Piccolo and A. Lieben, *Giorn. Sci. nat. econ. Palermo 2* (1886) 258.
350. F. Holm, *J. prakt. Chem. 100* (1867) 142.
351. J. L. W. Thudichum, *Proc. Roy. Soc.* (London) *17* (1869) 253.
352. H. H. Escher, *Z. physiol. Chem. 83* (1913) 198.
353. R. Kuhn and E. Lederer, *Z. physiol. Chem. 200* (1931) 246.
354. P. Karrer and W. Schlientz, *Helv. Chim. Acta 17* (1934) 55.
355. H.v. Euler and E. Klussmann, *Chem. Centr. 1932*, I, 3458.
356. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
357. H. v. Euler and E. Klussmann, *Chem. Centr. 1932*, I, 3458; *Chem. Centr. 1932*, II, 2202; *Biochem. Z. 250* (1932) 1.
358. H. v. Euler, B. Zondek and E. Klussmann, *Chem. Centr. 1933*, I, 801.
359. F. Maass, *Arch. mikr. Anat. 34* (1889) 452.
360. L. S. Palmer and C. H. Eckles, *J. biol. Chem. 17* (1914) 191.
361. L. S. Palmer, *J. Biol. Chem. 27* (1916) 27. — L. S. Palmer, C. Kennedy and H. L. Kempster, *J. biol. Chem. 46* (1921) 559.
362. L. S. Palmer and C. H. Eckles, *J. biol. Chem. 17* (1914) 237.
363. H. Paffrath and A. Consten, *Z. Kinderheilk. 42* (1926) 51; *B. ges. Physiol. 38* (1927) 816.
364. A. E. Gillam and M. S. El Ridi, *Biochem. J. 30* (1936) 1735.
365. F. Smith, *J. Physiol. 15* (1894) 162. — L. S. Palmer and C. H. Eckles, *J. biol. Chem. 17* (1914) 221.
366. A. F. Hess and V. C. Myers, *J. Am. Med. Assoc. 73* (1919) 1743.
367. Miura, *Keizo Dohi Commemoration Volume*, Tokyo 1917, 203.
368. Schüssler, *Munich med. Wschr. 66* (1919) 597. — H. Salomon, *Wiener Klin. Wschr. 32* (1919) 495.
369. A. A. Hymans, H. van den Bergh and J. Snapper, *Dtsch. Arch. klin. Med. 110* (1913) 540.
370. L. S. Palmer, *Carotenoids and related Pigments*, New York 1922.
371. H. v. Euler and H. Hellström, *Svensk Kem. Tidskr. 45* (1933) 203. — O. Brunner, E. Baroni and W. Kleinau, *Z. physiol. Chem. 236* (1935) 257. — G. Wald, *Nature London, 134* (1934) 65; G. Wald and A. B. Clark, *J. gen. Physiol. 21* (1938) 93.
372. W. J. Dann, *Biochem. J. 27* (1933) 1998; *30* (1936) 1644.
373. H. Wendt, *Klin. Wochschr. 15* (1936) 222.
374. E. Lönnberg, *Arkiv. Zool. 21*, A, (1930) No. 11, 1.
375. R. Kuhn, J. Stene and N. A. Sörensen, *Ber. 72* (1939) 1688.
376. G. Wald and H. Zussman, *J. biol. Chem. 122* (1938) 449.
377. Krukenberg, cf. *Physiol. Stud.* Ser. I, part 5 (1881) 72; Ser. II, part 1 (1882) 151; *vol. 2* (1882) 1; part 3 (1882) 128.
378. H. Brockmann and O. Völker, *Z. physiol. Chem. 224* (1934) 193.
379. E. Lönnberg, *Arkiv. Zool. 31*, A, (1938) No. 1.
380. E. Lönnberg, *Arkiv. Zool. 21*, A, (1929) No. 10, 1.
381. J. T. Cunningham, C. A. MacMunn, *Trans. Roy. Soc.* (London) Ser. B 184 (1893) 765.
382. E. Lönnberg, *Arkiv. Zool. 23* A (1931) No. 16, 1.
383. C. F. W. Krukenberg, cf. *Physiol. Stud.* Ser. II, part 2 (1882) 55; part 3, 138.
384. Tutyia, *Bull. Soc. Sci. Fisheries 3* (1935) 242.
385. E. Lederer, *Compt. rend. Soc. Biol. 118* (1935) 542.
386. A. Weissberger and H. Bach, *Naturwissenschaften 20* (1932) 350.
387. H. v. Euler and co-workers, *Z. physiol. Chem. 228* (1934) 77.
388. N. A. Sörensen, *Z. physiol. Chem. 235* (1935) 8.

389. C. F. W. Krukenberg, cf. *Physiol. Stud.*(1882) Ser. II, part 2, 1; part 3, 138.
390. R. T. Young and D. L. Fox, *Biol. Bull. 71* (1936) 217.
391. F. B. Sumner and D. L. Fox, *J. exper. Zool. 66* (1933) 263; *71* (1935) 101; *Proc. Nat. Acad. Sci 21* (1935) 330.
392. H. v. Euler and U. Gard, *Chem. Centr. 1932*, I, 1544.
393. E. Lederer, *Caroténoides des animaux inférieurs*, Paris 1938.
394. C.F.W.Krukenberg, cf. *Physiol. Stud.* (1882) Ser. II, part 2, 55; part 3, 138.
395. C. F. Krukenberg and H. Wagner, *Z. Biol. 21* (1885) 25.
396. N. A. Sörensen, *Tidskr. Kjemi Bergves 15* (1934) 12.
397. H. v. Euler and H. Hellström, *Z. physiol. Chem. 223* (1934) 89.
398. A. Emmerie, M. van Eekelen, B. Josephy and L. K. Wolff, *Acta brev. neerl. physiol. pharm. Microbiol. 4* (1934) 139.
399. E. Lederer, *Bull. Soc. Chim. Biol. 20* (1938) 554, 567.
400. St. Capranica, *Arch. Physiol.* (1877), 287.
401. W. Kühne, *J. Physiol. 1* (1878) 109. — G. Wald, *J. Gen. Physiol. 19* (1936) 351.
402. N. A. Sörensen, *Kgl. Norske Vid. Selsk. Forh. 6* (1933) 154; *Tidskr. Kjemi Bergves 15* (1934) 12.
403. C. F. W. Krukenberg, cf. *Physiol. Stud.* (1882) Ser. II, part 2, 43.
404. C. F. W. Krukenberg, cf. *Physiol. Stud.* (1882) Ser. II, part 2, 50.
405. A. Magnan, *Compt. rend. 144* (1907) 1130, 1068.
406. L. Zechmeister and P. Tuzson, *Z. physiol. Chem. 238* (1936) 197.
407. F. G. Dietel, *Klin. Wochschr. 12* (1933) 601.
408. C. Rand, *Biochem. Z. 281* (1935) 200. — O. Brunner and R. Stein, *Biochem. Z. 282* (1935) 47.
409. W. D. Halliburton, *J. Physiol. 7* (1886) 324.
410. G. Wald and H. Zussman, *J. Biol. Chem. 122* (1938) 449.
411. E. Lönnberg, *Arkiv. Zool. 30*, A, No. 6 (1938) 10.
412. Donald Hey, *Biochem. J. 31* (1937) 532.
413. P. Karrer and J. Rutschmann, *Helv. Chim. Acta 28* (1945) 1526.
414. P. Karrer and J. Rutschmann, *Helv. Chim. Acta 28* (1945) 1528.
415. A. E. Gillam and I. M. Heilbron, *Biochem. J. 29* (1935) 1064.
416. O. Baudisch and H. v. Euler, *Chem. Centr. 1935*, II, 1390.
417. G. H. Vansell and C. S. Bisson, *Chem. Centr. 1936*, II, 1085.
418. G. Lunde, *Chem. Centr. 1937*, II, 1894.
419. D. L. Fox, *Chem. Centr. 1937*, II, 3899.
420. V. M. Trikojus and J. C. Drummond, *Nature*, London, *139* (1937) 1105.
421. B. E. Bailey, *Chem. Centr. 1938*, II, 2669.
422. A. E. Gillam, M. S. El Ridi and R. S. Wimpenny, *Chem. Centr. 1939*, I, 2624.
423. S. Muraveisky and J. Chertok, *Chem. Centr. 1939*, II, 1458.
424. F. W. Quackenbush, H. Steenbock and W. H. Peterson, *Chem. Centr. 1939*, II, 2929.
425. H. H. Strain and W. M. Manning, *J. Am. Chem. Soc. 65* (1943) 2258.
426. P. Karrer and co-workers, *Helv. Chim. Acta 19* (1936) 29.
427. L. Zechmeister and L. Cholnoky, *J. biol. Chem. 135* (1940) 31.
428. W. C. Sherman, *Chem. Abstracts* 1940, 2089[8].
429. J. Tischer, *Z. physiol. Chem. 267* (1941) 14.
430. M. Nakamura and S. Tomita, *Chem. Centr. 1941*, I, 2195.
431. G. Mackinney and H. Milner, *J. Am. Pharm. Ass. 55* (1933) 4728.
432. W. Zirpel, *Z. Botan. 36* (1941) 538.
433. G. S. Fraps and A. R. Kemmerer, *Ind. Eng. Chem. Anal. Ed. 13* (1941) 806.
434. P. Karrer and E. Jucker, *Helv. Chim. Acta 30* (1947) 1774.
435. H. H. Strain, W. M. Manning, *J. Am. Chem. Soc., 64* (1942) 1235.
436. H. H. Strain, W. M. Manning, G. Hardin, *Biochem. Bull., 86* (1944) 169, cf. *J. biol. Chem., 148* (1943) 655.
437. R. Retosky, *Collection of Czechoslovak. Chem. Communications 13* (1948) 631.
438. H. J. Bielig and F. Graf Medem, *Experientia* 5 (1949) 11.
439. J. C. Sadana, *Biochem. J. 44* (1949) 401.
440. F. Haxo, *Arch. Biochem. 20* (1949) 400.

REFERENCES

1. H. v. Euler, V. Demole, P. Karrer and O. Walker, *Helv. Chim. Acta 13* (1930) 1078. — R. Willstätter and A. Stoll, *Untersuchungen über das Chlorophyll*, Berlin 1913; *Untersuchungen über die Assimilation der Kohlensäure*, Berlin 1918. — R. Kuhn and H. Brockmann, *Ber. 64* (1931) 1859. — P. Karrer and W. Schlientz, *Helv. Chim. Acta 17* (1934) 7. — R. Kuhn, A. Winterstein and E. Lederer, *Z. physiol. Chem. 197* (1931) 141. — G. Mackinney. *J. Biol. Chem. 111* (1935) 75. — H. H. Strain, *Leaf Xanthophylls*, Washington 1938.
2. P. Karrer, E. Jucker, J. Rutschmann and K. Steinlin, *Helv. Chim. Acta 28* (1945) 1146. — P. Karrer, E. Krause-Voith, K. Steinlin, *Helv. Chim. Acta 31* (1948) 113.
3. R. Willstätter and A. Stoll, cf. footnote 1.
4. H. v. Euler, V. Demole, P. Karrer and O. Walker, *Helv. Chim. Acta 13* (1930) 1078.
5. Sorby, *Quart. J. Microscop. Sci. 19* (1871) 215. — Kraus, *Zur Kenntnis der Chlorophyllfarbstoffe und ihrer Verwandten*, Stuttgart, 1872, p. 1–131. — Arnaud, *Compt. rend. 109* (1889) 911. — Immendorf, *Landw. Jahrb. 18* (1889) 507. — H. Molisch, *Ber deut. Botan. Ges. 14* (1896) 18. — T. Tammes, *Flora 87* (1900) 205. — F. G. Kohl, *Untersuchungen über das Carotin und seine physiologische Bedeutung in den Pflanzen*, Berlin, 1902, p. 1–165. — C. A. Schunck, *Proc. Roy. Soc.* (London) *72* (1903) 165. — R. Willstätter and A. Stoll, *Untersuchungen über die Assimilation der Kohlensäure*, Berlin, 1918.
6. Older literature: J. L. W. Thudichum, *Proc. Roy. Soc.* (London) *17* (1869) 253. — L. Dippel, *Flora 61* (1878) 17. — T. Tammes, cf. ref. 5. — F. G. Kohl, cf. ref. 5. — C. van Wisselingh, *Flora 107* (1914) 371.
7. Wheldale, *The Anthocyanine Pigments of Plants*, Cambridge, 1916.
8. R. Willstätter and A. Stoll, *Untersuchungen über die Assimilation der Kohlensäure*, 1918.
9. E. Goerrig, *Beih. bot. Zbl. 35* (1917) 342.
10. Immendorf, *Landw. Jahrb. 18* (1889) 507. — F. G. Kohl, *Untersuchungen über das Carotin und seine physiologische Bedeutung in Pflanzen*, Berlin, 1902, p. 1–165. — C. A. Schunck, *Proc. Roy. Soc.* (London) *72* (1903) 165. — M. Tswett, *Ber. deut. Botan. Ges. 26* (1908) 88. — R. Willstätter and A. Stoll, cf. footnote 3. — H. v. Euler, V. Demole, A. Weinhagen and P. Karrer, *Helv. Chim. Acta 14* (1931) 831.
11. M. Tswett, *Ber. deut. Botan. Ges. 26* (1908) 88.
12. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
13. R. Willstätter and A. Stoll, footnote 8.
14. P. Karrer and O. Walker, *Helv. Chim. Acta 17* (1934) 43.
15. H. Kylin, *Chem. Abstracts* 1940, 7994 (*Kgl.. Fysiograf. Sällskap. Lund Förh. 9* (1939) 213.
16. I. M. Heilbron, *J. Chem. Soc.* 1942, 79.
17. E. Lönnberg, *Arkiv. Zool. 22*, A, (1931) No. 14; *23*, A, (1931), No. 15; 25, A, (1932), No. 1; 26, A, No. 7.
18. O. Walker, *Dissertation*, Zürich, 1935.
19. In this connection, reference should be made to the detailed investigation of H. Brockmann and O. Völker, *Z. physiol. Chem. 224* (1934) 193.
20. Dr. Sauermann, *Arch. intern. physiol.* 1889, 543.
21. M. Bogdanow, *Compt. rend. 46* (1858) 780.
22. W. Kühne, *J. Physiol.* (London) *1* (1878) 109.
23. E. Lönnberg, *Arkiv. Zool. 21*, A, (1930) No. 11, 1.
24. C. F. W. Krukenberg, *Physiol. Stud.* (1881) Ser. I, part 5, 72; (1882) Ser. II, part 1, 151; Ser. II, part 2, 1; Ser. II, part 3, 128.
25. E. Lönnberg, *Arkiv. Zool. 21*, A, (1930) No. 11, 1.
26. E. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
27. N. S. Capper, J. M. W. McKibbin and J. H. Prentice, *Biochem. J. 25* (1931) 265.
28. W. D. Halliburton, *J. Physiol.* (London) 7 (1886) 324. — C. A. Schunck, *Proc. Roy. Soc.* (London) *72* (1903) 165.

29. N. S. Capper, J. M. W. McKibbin and J. H. Prentice, *Biochem. J. 25* (1931) 265. — H. v. Euler and E. Klussmann, *Biochem. Z. 256* (1932) 11.
30. G. Städeler, *J. prakt. Chem. 100* (1867) 148.
31. J. L. W. Thudichum, *Proc. Roy. Soc.* (London) *17* (1869) 253.
32. C. A. Schunck, *Proc. Roy. Soc.* (London) *72* (1903) 165.
33. R. Willstätter and H. H. Escher, *Z. physiol. Chem. 76* (1912) 214.
34. R. Kuhn, A. Winterstein and E. Lederer, *Z. physiol. Chem. 197* (1931) 141.
35. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
36. R. Willstätter and H. H. Escher, *loc. cit.*; L. S. Palmer, *J. Biol. Chem. 23* (1915) 261. — H. v. Euler and H. Hellström, *Biochem. Z. 211* (1931) 252. — R. Kuhn and, H. Brockmann loc. cit.
37. E. Lönnberg, *Ark. Zool. 32*, A (1939) No. 8.
38. W. Kühne, *J. Physiol.* (London) *1* (1878) 109. — C. F. W. Krukenberg, cf. *Physiol. Stud. 1882* Ser. II part 2, 50.
39. C. F. W. Krukenberg, *loc. cit.* — W. D. Halliburton, *J. Physiol. 7* (1886) 324.

SPECIAL PART

CHAPTER X

Carotenoid hydrocarbons of known constitution

I. LYCOPENE $C_{40}H_{56}$

History

1873 HARTSEN[1] isolates a dark-red crystalline pigment, later identified as lycopene, from *Tamus communis* L.

1875 MILLARDET[2] obtains impure lycopene, which he terms solanorubin, from tomatoes.

1903 SCHUNCK[3] shows that the pigment from tomatoes, which he terms lycopene, has an absorption spectrum different from that of carotene.

1910 WILLSTÄTTER and ESCHER[4] make a detailed investigation of lycopene. They determine the correct molecular formula $C_{40}H_{56}$, and recognise that lycopene is an isomer of carotene.

1928–31 KARRER and co-workers[5] elucidate the constitution of lycopene.

1932 KUHN and GRUNDMANN[6] carry out the chromic acid oxidation of lycopene and obtain long-chain degradation products, the constitution of which confirms the formula of lycopene.

Occurrence

Recent investigations employing the highly refined chromatographic method of separation have shown that the tomato pigment is much more widely distributed in nature than was formerly believed. The frequent occurrence of lycopene in ripe fruit is especially striking (cf. p. 119 concerning the formation of the pigment during the ripening process). Lycopene is also found in other parts of plants and in animal sources, though often only in small quantities.

TABLE 29

VEGETABLE AND ANIMAL SOURCES FROM WHICH LYCOPENE HAS BEEN ISOLATED

Source	References
a) Fruit of:	
Actinophloeus Macarthurii	J. ZIMMERMAN, *Rec. Trav. chim. 51* (1932) 1001.
Bryonia dioica Jacq.	A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem. 207* (1932) 25, 32.

References p. 165–170.

Source	References
Citrullus vulgaris Schrd.	L. ZECHMEISTER and P. TUZSON, *Ber.* ***63*** (1931) 2881. — L. ZECHMEISTER and A. POLGÁR, *J. biol. Chem.* ***139*** (1941) 193.
Citrus decumana L.	M. B. MATLACK, *Chem. Centr.* ***1928***, I, 2948.
Citrus grandis Osb.	M. B. MATLACK, *Chem. Centr.* ***1935***, I, 95; *J. biol. Chem.* ***110*** (1935) 249.
Convallaria majalis L.	A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem.* ***207*** (1932) 25, 32.
Diospyros costata	K. SCHÖN, *Biochem. J.* ***29*** (1935) 1779.
Diospyros Kaki L.	P. KARRER and co-workers, *Helv. Chim. Acta* ***15*** (1932) 490.
Erythroxylon novogranatense	J. ZIMMERMAN, *Rec. Trav. chim.* ***51*** (1932) 1001.
Palm oil	R. F. HUNTER and A. D. SCOTT, *Biochem. J.* ***35*** (1941) 31.
Passiflora coerulea	P. KARRER and co-workers, *Helv. Chim. Acta* ***19*** (1936) 28.
Prunus armeniaca L.	H. BROCKMANN, *Z. physiol. Chem.* ***216*** (1933) 47.
Ptychosperma elegans	J. ZIMMERMAN, *Rec. Trav. chim.* ***51*** (1932) 1001.
Rosa canina	H. H. ESCHER, *Helv. Chim. Acta* ***11*** (1928) 753. — P. KARRER and R. WIDMER, *Helv. Chim. Acta* ***11*** (1928) 751.
Rosa rubiginosa	R. KUHN and C. GRUNDMANN, *Ber.* ***67*** (1934) 339.
Rosa rugosa Thumb.	H. WILLSTAEDT, *Chem. Centr.* ***1935***, II, 707; *Svensk Kem. Tidskr.* ***47*** (1935) 112.
Rubus Chamaemorus	H. WILLSTAEDT, *Skand. Arch. Physiol.* ***75*** (1936) 155.
Solanum Balbisii L.	H. KYLIN, *Z. physiol. Chem.* ***163*** (1927) 229. — L. ZECHMEISTER and L. v. CHOLNOKY, *Ber.* ***63*** (1930) 787.
Solanum Dulcamara L.	do.
Solanum Lycopersicum	C. A. SCHUNCK, *Proc. Roy. Soc.* (London) ***72*** (1903) 165. — R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem.* ***64*** (1910) 47. — R. KUHN and C. GRUNDMANN, *Ber.* ***65*** (1932) 1886. — H. v. EULER, P. KARRER, E. v. KRAUSS and O. WALKER, *Helv. Chim. Acta* ***14*** (1931) 154.
Tamus communis L.	L. ZECHMEISTER and L. v. CHOLNOKY, *Ber.* ***63*** (1930) 423.

b) Other vegetable and animal sources:

Source	References
Bacillus Grasberger	E. CHARGAFF and E. LEDERER, *Chem. Centr.* ***1936***, I, 3159.
Bacillus Lombardo Pellegrini	E. CHARGAFF and E. LEDERER, *Chem. Centr.* ***1936***, I, 3159.
Bact. Sarcina aurantica	V. READER, *Biochem. J.* ***19*** (1926) 1039.
Calendula officinalis	L. ZECHMEISTER and L. v. CHOLNOKY, *Z. physiol. Chem.* ***208*** (1932) 28. — P. KARRER and A. NOTTHAFT, *Helv. Chim. Acta* ***15*** (1932) 1196.
Chara (Anther)	P. KARRER and co-workers, *Helv. Chim. Acta* ***26*** (1943) 2121.

Source	References
Cuscuta salina	G. MACKINNEY, *J. biol. Chem.* ***112*** (1935/36) 421.
Cuscuta subinclusa	G. MACKINNEY, *J. biol. Chem.* ***112*** (1935/36) 421.
Dimorphotheca aurantiaca	P. KARRER and A. NOTTHAFFT, *Helv. Chim. Acta* ***15*** (1932) 1196.
Gazania rigens	L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc.* ***65*** (1943) 1535.
Gonocaryum obovatum and Gonocaryum pyriforme	V. N. LUBIMENKO, *Rev. gén. botan.* ***25*** (1914) 474. — A. WINTERSTEIN, *Z. physiol. Chem.* ***215*** (1933) 51; ***219*** (1933) 249.
Human liver	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem.* ***234*** (1935) 241. — H. WILLSTAEDT and T. LINDQVIST, *Z. physiol. Chem.* ***240*** (1936) 10.
Mimulus longiflorus	L. ZECHMEISTER and W. A. SCHROEDER, *Arch. Biochem.* ***1*** (1943) 231.
Saffron	R. KUHN and A. WINTERSTEIN, *Ber.* ***67*** (1934) 344.
Thiocystis-bacteria	P. KARRER and U. SOLMSSEN, *Helv. Chim. Acta* ***19*** (1936) 1019; ***18*** (1935) 25.
Vicia	P. KARRER and A. NOTTHAFFT, *Helv. Chim. Acta* ***15*** (1932) 1196.

TABLE 30

VEGETABLE AND ANIMAL SOURCES IN WHICH LYCOPENE HAS BEEN DETECTED

Source	References
a) Fruit of:	
Actinophloeus angustifolia	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Aglaonema nitidum	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Aglaonema oblongifolium	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Aglaonema oblongifolium Var. Curtisii	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Aglaonema simplex	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Arbutus Unedo	K. SCHÖN, *Biochem. J.* *29* (1935) 1779.
Archontophoenix Alexandrae	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Areca Alicae	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Arum italicum	V. N. LUBIMENKO, *Chem. Abstracts* *14* (1920) 1697.
Arum maculatum	H. KYLIN, *Z. physiol. Chem.* *163* (1927) 229.
Arum orientale	V. M. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Calyprocalyx spicatus	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.
Citrus aurantium (?)	L. ZECHMEISTER and P. TUZSON, *Naturwissenschaften* *19* (1931) 307.
Cranberries	H. WILLSTAEDT, *Chem. Centr.* *1937*, I 3658.
Elaeis guineensis (?)	A. H. GILL, *J. Ind. Eng. Chem.* *9* (1917) 136; *10* (1918) 612.
Elaeis melanococca	A. H. GILL, *J. Ind. Eng. Chem.* *9* (1917) 136; *10* (1918) 612.
Erythroxylon coca	V. N. LUBIMENKO, *Rev. gén. botan.* *25* (1914) 474.

Source	References
Evonymus japonicus (Arillus)	V. N. LUBIMENKO, *Rev. gén. botan. 25* (1914) 474.
Momordica Balsamina (Arillus)	G. and F. TOBLER, *Ber. deutsch. botan. Ges. 28* (1910) 365, 496. — B. M. DUGGAR, *Washington Univ. Stud. 1* (1913) 22.
Momordica Charantia (Arillus)	G. and F. TOBLER, *Ber. deutsch. botan. Ges. 28* (1910) 365, 496. — B. M. DUGGAR, *Washington Univ. Stud. 1* (1913) 22.
Nertera depressa	V. N. LUBIMENKO, *Rev. gén. botan. 25* (1914) 474.
Palm oil	P. KARRER, H. v. EULER and H. HELLSTRÖM, *Chem. Centr. 1932*, I, 1800. — R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 200* (1931) 255.
Pandanus polycephalus	V. N. LUBIMENKO, *Rev. gén. botan. 25* (1914) 474.
Solanum decasepalum	V. N. LUBIMENKO, *Rev. gén. botan. 25* (1914) 474.
Synaspadix petrichiana	V. N. LUBIMENKO, *Rev. gén. botan. 25* (1914) 474.
Tabernaemontana pentasticta	V. N. LUBIMENKO, *Rev. gén. botan. 25* (1914) 474.
Taxus baccata	R. KUHN and H. BROCKMANN, *Chem. Centr. 1933*, II, 553.
Trichosanthes-species	N. A. MONTEVERDE and N. V. LUBIMENKO, *Bull. Acad. Sci. Petrograd.* Ser. 6, *7*, II, 1105.

b) Other vegetable and animal sources:

Source	References
Butter	A. E. GILLAM and I. M. HEILBRON, *Biochem. J. 29* (1935) 834.
Cantharellus cibarius	H. WILLSTAEDT, *Chem. Centr. 1938*, II, 2272.
Cantharellus lutescens	H. WILLSTAEDT, *Chem. Centr. 1938*, II, 2272.
Cantharellus infundibiliformis	H. WILLSTAEDT, *Chem. Centr. 1938*, II, 2272.
Coccinella septempunctata	E. LEDERER, *Chem. Centr. 1936*, I, 3853.
Human serum	E. v. DÁNIEL and G. J. SCHEFF, *Proc. Soc. Exp. Biol. Med. 33* (1935) 26. — E. v. DÁNIEL and T. BÉRES, *Z. physiol. Chem. 238* (1936) 160.
Vaccinium vitis idaea	H. WILLSTAEDT, *Svensk. Chem. Tid. 48* (1936) 212.

Isolation

For the isolation of lycopene, it is convenient to start from tomato preserves rather than from the fresh fruit which contain about 97% water. WILLSTÄTTER and ESCHER[7] worked up 75 kg of "Purée di pomidori concentrata" and obtained 11 g of once recrystallised lycopene.

The preserves are shaken in bottles in portions of about 8 kg with 4 l (or preferably more) 95% ethanol. The mixture is then pressed through a fine cloth under slight pressure. The operation is repeated using about 3 l of ethanol. The red

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residue is dried at 40–50°, finely ground and continuously extracted with carbon disulphide. The solvent is removed by distillation, finally under reduced pressure at 40° in an atmosphere of dry carbon dioxide, which is passed in through a capillary. The dark reddish brown paste which remains is diluted with 3 l ethanol when crystallisation immediately sets in. After a short time the crystalline mass is filtered and the residue washed with cold petroleum ether. The crude lycopene can be purified either by dissolution in carbon disulphide and precipitation with ethanol, or by recrystallisation from a large volume of petroleum ether (b.p. 50–80°, 4–5 l per 1 g of lycopene). For analysis, the material is first crystallised from petroleum ether, any sparingly soluble components being rejected, and then recrystallised from carbon disulphide, or a mixture of carbon disulphide and ethanol, without further fractionation. Using this procedure, 11 g of once recrystallised lycopene can be obtained from 75 kg of preserves, whereas WILLSTÄTTER and ESCHER obtained 2.7 g of pigment from 135 kg of fresh tomatoes. Thus 1 kg of fresh fruit yields 0.02 g of lycopene, while 1 kg of preserves yields 0.15 g of lycopene. Some commercial preserves contain acidic materials which have a deleterious effect during the isolation of lycopene. By addition of potassium carbonate to the tomato purée, KUHN and GRUNDMANN[8] were able to improve the yield of lycopene considerably. From 1 kg of preserves they obtained 0.265 g of pigment.

According to ZECHMEISTER and VON CHOLNOKY[2], lycopene can also be isolated from *Tamus communis* berries.

Chemical Constitution

```
CH3  CH3                                                                                CH3  CH3
  \  /                                                                                    \  /
   C          CH3            CH3              CH3              CH3                         C
  //          |              |                |                |                            \\
 CH    CH·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CH·CH                                  CH
 |     ||                                                      ||                           |
CH2    C·CH3                                               H3C·C                           CH2
  \   /                       Lycopene                            \                        /
   CH2                                                             \_______ CH2 __________/
```

WILLSTÄTTER and ESCHER[10] recognised that lycopene is an isomer of carotene and determined the correct empirical formula, $C_{40}H_{56}$. The constitution of the pigment was elucidated by KARRER and his co-workers[5]. The results of these investigations can be briefly summarised as follows.

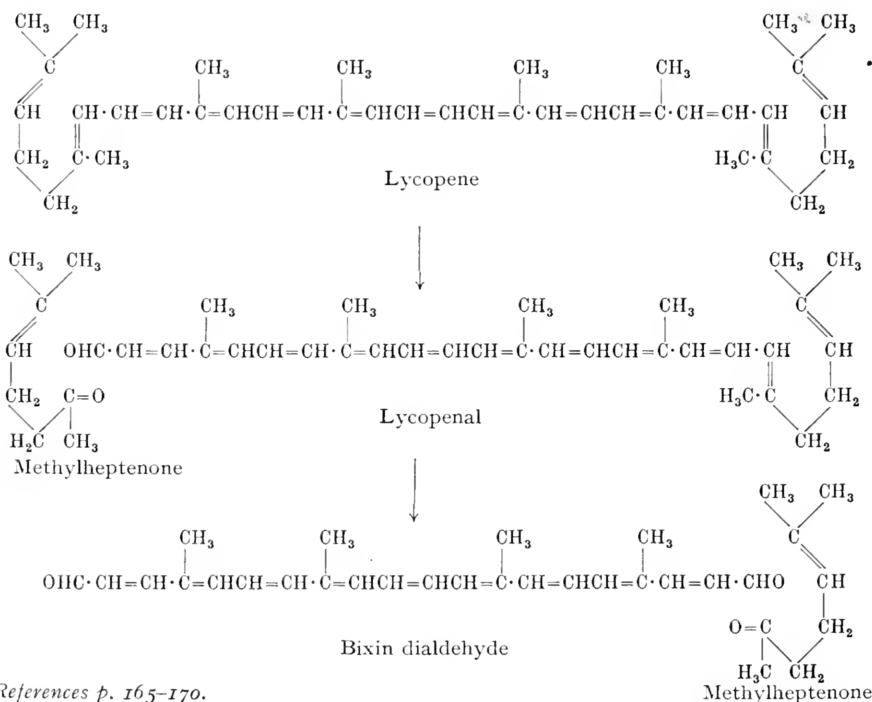
The deep red colour of lycopene suggested the presence of numerous double bonds. KARRER and ROSE WIDMER[11] found that 13 mols of hydrogen were absorbed during the catalytic hydrogenation of the pigment. The empirical formula of perhydrolycopene was $C_{40}H_{82}$, which showed that lycopene must have an open-chain structure (cf. the corresponding discussion of carotene). According to PUMMERER, REBMANN and REINDEL[12], lycopene absorbs 13 mols of iodine chloride, thus confirming the presence of thirteen ethylenic bonds. KARRER and BACHMANN[13] and KARRER, HELFENSTEIN, PIEPER and WETTSTEIN[14] found that ozonisation affords considerable quantities of acetone. One mol of the pigment gave 1.6 mols of ketone, suggesting the presence of

References p. 165–170.

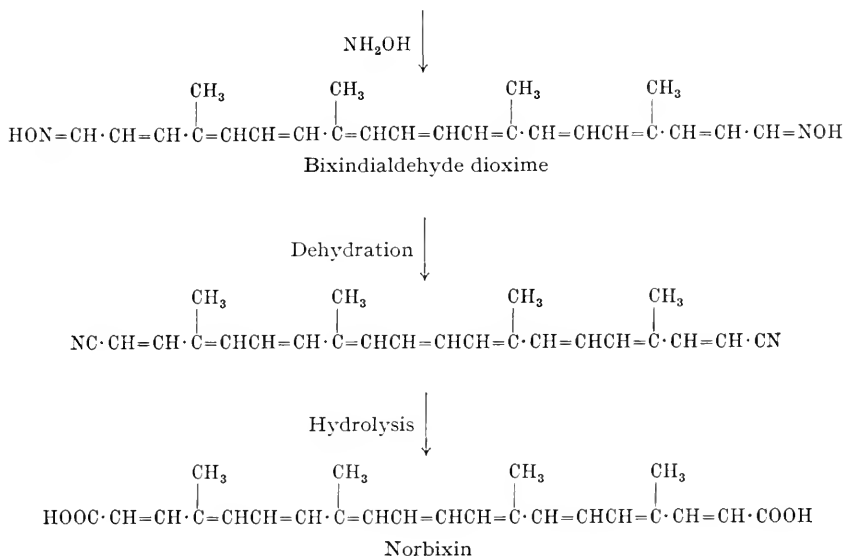
2 *iso*propylidene groupings $(CH_3)_2C=$. Ozonisation also furnished succinic acid, but no higher fatty acids were obtained. The succinic acid is derived from the grouping

By means of oxidative degradation of lycopene with permanganate and chromic acid, KARRER, HELFENSTEIN, WEHRLI and WETTSTEIN[16] proved the presence of 6 side-chain methyl groups. Finally, KARRER, HELFENSTEIN and WIDMER[17] synthesized perhydrolycopene from dihydrophytol and showed that it was identical in all respects with the product obtained by the catalytic hydrogenation of lycopene. As the result of these investigations the formula for lycopene shown above was put forward by KARRER, HELFENSTEIN, PIEPER and WETTSTEIN[18].

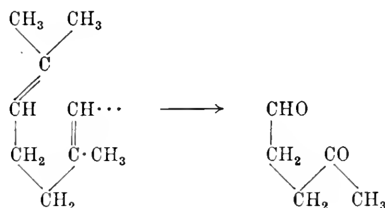
This formula has been confirmed by later investigations by KUHN and co-workers. KUHN and WINTERSTEIN[19] isolated toluene and m-xylene from the products of the thermal decomposition of the pigment and KUHN and GRUNDMANN[20] obtained lycopenal, a complex degradation product, together with methyl heptenone from the oxidation of the pigment with chromic acid. Further oxidation of lycopenal with chromic acid gave bixin dialdehyde and methyl heptenone. The structure of lycopenal was established by the conversion of bixin dialdehyde into norboxin, the constitution of which had been elucidated earlier by KARRER and co-workers.



References p. 165–170.



The observation of STRAIN[21], that the ozonisation of lycopene also gives rise to levulinic aldehyde and levulinic acid, is also in accord with the formula given above.



Formation

Numerous early investigations deal with the formation of carotenoids in the plant during the ripening process. Thus DUGGAR[22] showed in 1913 that the red tomato pigment is no longer formed at temperatures above 30°, a yellow pigment, probably a flavone or flavanol, being produced instead. The agents responsible for the formation of lycopene are not, however, destroyed at 30° since yellow tomatoes ripened at 30° again acquire a red colour due to lycopene on being restored to a lower temperature. Some authors consider light to be a necessary agent for the ripening process[23]; according to DUGGAR, however, the ripening process is independent of light but requires the presence of oxygen. The investigations of DUGGAR were repeated and confirmed by KARRER and co-workers[24].

KUHN and GRUNDMANN[25], in 1932, examined the tomato pigment at different stages of ripening by means of adsorption analysis. They obtained the following figures for fresh fruit grown in the open.

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TABLE 31

COMPOSITION OF THE TOMATO PIGMENTS (KUHN AND GRUNDMANN)

	Mg of pigment in 100 g of fresh fruit		
	green	half ripened	fully ripened
Lycopene	0.11	0.84	7.85
β-Carotene (isolated)	0.16	0.43	0.73
Xanthophylls, free	0.02	0.03	0.06
Xanthophylls, esterified	0.00	0.02	0.10

Properties and physical constants

Crystalline form: Lycopene crystallises from a mixture of carbon disulphide and ethanol in long red needles. From petroleum ether it crystallises in characteristic felted hair-like needles, or occasionally in long dark red-violet prisms. In powder form, it is a dark reddish-brown. In contrast to most carotenoids, crystals of lycopene show little metallic lustre. (For X-ray diffraction pattern, see MACKINNEY[26].)

Melting point: 170° (uncorr.)[27]; 173° (uncorr.)[28]; 174° (corr.)[29]; 175° (corr.)[30].

Solubility:

Ethanol, cold	almost insoluble
Ethanol, hot	very sparingly soluble
Methanol	almost insoluble
Benzene, cold	fairly soluble
Benzene, hot	very soluble
Chloroform, cold	easily soluble
Chloroform, hot	very easily soluble
Carbon disulphide	very easily soluble

1 g of lycopene is soluble in 50 ml of cold carbon disulphide, 3 l of boiling ether, 10–12 l of boiling petroleum ether, or 14 l of hexane of 0°[31].

Spectral properties:

Solvent:	*Absorption maxima:*		
Carbon disulphide	548	507.5	477 mμ
Chloroform	517	480	453 mμ
Benzene	522	487	455 mμ
Petroleum ether	506	475.5	447 mμ
Ethanol	503	472	443 mμ
Hexane	504	472	443 mμ

(cf. Fig. 4, p. 349, and Fig. 31, p. 361)

Colour of solutions:

In carbon disulphide	blue with a red tinge
In ether (saturated solution) . . .	bluish-red
In ethanol (hot saturated solution)	dark yellow

References p. 165–170.

Optical activity: Lycopene is optically inactive.

Colour reactions: Lycopene dissolves in concentrated sulphuric acid with an indigo blue colour. With fuming nitric acid, it yields a purple colouration which rapidly disappears[32]. On adding a solution of antimony trichloride in chloroform to a solution of lycopene in chloroform, an intense unstable blue colour is produced[33].

Partition test: Lycopene is completely epiphasic.

Chromatographic behaviour: Lycopene is adsorbed six times more strongly than carotene on alumina from petroleum ether solution. Similar behaviour is shown on adsorption on calcium oxide or calcium hydroxide. Petroleum ether containing a little methanol is used for elution[34]. Like other carotenoid hydrocarbons, lycopene is only weakly adsorbed on calcium or zinc carbonate and can thus be separated from phytoxanthins[35].

Detection and estimation: After saponification of the whole extract, lycopene, together with the carotenes, is present in the epiphasic fraction. It is best identified by chromatographic separation on calcium hydroxide, followed by a determination of the absorption maxima.

CONNELL[36] recommends a solution of potassium dichromate and cobalt sulphate as standard for colorimetric determinations. According to KUHN and BROCKMANN[37], an alcoholic solution of azobenzene can also be employed.

Physiological properties: As would be expected from its structure, lycopene possesses no vitamin A potency.

Derivatives

Perhydrolycopene $C_{40}H_{82}$

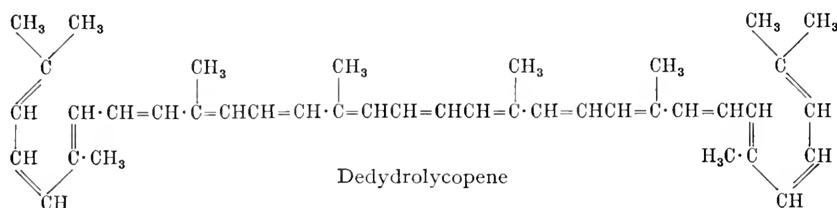
Perhydrolycopene is formed by the catalytic hydrogenation of lycopene[38]. It has also been synthesized by treating dihydrophytol with phosphorus pentabromide and heating the dihydrophytyl bromide (16-bromo-2:6:10:14:-tetramethylhexadecane) with potassium at 130–140°[39].

Perhydrolycopene is a colourless oil. B.p. 238–240°/0.3 mm.; 212–214°/0.02 mm[40]. d_4^{18} 0.822 from lycopene, 0.824 from phytol[40], n^{18} 1.4560 from lycopene; 1.4567 from phytol.

Dehydrolycopene $C_{40}H_{52}$

Lycopene is dehydrogenated by the action of bromsuccinimide (2 mols) and converted into dehydrolycopene (KARRER and RUTSCHMANN[41]):

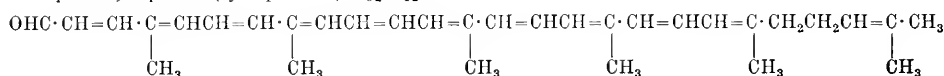
References p. 165–170.



Dehydrolycopene contains 15 conjugated double bonds. It can only be crystallised from pyridine, being almost insoluble in most other solvents. The crystals appear dark violet to black in direct light and are slowly discoloured and decomposed on heating in an evacuated tube above 200°, no definite melting point being observed. With a solution of antimony trichloride in chloroform, dehydrolycopene gives a fairly stable blue colouration, the absorption spectrum of which exhibits a sharp band with a maximum at 472 mμ and continuous absorption above 640 mμ.

Solvent:	*Absorption maxima:*		
Carbon disulphide	601	557	520 mμ
Hexane	542	504	476 mμ
Benzene	570	531	493 mμ
Pyridine	574	535	498 mμ
Chloroform	567	528	493 mμ

Apo-2-lycopenal (lycopenal*) $C_{32}H_{42}O$:



Lycopenal is obtained by the chromic acid oxidation (3 atoms O) of lycopene in a mixture of acetic acid and benzene[42]. It crystallises from a mixture of benzene and ethanol in deep-red plates, melting point 147° (corr.). It is easily soluble in chloroform, carbon disulphide and benzene, but only sparingly in ethanol. It is entirely epiphasic in the partition test.

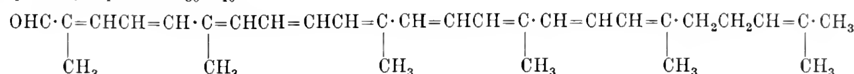
Solvent:	*Absorption maxima:*		
Carbon disulphide	569	528.5	493.5 mμ
Petrol	525.5	490.5	455.5 mμ

Lycopenal is very easily oxidised in the solid state or in solution. On treatment with chromic acid (3 atoms O), 2-methylhept-2-en-6-one and apo-2:12-lycopene dialdehyde (bixin dialdehyde)[43] are formed. With free hydroxylamine, apo-2-lycopenal gives apo-2-lycopenal oxime wich crystallises from pyridine in lustrous blue-violet prisms, 198° (corr., evacuated capillary).

* The old name for apo-2-lycopenal is lycopenal.

References p. 165–170.

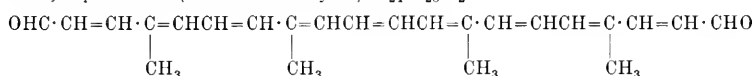
Apo-3-lycopenal $C_{30}H_{40}O$:



Apo-3-lycopenal was obtained by KARRER and JAFFÉ[44] by the mild oxidation of lycopene with potassium permanganate. It separates from petroleum ether in brown-black crystals, m.p. 138°.

Solvent:	*Absorption maxima:*		
Carbon disulphide	545	508	ca. 478 mμ
Petrol	502	473	mμ
Benzene	518	488	mμ

Apo-2:12-lycopenedial (bixindialdehyde) $C_{24}H_{28}O_2$:



Bixindialdehyde is formed by the oxidation of lycopene or lycopenal (apo-2-lycopenal) with chromic acid[45]. It crystallises from pyridine in lustrous blue prisms, m.p. 220° (corr.). On heating in air, it decomposes at 180° without melting. Bixindialdehyde is appreciably soluble in pyridine and chloroform, and sparingly soluble in hot benzene, but only dissolves with great difficulty in petrol, alcohols, carbon disulphide, ether, acetone, or dioxan.

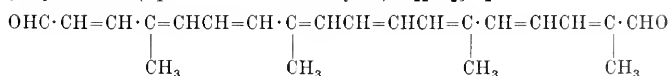
Solvent:	*Absorption maxima:*		
Carbon disulphide	539.5	502	467.5 mμ
Petroleum ether	502	468	437.5 mμ
Pyridine	534.5	494	mμ
Chloroform	528	490	mμ

(cf. Fig. 17, p. 355)

Bixindialdehyde dioxime crystallises from pyridine in needles which decompose above 250° without melting[46]. It is soluble only in pyridine.

Solvent:	*Absorption maxima:*		
Pyridine	514	482	452 mμ

Apo-3:12-lycopenedial (apo-1-bixindialdehyde) $C_{22}H_{26}O_2$:



Apo-1-bixindialdehyde was obtained by KARRER and JAFFÉ by the chromic oxidation of lycopene[47]. It separates from methanol in dark crystals, m.p. 168° (uncorr.).

References p. 165–170.

Solvent:	*Absorption maxima:*		
Carbon disulphide	517	484	453 mμ
Petroleum ether	480	452	mμ

With free hydroxylamine, apo-3:12-lycopenedial forms a dioxime which separates in lustrous red crystals which sinter above 210°.

Solvent:	*Absorption maxima:*	
Carbon disulphide	510	480 mμ
Ethanol	481	449 mμ

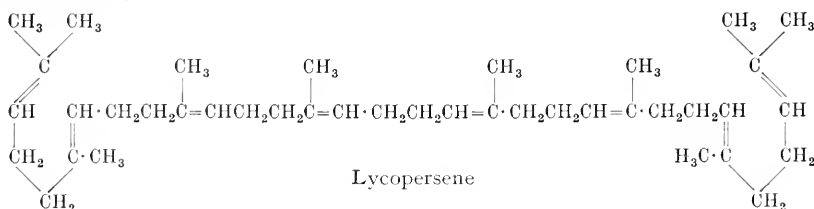
Neo-lycopene $C_{40}H_{56}$:

In the presence of small quantities of iodine, on standing at elevated temperatures, or merely on standing in solution at room temperature for 1 or 2 days, lycopene is partly converted into an isomer, neolycopene (ZECHMEISTER and TUZSON[48]). It has not yet been possible to obtain this pigment in a crystalline state. During chromatography on calcium hydroxide it gives rise to a brown-red loosely adsorbed zone below that of lycopene. A *cis-trans* change appears to be involved in this isomerisation.

Solvent:	*Absorption maxima:*			
Carbon disulphide	536	498	466	mμ
Benzene	512	479	450	mμ
Chloroform	512	478	447.5	mμ
Acetone	499.5	468	439	mμ
Petroleum ether	499.5	468	439	mμ
Ethanol	500	469	439	mμ

Neolycopene is more easily soluble in organic solvents than lycopene.

Lycopersene $C_{40}H_{66}$:



Lycopersene

Lycopersene has been prepared by KARRER and KRAMER[49] by reacting geranyl-geranyl bromide with sodium, a method analogous to that employed in the synthesis of squalene[50].

Lycopersene is a rather viscous oil, which can be distilled at 0.02 mm pressure in an air bath at 225–228°. It is a colourless compound. It absorbs 8 molecules of hydrogen chloride, forming a crystalline octa-hydrochloride $C_{40}H_{74}Cl_8$, which after recrystallisation from acetone melts at 126°.

References p. 165–170.

2. PROLYCOPENE $C_{40}H_{56}$

In 1941, ZECHMEISTER and collaborators[51] discovered a new polyene pigment in the "tangerine tomato", a variety of *lycopersicum esculentum.* They proposed the name prolycopene for this new pigment which has also been found in several other plants including *Butia capitata*[51], *Butia eriospatha* Becc.[52], *Pyrocantha angustifolia*[53], *Evonymus fortunei*[53] and *Mimulus longiflorus* Grant[55].

According to ZECHMEISTER and collaborators[51,56] prolycopene is to be regarded as a naturally occurring stereoisomer of lycopene. The chromophoric system of this pigment is assumed to contain 5 to 7 *cis*-double bonds in contrast to lycopene which is believed to have an exclusively *trans*-arrangement of double bonds. Under the catalytic influence of iodine, prolycopene is converted into a complicated mixture of stereoisomers which includes the natural (*trans*)-lycopene.

Prolycopene crystallises from petroleum ether or ethanol in plates, m.p. 111°. In other solvents, this pigment is more easily soluble than lycopene. On chromatography on calcium hydroxide it gives rise to a zone below that of lycopene.

Solvent:	*Absorption maxima:*	
Carbon disulphide	500.5	469.5 mμ
Benzene	485	455.5 mμ
Chloroform	484	453.5 mμ
Ethanol	(471)	(445) mμ
Petroleum ether	470	443.5 mμ

ZECHMEISTER and PINCKARD[57] discovered 6 new lycopene isomers, believed to contain 4 to 7 *cis*-double bonds, in ripe berries of *pyracantha angustifolia (Schneid)*. They were designated according to decreasing strength of adsorption as poly-*cis*-lycopenes I–VI. Three of these compounds could be crystallised.

	M.p.	*Absorption maxima in hexane solution*
Poly-cis-lycopene I	93–95°	444–445 mμ
Poly-cis-lycopene II	85–87°	441–442 mμ
Poly-cis-lycopene III	105–106°	443–446 mμ
Poly-cis-lycopene IV		426 mμ
Poly-cis-lycopene V		431–432 mμ
Poly-cis-lycopene VI		433 mμ

References p. 165–170.

3. β-CAROTENE $C_{40}H_{56}$

History

1831 WACKENRODER discovers carotene* in the roots of the carrot (*Daucus Carota*).
1847 ZEISE[58] describes the new pigment in more detail and determines the empirical formula C_5H_8.
1866 ARNAUD[59] establishes that carotene is a hydrocarbon.
1907 WILLSTÄTTER and MIEG[60] prove the identity of carotene from leaves and carrots. They establish the correct molecular formula $C_{40}H_{56}$.
1928 ZECHMEISTER, VON CHOLNOKY and VRABÉLY establish the presence of 11 double bonds and 2 ring systems in carotene[61].
1929–31 KARRER and collaborators[62] elucidate the constitution of β-carotene.
1932–35 KUHN and BROCKMANN[63] carry out extensive investigations on β-carotene and obtain long-chain degradation products which confirm the formula assigned to β-carotene.

Occurrence

β-Carotene is very widely distributed in nature. All green parts of plants (leaves[64], stalks, etc.) contain this pigment which invariably accompanies chlorophyll together with xanthophyll, xanthophyll epoxide and frequently α-carotene[65].

Autumnal leaves also contain β-carotene[66]. In fact, numerous investigations have shown that β-carotene is to be found almost throughout the whole of the vegetable and animal kingdoms. The following summary will give some indication of the variety of β-carotene sources.

TABLE 32

VEGETABLE SOURCES FROM WHICH β-CAROTENE HAS BEEN ISOLATED

Source	References
a) Fruit:	
Arbutus	K. SCHÖN, *Biochem. J. 29* (1935) 1779.
Capsicum frutescens jap. (skin)	L. ZECHMEISTER and L. V. CHOLNOKY, *Ann. 489* (1931) 1.
Capsicum japonicum (skin)	L. ZECHMEISTER and L. V. CHOLNOKY, *Ann. 454* (1927) 54; *455* (1927) 70; *509* (1934) 269.

* WACKENRODER, *Geigers Magazin Pharm. 33* (1831) 141. For nearly 100 years, carotene as isolated from the carrot was regarded as a homogenous compound. It was only by means of modern chemical and physical methods of separation (particularly chromatography) that the complex nature of carotene was established. It was recognised by several investigators simultaneously that the carrot pigment is a mixture of several isomers in which β-carotene predominates. Investigations in which several times recrystallised carotene were employed therefore relate to materials consisting mainly of β-carotene.

References p. 165–170.

Source	References
Citrullus vulgaris Schrad.	L. ZECHMEISTER and P. TUZSON, *Ber. 63* (1930) 2883.
Citrus aurantium Risso.	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 221* (1934) 279.
Citrus madurensis Lour.	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 221* (1934) 279.
Citrus poonensis hort.	R. YAMAMOTO and S. TIN, *Chem. Centr. 1934*, I, 1660.
Convallaria majalis	A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem. 207* (1932) 31.
Cucurbita maxima Duch.	H. SUGINOME and K. UENO, *Chem. Centr. 1931*, II, 2892. — L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 824.
Diospyros costata	K. SCHÖN, *Biochem. J. 29* (1935) 1779.
Gonocaryum pyriforme (skin)	A. WINTERSTEIN, *Z. physiol. Chem. 215* (1933) 52; *219* (1933) 249.
Mangifera indica	R. YAMAMOTO, Y. OSIMA and T. GOMA, *Chem. Centr. 1933*, I, 441.
Pirus aucuparia	R. KUHN and E. LEDERER, *Ber. 64* (1931) 1354.
Prunus armeniaca	H. BROCKMANN, *Z. physiol. Chem. 216* (1933) 45.
Rosa canina	R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 341.
Rosa damascena	R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 341.
Rosa rubiginosa	R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 341.
Solanum Lycopersicum	R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 49.
Taxus baccata	R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 834.

b) Blossoms:

Source	References
Acacia decurrens	J. M. PETRIE, *Biochem. J. 18* (1924) 957.
Acacia discolour, Acacia linifolia, Acacia longifolia	do.
Calendula officinalis	L. ZECHMEISTER and L. v. CHOLNOKY, *Z. physiol. Chem. 208* (1932) 29.
Caltha palustris	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta 15* (1932) 1195.
Gazania rigens	K. SCHÖN, *Biochem. J. 32* (1938) 1566. — L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc. 63* (1943) 1535.
Genista tridentata	K. SCHÖN and B. MESQUITA, *Biochem. J. 30* (1936) 1966.
Kerria japonica DC	P. KARRER and E. JUCKER, *Helv. chim. Acta 29* (1946) 1539.
Laburnum anagyroides	do.
Ranunculus	P. KARRER and A. NOTTHOFFT, *Helv. chim. Acta 15* (1932) 1195. — P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta 28* (1945) 1146.
Sarothamnus scoparius	P. KARRER and E. JUCKER, *Helv. chim. Acta 27* (1944) 1585.
Tragopogon pratensis	see *Ranunculus*.

Source	References
Trollius europaeus	P. Karrer and E. Jucker, *Helv. chim. Acta 29* (1946) 1539.
Ulex europaeus	K. Schön, *Biochem. J. 30* (1936) 1960.
Ulex Gallii	do.

c) Other vegetable sources:

Oil from *Acacia acuminata*	T. M. Trikoyus and J. C. Drummond, *Nature 139* (1937) 1105.
Aphanizomenon flosaquae	J. Tischer, *Z. physiol. Chem. 251* (1938) 109.
Bacillus Grasberger	E. Chargaff and E. Lederer, *Chem. Centr. 1936, I,* 3159.
Bacillus Lombardo Pellegrini	do.
Brown algae	H. Kylin, *Z. Physiol. Chem. 82* (1912) 224. — R. Willstätter and H. J. Page, *Ann. 404* (1914) 251; P. W. Carter, L. C. Cross, I. M. Heilbron and E. R. H. Jones, *Biochem. J. 43* (1948) 349.
Cladophora Sauteri	I. M. Heilbron, E. G. Parry and R. F. Phipers, *Biochem. J. 29* (1935) 1376.
Crocus sativus	R. Kuhn and A. Winterstein, *Ber. 67* (1934) 349.
Cuscuta salina	G. Mackinney, *J. biol. Chem. 112* (1935) 421.
Cuscuta subinclusa	do.
Diatomea	F. G. Kohl, *Chem. Centr. 1906*, I, 1669.
Fucus vesiculosus	I. M. Heilbron and R. F. Phipers, *Biochem. J. 29* (1935) 1369.
Haematococcus pluvialis	J. Tischer, *Z. physiol. Chem. 250* (1937) 147; *252* (1938) 225.
Lycogala epidendron	E. Lederer, *Chem. Centr. 1939*, I, 2991.
Nitella opaca	I. M. Heilbron, E. G. Parry and R. F. Phipers, *Biochem. J. 29* (1935) 1376.
Oedogonium	do.
Palm oil	R. Kuhn and H. Brockmann, *Z. physiol. Chem. 200* (1931) 255.
Rhodymenia palmata	I. M. Heilbron, E. G. Parry and R. F. Phipers, *Biochem. J. 29* (1935) 1376.
Torula rubra	E. Lederer, *Compt. rend. 197* (1933) 1694.
Trentepohlia aurea	E. Lederer, *Chem. Centr. 1939*, I, 2991.
Yellow maize	R. Kuhn and C. Grundmann, *Ber. 67* (1934) 593.

TABLE 33

OCCURRENCE OF β-CAROTENE IN THE ANIMAL ORGANISM

Source	References
Bloodserum	H. v. EULER, B. v. EULER and H. HELLSTRÖM, *Biochem. Z. 202* (1930) 370. — H. WILLSTAEDT and T. LINDQVIST, *Z. physiol. Chem. 240* (1936) 10.
Corpus luteum of cows and sheep	H. H. ESCHER, *Z. physiol. Chem. 83* (1913) 198. — R. KUHN and E. LEDERER, *Z. physiol. Chem. 200* (1931) 246. — P. KARRER and W. SCHLIENTZ, *Helv. chim. Acta 17* (1934) 55.
Corpora rubra of cows	R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 41.
Faeces of sheep and cows	P. KARRER and A. HELFENSTEIN, *Helv. chim. Acta 13* (1930) 86.
Fat tissues of mammals	L. S. PALMER and C. H. ECKLES, *J. Biol. chem. 17* (1914) 211. — H. VAN DEN BERGH, P. MULLER and J. BROEKMEYER, *Biochem. Z. 108* (1920) 279. — C. L. CONNOR, *J. biol. Chem. 77* (1928) 619. — L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 154.
Fish roe	H. v. EULER, U. GARD and H. HELLSTRÖM, *Svensk. Kem. Tidskr. 44* (1932) 191; *Chem. Centr. 1932*, I, 1504.
Gallstones of oxen	G. FISCHER and H. RÖSE, *Z. physiol. Chem. 88* (1913) 331. — H. FISCHER and R. HESS, *Z. physiol. Chem. 187* (1930) 133.
Human milk	L. S. PALMER and C. H. ECKLES, *J. biol. Chem. 17* (1914) 237.
Human *placenta*	R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 41.
Integuments of insects	E. LEDERER, *Chem. Centr. 1936*, I, 3853.
Kidneys of mammals	H. VAN DEN BERGH, P. MULLER and J. BROEKMEYER, *Biochem. Z. 108* (1920) 279. — O. BAILLY, *Chem. Centr. 1935*, I, 3806.
Livers of mammals	H. v. EULER and E. VIRGIN, *Biochem. Z. 245* (1932) 252. — H. v. EULER and E. KLUSSMANN, *Biochem. Z. 256* (1932) 11. — H. WILLSTAEDT and T. LINDQVIST, *Z. physiol. Chem. 240* (1936) 10.
Milk fat	A. E. GILLAM and M. S. EL RIDI, *Biochem. J. 31* (1937) 251. — L. S. PALMER and C. H. ECKLES, *J. biol. Chem. 17* (1914) 191.
Salmon flesh	H. v. EULER, H. HELLSTRÖM and M. MALMBERG, *Svensk. Kem. Tidskr. 45* (1933) 151.

TABLE 34

SOURCES FOR THE PREPARATION OF β-CAROTENE

Source	Yield of carotene from 1 kg of material	Literature references
Carrots	1 g (max.)	R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 47. — R. KUHN and E. LEDERER, *Ber. 64* (1931) 1349. — H. N. HOLMES and H. M. LEICESTER, *J. Am. Chem. Soc. 54* (1932) 716. — N. T. DELEANO and J. DICK, *Biochem. Z. 259* (1933) 110.
Cucurbita maxima Duch. (Fruit)	0.1 g	L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 824.
Palm oil	1.5–2.0 g	P. KARRER, H. v. EULER and H. HELLSTRÖM, *Ark. Kemi. B. 10* (1931) No. 15. — O. UNGNADE, *Chem. Ztg. 63* (1939) 9.
Paprica skin	0.3 g	L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 455* (1927) 70.
Stinging nettles (ground)	0.15–0.2 g	R. WILLSTÄTTER and A. STOLL, *Untersuchungen über Chlorophyll*, Berlin 1934, Julius Springer, p. 237. — R. WILLSTÄTTER and W. MIEG, *Ann. 355* (1907) 12.

TABLE 35

α-CAROTENE CONTENT OF DIFFERENT CAROTENE PREPARATIONS

(KUHN AND LEDERER[67], KUHN AND BROCKMANN[68])

Palm oil	30–40%	Green stinging nettles	traces*
Green chestnut leaves	25%	Paprica	traces
Red berries	15%	Spinach	traces*
Large pumpkin[69]	1%	Grass	None

* P. KARRER and W. SCHLIENTZ[70].

Preparation

Following WILLSTÄTTER and ESCHER[71], finely cut carrots are dried, ground and continuously extracted with petroleum ether at room temperature. The combined extracts are concentrated as far as possible at 30–40° under reduced pressure and diluted with an equal volume of carbon disulphide. From this solution the crude

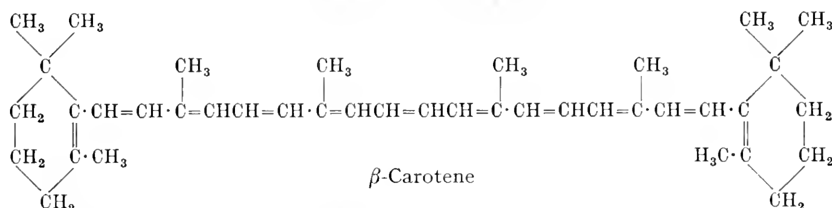
References p. 165–170.

carotene is precipitated with ethanol. Spirits of wine are added in small portions every 2 to 5 minutes. At first only colourless materials separate. As soon as the first carotene crystals are formed, the colourless materials are separated by rapid filtration. The mother liquors are diluted with the remainder of the alcohol (about 3–6 volumes of the original carotene solution are required) and allowed to stand for 20 hours at −10°. After this time the crude carotene is filtered off, dissolved in carbon disulphide, precipitated with ethanol, extracted with a little warm petroleum ether to remove remaining impurities, and finally recrystallised from a large volume of petroleum ether.

In order to obtain the pure β-isomer from crude carotene, the latter is chromatographed from petroleum ether on calcium hydroxide[72]. KARRER and WALKER[72] obtained 17 g of pure β-carotene and 2.5 g of pure α-carotene from 35 g of crude carotene.

In contrast to WILLSTÄTTER and ESCHER[73], KUHN and LEDERER[74] submit the shredded carrots to a preliminary extraction with methanol.

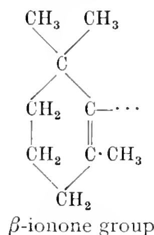
Chemical Constitution



β-Carotene

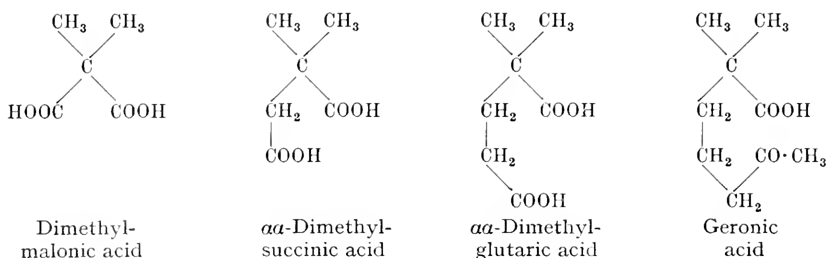
In 1907, WILLSTÄTTER and MIEG[75] established the correct molecular formula $C_{40}H_{56}$ for carotene. ZECHMEISTER, VON CHOLNOKY and VRABÉLY[76] showed that carotene contains 11 double bonds which can be saturated by hydrogenation. The formula of perhydrocarotene, $C_{40}H_{78}$ proves the presence of 2 ring systems. According to PUMMERER and REBMANN[77] carotene absorbs 11 molecules of iodine chloride, thus confirming the presence of 11 carbon-carbon double bonds.

KARRER and co-workers[78] oxidised β-carotene with permanganate and with ozone and obtained α:α-dimethylglutaric acid, α:α-dimethylsuccinic acid, dimethylmalonic acid and geronic acid (α:α-dimethyl-δ-acetylvaleric acid), the latter being a particularly characteristic degradation product. All these compounds are also formed by the oxidation of β-ionone, in comparable yield. Thus by the oxidation of pure β-carotene, KARRER and MORF obtained geronic acid in 16% yield, while β-ionone afforded the acid in 19.4% yield. It was thus concluded that β-carotene contains two β-ionone groupings.

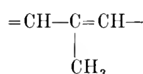


β-ionone group

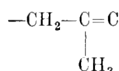
References p. 165–170.



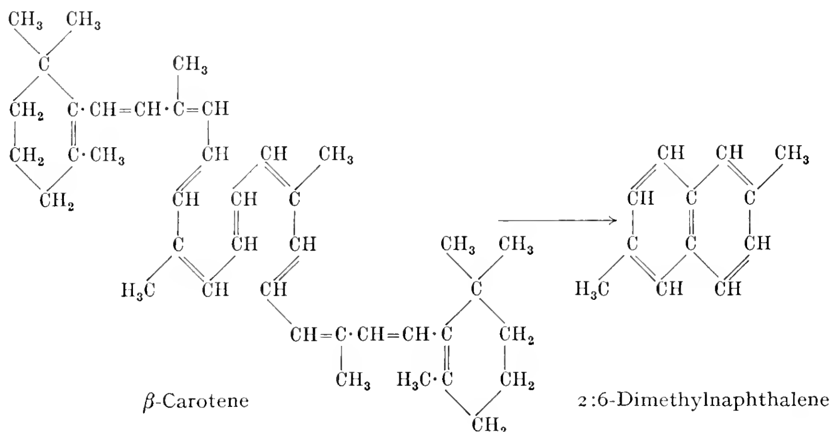
KARRER and HELFENSTEIN[79] quantitatively estimated the acetic acid formed by the permanganate oxidation of carotene and thus established the presence of 4 groupings of the type



It was therefore concluded that the two β-ionone rings are joined by a chain of 4 isoprene residues. Furthermore, according to KUHN and EHMANN[80] and KUHN and L'ORSA[81], the results of chromic acid oxidation indicated the presence of 2 groupings of the type

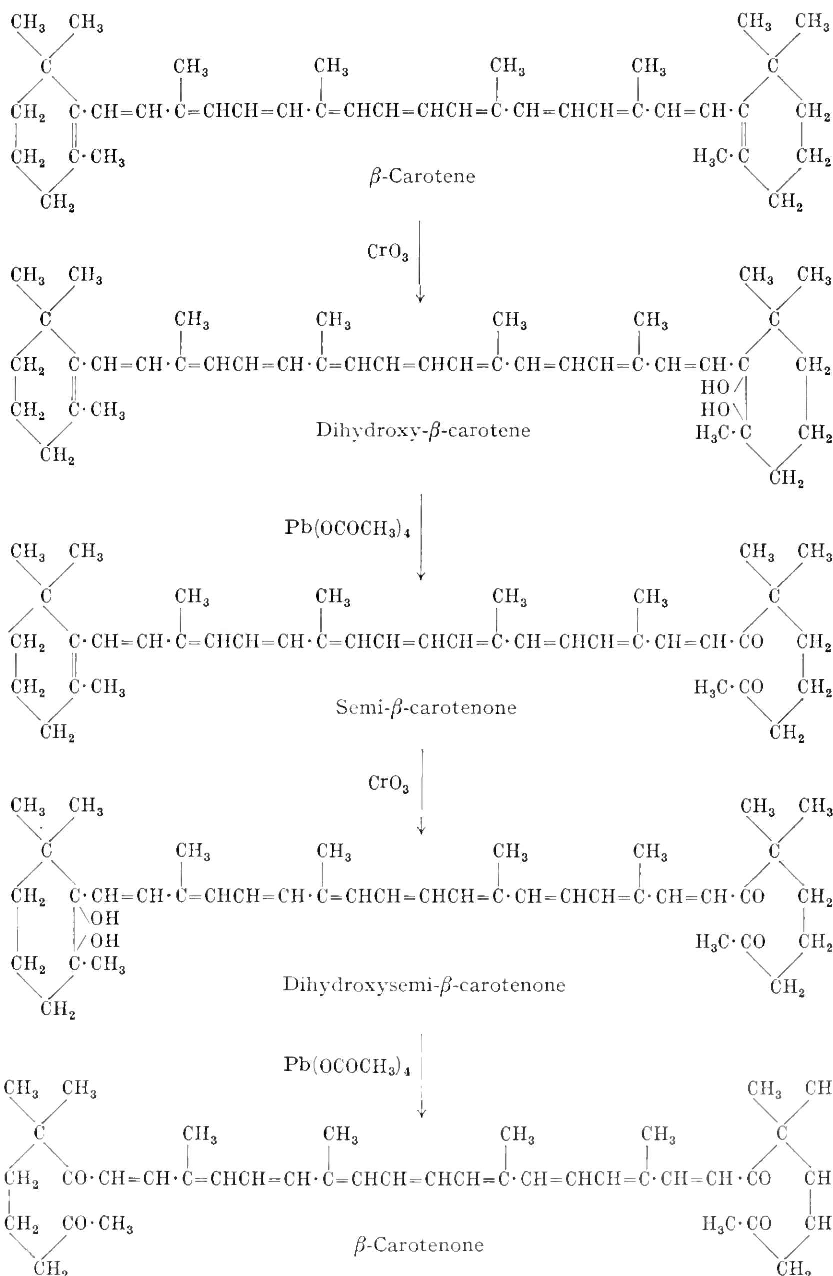


These results led KARRER, HELFENSTEIN, WEHRLI and WETTSTEIN[82] and KARRER and MORF[83] to propose the now accepted formula for β-carotene. The structure of β-carotene was confirmed by the later investigations of PUMMERER, REBMANN and REINDEL[84], STRAIN[85] and KUHN and WINTERSTEIN[86]. The latter authors showed that thermal decomposition of the pigment gives rise to 2:6-dimethylnaphthalene.

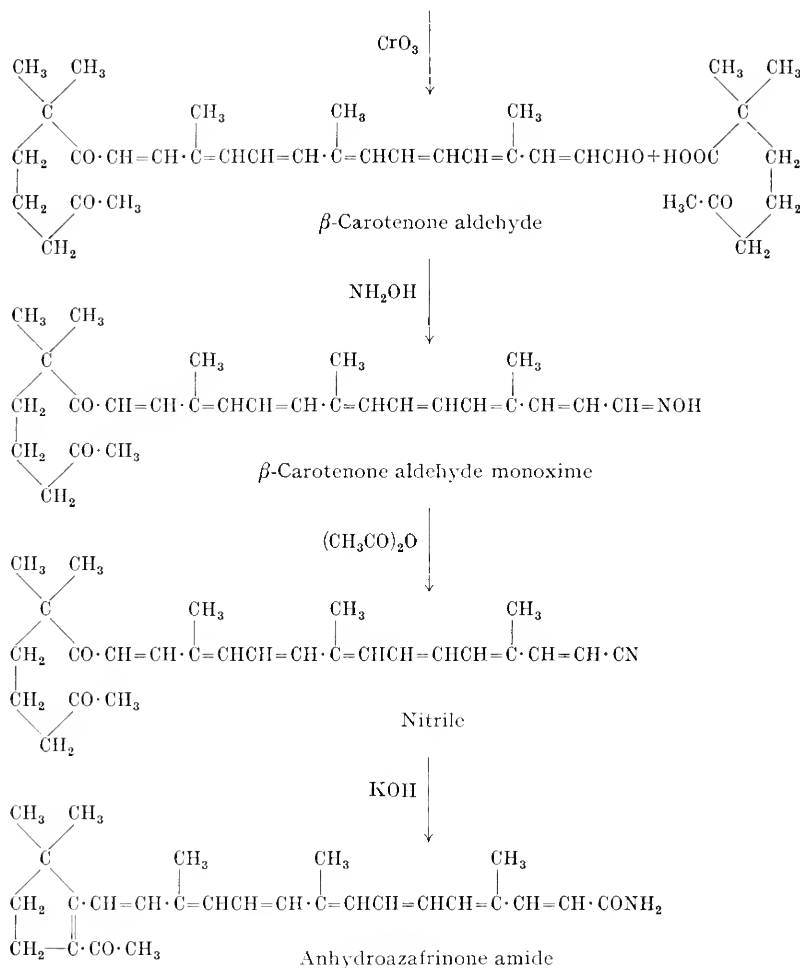


References p. 165–170.

Further confirmation of the β-carotene formula was provided by the investigations of KUHN and BROCKMANN[87] in which β-carotene was related to azafrin through mild stepwise degradation reactions.



References p. 165–170.



As anhydroazafrinone amide can also be obtained from azafrin via azafrinone amide the relation between β-carotene and azafrin is established. (For the individual compounds involved in these reactions see p. 284).

Formation

P. KARRER and E. JUCKER[88] obtained β-carotene by the action of sodium ethoxide on α-carotene. Up to the present time this is the only way in which this pigment has been obtained synthetically*.

* P. KARRER and S. SCHWYZER, *Helv. Chim. Acta 31* (1948) 1055, have recently described the formation of traces of a carotenoid pigment, probably β-carotene, in the reaction of vitamin A *p*-toluenesulphonic ester and sodium iodide in acetone. The main product is anhydro-vitamin A.

Properties

Crystalline form: Dark violet hexagonal prisms from benzene-methanol. Red, rhombic, almost quadratic plates from petroleum ether.

Melting point: 181–182° (corr.)[89]; 181–182° (uncorr., KARRER and co-workers[90]); 183° (corr., evacuated capillary, KUHN and BROCKMANN[91]); 187.5° (MILLER[92]).

Solubility: β-Carotene is less soluble than the α-isomer, so that the latter is concentrated in the mother liquors during the crystallisation of carotene. β-Carotene is easily soluble in carbon disulphide, benzene and chloroform, and fairly easily soluble in ether and petroleum ether. 100 ml of *n*-hexane dissolve 109 mg of β-carotene at 0°. The pigment is almost insoluble in ethanol and methanol.

Spectral properties:

Solvent:	*Absorption maxima:*		
Carbon disulphide	520	485	450 mμ
Chloroform	497	466	mμ
Petrol	483.5	452	426 mμ
Hexane	477	450	425 mμ

(cf. Fig. 6, p. 350 and Fig. 31, p. 361)

Quantitative extinction measurements: HAUSSER and SMAKULA[93].
Raman spectrum: VON EULER and HELLSTRÖM[94].

Colour reactions: On dissolving 1–2 mg of β-carotene in 2 ml of chloroform and adding concentrated sulphuric acid, the acid layer is coloured blue. On dissolving the pigment in chloroform and adding one drop of fuming nitric acid, an immediate blue colouration is first produced which then turns green and finally dirty yellow. On dissolving 1–2 mg of β-carotene in chloroform and adding a solution of antimony trichloride in chloroform a dark blue colouration is produced which has an absorption maximum at 590 mμ. α-Carotene behaves differently; cf. VON EULER, KARRER and RYBDOM[95].

Hydrogen chloride in ether or methanol solution produces no colouration. (For further data, see ZECHMEISTER[96]).

Optical activity: β-Carotene has a symmetrical structure and is optically inactive.

Partition test: On partition between petroleum ether and 90% aqueous methanol, the concentration of β-carotene in the former is 660 times as great as in the latter (KUHN and BROCKMANN[97]).

Chromatographic properties: β-Carotene is fairly strongly adsorbed on calcium hydroxide from petroleum ether solution. It is found below γ-carotene and above α-carotene on the chromatographic column[98]. Elution can be effected

by means of ether containing about 5 % methanol. β-Carotene is only very weakly adsorbed on zinc carbonate and calcium carbonate, and is washed through during the development of a chromatogram on these adsorbents.

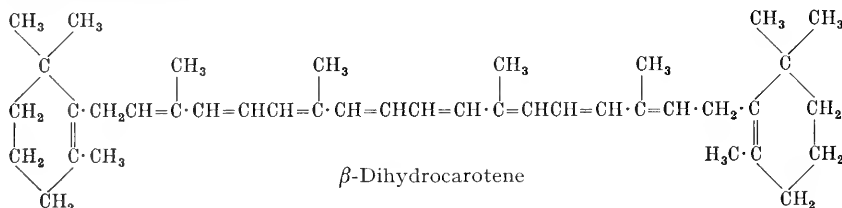
Behaviour towards oxygen: On standing in air, carotene absorbs oxygen with increasing rate with the formation of colourless products[99]. According to von Euler etc.[100] the autoxidation of very pure preparations only begins after several days' contact with air, formaldehyde being formed[101]. On shaking a solution of β-carotene in carbon tetrachloride in oxygen, a little glyoxal is formed[102].

Detection and estimation: β-carotene can be separated from the other carotenoid hydrocarbons by chromatographic adsorption on calcium hydroxide from petroleum ether. It is identified by its absorption maxima. According to Kuhn and Brockmann[103] an alcoholic solution of azobenzene can be used as standard for colourimetric determinations.

Physiological behaviour: β-Carotene possesses high vitamin A potency which has been studied in detail by von Euler, Karrer and co-workers[104] (cf. p. 11).

Derivatives

β-Dihydrocarotene $C_{40}H_{58}$:



β-Dihydrocarotene is formed together with other products by the reduction of β-carotene with aluminium amalgam[105]. Pure β-dihydrocarotene was isolated by Karrer and Rüegger[106] using the refined chromatographic method of separation. The constitution shown above was derived by these authors.

Dihydrocarotene crystallises from petroleum ether in salmon-red plates, m.p. 182°. It is vitamin A-inactive even in high doses.

Solvent:	*Absorption maxima*	
Carbon disulphide	461	432 mμ

(cf. Fig. 30, p. 360 and Fig. 31, p. 361)

Perhydrocarotene $C_{40}H_{78}$:

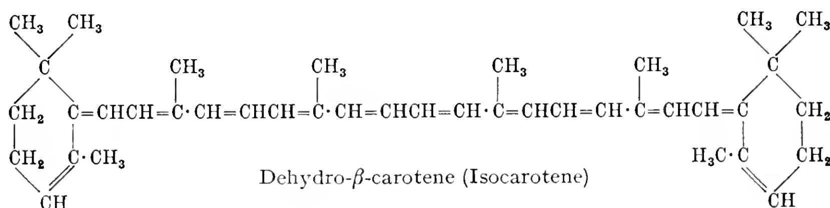
Perhydrocarotene is obtained by the hydrogenation of β-carotene in the presence of colloidal platinum as catalyst[107]. Perhydrocarotene is a very viscous, distillable oil. It is very soluble in cyclohexane, easily soluble in ben-

zene and ether, but only sparingly soluble in cold methanol and ethanol. Perhydrocarotene is optically inactive. According to VON EULER, DEMOLE, KARRER and WALKER[108] it possesses no biological activity.

Dehydro-β-carotene (Isocarotene) $C_{40}H_{54}$:

This hydrocarbon is formed by the decomposition of iodine addition products of β-carotene with thiosulphate, acetone, mercury or finely divided silver[109].

According to KARRER and SCHWAB[110], dehydro-β-carotene has the following constitution*:



Dehydro-β-carotene (Isocarotene)

Dehydro-β-carotene crystallises from petroleum ether in glistening violet-blue needles or plates, and from a mixture of benzene and methanol in violet prisms, m.p. 192–193° (corr., KARRER, SCHÖPP and MORF)[111]. It is very sparingly soluble in petroleum ether, but easily soluble in benzene and chloroform. It is practically insoluble in alcohols.

Solvent:	*Absorption maxima:*		
Carbon disulphide	543	504	472 mμ
Petroleum ether	504	475	447 mμ
Chloroform	518	485	455 mμ

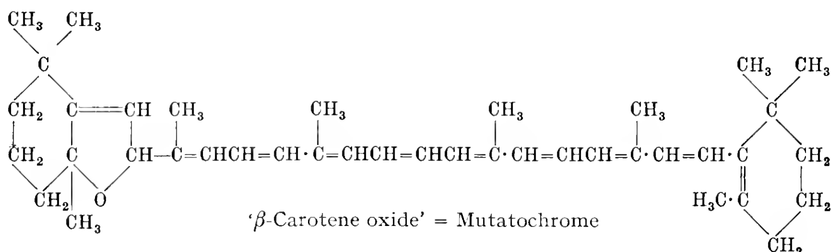
(cf. Fig. 31, p. 361)

Quantitative extinction measurements have been recorded by HAUSSER and SMAKULA[112]. With antimony trichloride in chloroform, isocarotene gives a stable blue colouration. It exhibits no vitamin A activity.

'β-Carotene oxide' $C_{40}H_{56}O$:

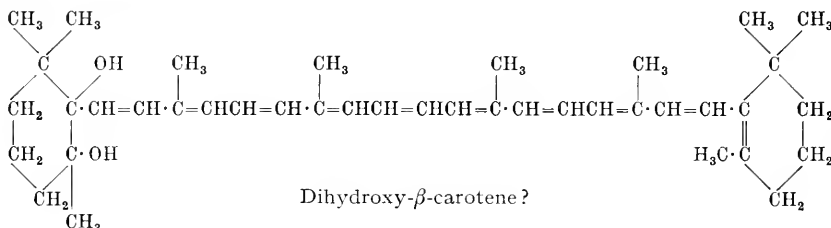
This compound was obtained by VON EULER, KARRER and WALKER[113] by the oxidation of β-carotene with perbenzoic acid. It is not an epoxide as was at first assumed, but a furanoid oxide of the following constitution (KARRER and JUCKER[114]).

* According to H. v. EULER, P. KARRER and O. WALKER, *Helv. chim. Acta* 15 (1932) 1507, small amounts of isocarotene are formed by the oxidation of β-carotene with perbenzoic acid.


'β-Carotene oxide' = Mutatochrome

For the properties and reactions of mutatochrome see p. 147.

Dihydroxy-β-carotene $C_{40}H_{58}O_2$*:


Dihydroxy-β-carotene ?

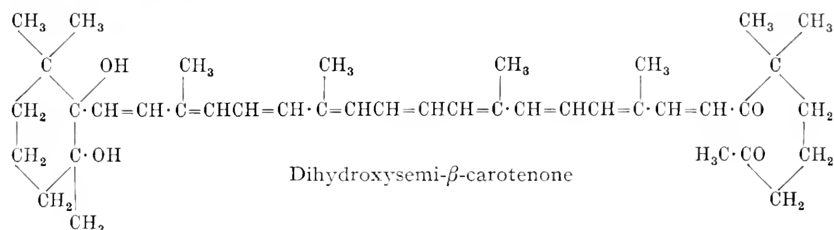
Dihydroxy-β-carotene is formed during the careful oxidation of β-carotene with aqueous 0.1 N-chromic acid (1.5 atoms O). It crystallises from a mixture of petrol and methanol in orange-red needles, m.p. 184° (KUHN and BROCKMANN[115]). Dihydroxy-β-carotene is easily adsorbed on aluminium oxide from benzene solution, but it is not adsorbed on calcium carbonate. (The lack of adsorption on calcium carbonate is unexpected for a compound assumed to contain two hydroxyl groups). Dihydroxy-β-carotene is easily soluble in benzene, chloroform and carbon disulphide, sparingly soluble in petrol, and insoluble in alcohols. On partition between petroleum ether and 90% methanol, it is found almost entirely in the upper layer. (The insolubility in alcohols and the epiphasic character of the compound are irreconcilable with the proposed formula).

Solvent:	*Absorption maxima:*		
Carbon disulphide	508	475	446 mμ
Chloroform	487	456	429 mμ
Petrol	478	448	420 mμ
Hexane	476	446	419 mμ
Benzene	489	457	428 mμ

Dihydroxy-β-carotene shows vitamin A activity.

* Concerning the molecular formula of dihydroxy-β-carotene see R. KUHN and H. BROCKMANN, *Ber.* 67 (1934) 1408 and *Ann.* 516 (1935) 99.

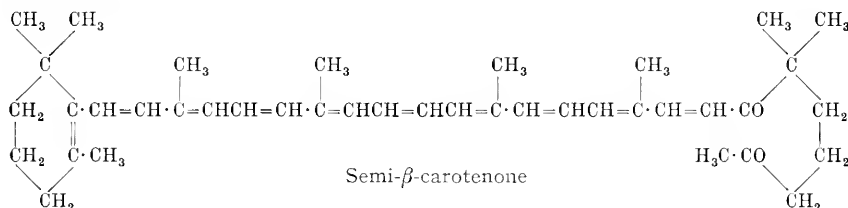
Dihydroxysemi-β-carotenone $C_{40}H_{58}O_4$:



Dihydroxysemi-β-carotenone is prepared by the oxidation of dihydroxy-β-carotene with 0.1 N chromic acid[116]. The pigment crystallises from a mixture of benzene and petroleum ether in dark red prisms with a bluish lustre, m.p. 172°. It is readily soluble in chloroform, somewhat less soluble in benzene and ethanol and hardly soluble in petroleum ether. Dihydroxysemi-β-carotenone is hypophasic in the partition test.

Solvent:	*Absorption maxima:*		
Carbon disulphide	534	495	464 mμ
Petroleum ether	497	468	440 mμ
Benzene	512	481	452 mμ
Ethanol	(498)	(471)	mμ
Chloroform	510	479	452 mμ

Semi-β-carotenone $C_{40}H_{56}O_2$:



Semi-β-carotenone was prepared by KUHN and BROCKMANN[117] by the oxidation of β-carotene with 0.1 N chromic acid. It is also formed on treating a solution of dihydroxy-β-carotene in benzene with lead tetra-acetate in glacial acetic acid[118]. It crystallises from methanol in square, scarlet plates, m.p. 118–119° (corr., evacuated capillary). Semi-β-carotenone is fairly soluble in petroleum ether and less soluble in ethanol. It is epiphasic in the partition test. For further information, cf. KUHN and BROCKMANN[117].

Solvent:	*Absorption maxima:*		
Carbon disulphide	538	499 mμ	
Chloroform	(519)	(487) mμ (diffuse)	
Petroleum ether	501	470	446 mμ
Benzene	518	486	458 mμ
Hexane	500	469	443 mμ

References p. 165–170.

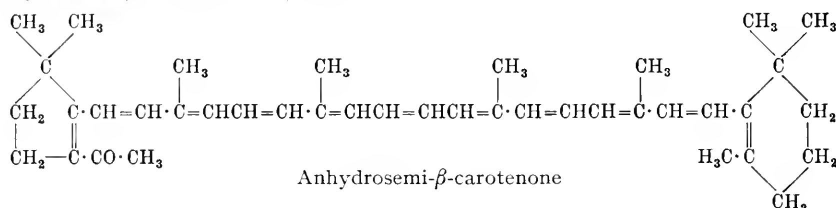
Semi-β-carotenone oxime crystallises from 90% ethanol in scarlet needles, m.p. 134–135° (evacuated capillary). The absorption maxima of the oxime are almost identical in wavelength location with those of the parent compound.

Neosemi-β-carotenone $C_{40}H_{56}O_2$:

KARRER and SOLMSSEN[119] observed that instead of semi-β-carotenone a different compound, neosemi-β-carotenone, can be formed by the oxidation of β-carotene with aqueous 0.1 N chromic acid. This compound separates from methanol in almost black crystals, m.p. 143°.

Solvent:	*Absorption maxima:*	
Carbon disulphide	510	479 mμ

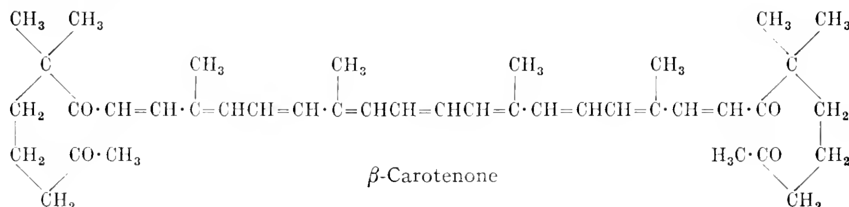
Anhydrosemi-β-carotenone $C_{40}H_{54}O$:



This compound is formed by splitting off a molecule of water from semi-β-carotenone by treatment with methanolic potassium hydroxide[120]. The pigment crystallises from a mixture of benzene and methanol in almost black prisms, with a green lustre, m.p. 177°. It is readily soluble in carbon disulphide, chloroform and benzene, and sparingly soluble in petroleum ether and absolute ethanol. It is entirely epiphasic in the partition test.

Solvent:	*Absorption maxima:*		
Carbon disulphide	547	509	481 mμ
Chloroform	524	489	459 mμ
Benzene	528	490	459 mμ
Petroleum ether	512	480	452 mμ

β-Carotenone $C_{40}H_{56}O_4$:



KUHN and BROCKMANN[121] prepared β-carotenone by the oxidation of β-carotene with chromic acid. It is also formed by the oxidation of semi-

References p. 165–170.

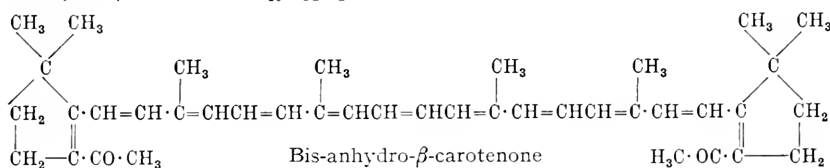
β-carotenone with chromic acid[122]. β-Carotenone crystallises from a mixture of benzene and light petroleum in scarlet, hexagonal flakes with a blue lustre, m.p. 174–175° (corr., evacuated capillary[123]). The pigment can be adsorbed on aluminium oxide or calcium carbonate from petroleum ether solution. On partition between petroleum ether and 90 % methanol it is found mainly in the lower layer. β-Carotenone is easily soluble in chloroform, carbon disulphide and benzene, sparingly soluble in cold methanol and ethanol, and very sparingly soluble in petroleum ether.

Solvent:	*Absorption maxima:*		
Carbon disulphide	538	499	466 mμ
Chloroform	527	489	454 mμ
Petroleum ether	502	468	440 mμ
Benzene	522	486	453 mμ
Hexane	500	466	436 mμ

(cf. Fig. 29, p. 360)

Quantitative extinction measurements in hexane have been recorded by HAUSSER and SMAKULA[124]. β-Carotenone forms a *dioxime*, m.p. 198° (corr., evacuated capillary).

Bis-anhydro-β-carotenone $C_{40}H_{52}O_2$:

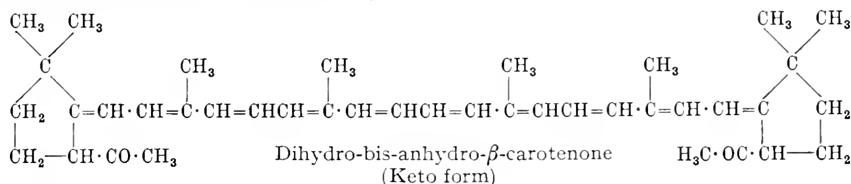


Bis-anhydro-β-carotenone

This derivative was obtained by KUHN and BROCKMANN[125] by treating β-carotenone with methanolic potassium hydroxide. It crystallises from a mixture of benzene and methanol in steel-blue prisms or plates, m.p. 209°. It is almost entirely epiphasic in the partition test. Bis-anhydro-β-carotenone is soluble in chloroform, benzene and carbon disulphide, but almost insoluble in petrol, methanol and petroleum ether.

Solvent:	*Absorption maxima:*		
Carbon disulphide	(567)	(525)	(477) mμ
Chloroform	(545)	(506)	(481) mμ
Benzene	(545)	(505)	(478) mμ
Petrol	530	494	462 mμ

Dihydro-bis-anhydro-β-carotenone $C_{40}H_{54}O_2$:



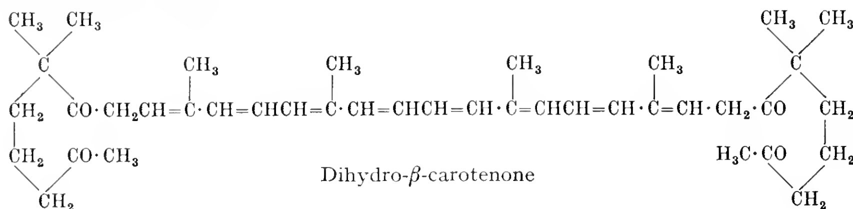
Dihydro-bis-anhydro-β-carotenone
(Keto form)

References p. 165–170.

This compound is formed by shaking bis-anhydro-β-carotenone with zinc dust in a mixture of pyridine and glacial acetic acid[126]. The pigment crystallises from aqueous pyridine in brilliant red needles, m.p. 217° (corr., evacuated capillary). Dihydro-bis-anhydro-β-carotenone is readily soluble in carbon disulphide, benzene, chloroform and pyridine, but only sparingly soluble in petrol and petroleum ether. It is rapidly oxidised by air in alkaline alcoholic solution to bis-anhydro-β-carotenone.

Solvent:	*Absorption maxima:*		
Carbon disulphide	510	478	448 mμ
Chloroform	490	459	430 mμ
Benzene	492	460	430 mμ
Petrol	479	448	421 mμ

Dihydro-β-carotenone $C_{40}H_{58}O_4$:



Dihydro-β-carotenone is formed by the reduction of β-carotenone with zinc dust in pyridine and glacial acetic acid[127]. The pigment crystallises from a mixture of petrol and benzene in golden-yellow needles, m.p. 130° (corr. evacuated capillary). It is readily soluble in pyridine, chloroform and benzene, but only sparingly soluble in petroleum ether and alcohols. It is almost entirely hypophasic in the partition test.

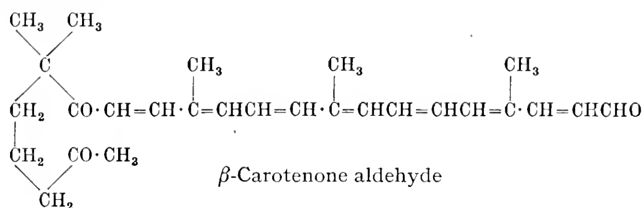
Solvent:	*Absorption maxima:*	
Carbon disulphide	454.5	426 mμ
Chloroform	435	411 mμ
Hexane	426	mμ
Petrol	429	mμ
Benzene	436	411 mμ
Petroleum ether	424	mμ

On treatment with hydroxylamine, dihydro-β-carotenone forms a dioxime $C_{40}H_{60}O_4N_2$, which crystallises from hot benzene in golden-yellow plates, m.p. 151° (corr., evacuated capillary). The dioxime is less readily soluble in petrol and benzene and more readily soluble in alcohol than dihydro-β-carotenone.

Solvent:	*Absorption maxima:*	
Carbon disulphide	454.5	426 mμ
Petrol	429	mμ
Ethanol	426	mμ

References p. 165–170.

β-Carotenone aldehyde $C_{27}H_{36}O_3$:



This aldehyde is formed by the oxidation of β-carotene or β-carotenone with chromic acid[128]. It crystallises from a mixture of benzene and petrol in yellow-red needles with a bluish lustre, m.p. 146–147° (corr. evacuated capillary). The compound is readily soluble in chloroform, carbon disulphide, benzene and hot methanol, but only sparingly soluble in cold petrol and petroleum ether.

Solvent:	*Absorption maxima:*		
Carbon disulphide	491	459	430 mμ
Chloroform	482	450	423 mμ
Petroleum ether	457	430	404 mμ
Petrol	461	432	406 mμ
Benzene	476	446	420 mμ
Hexane	458	431	405 mμ
Ethanol	(473)	(442)	mμ

On prolonged treatment with excess hydroxylamine, β-carotenone aldehyde forms a dioxime. With regard to its constitution, compare KUHN and BROCKMANN[128]. It crystallises from dilute methanol in yellow-red plates, m.p. 183–184°. It is sparingly soluble in petroleum ether, petrol and cold benzene, but more easily soluble in ethanol.

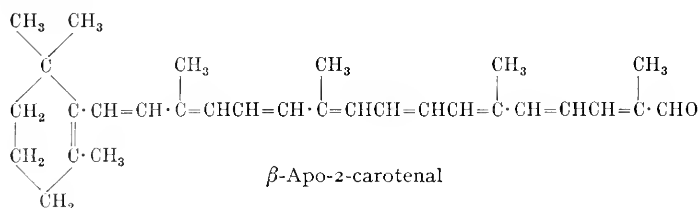
Solvent:	*Absorption maxima:*		
Carbon disulphide	492	460	431 mμ
Chloroform	478	448	420 mμ
Petrol	462	435	mμ
Benzene	477	447	419 mμ

Using molecular proportions of hydroxylamine and β-carotenone aldehyde, a monoxime is obtained which is a mixture of aldoxime and ketoxime.

The monoxime crystallises from methanol in yellow-red plates or needles, m.p. 174°. (Concerning the conversion of the aldoxime into anhydroazafrinone amide, see p. 134 and KUHN and BROCKMANN loc. cit.).

References p. 165–170.

β-Apo-2-carotenal $C_{30}H_{40}O$:



β-Apo-2-carotenal

This aldehyde is formed by the potassium permanganate oxidation of β-carotene[129]. β-Apo-2-carotenal crystallises from methanol in violet plates, m.p. 139°. On addition of concentrated hydrochloric acid to an ethereal solution of the pigment an intense stable blue colouration is produced. The aldehyde shows strong vitamin A activity.

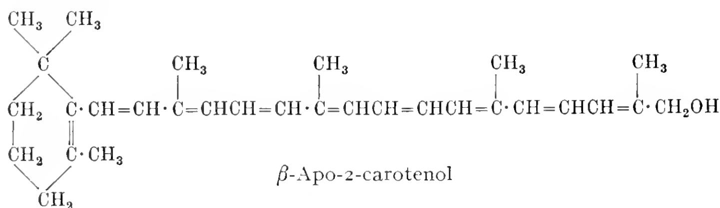
Solvent:	*Absorption maxima:*	
Carbon disulphide	525	490 mμ
Petroleum ether	484	454 mμ
Ethanol	(498 . . .	447)mμ

β-Apo-2-carotenal oxime crystallises in glistening violet rhombs or prisms, m.p. 180°.

Solvent:	*Absorption maxima:*	
Carbon disulphide	507	473 mμ
Petroleum ether	471	441 mμ
Ethanol	475	445 mμ

β-Apo-2-carotenal semicarbazone melts at 212° (sinters above 205°).

β-Apo-2-carotenol $C_{30}H_{42}O$:



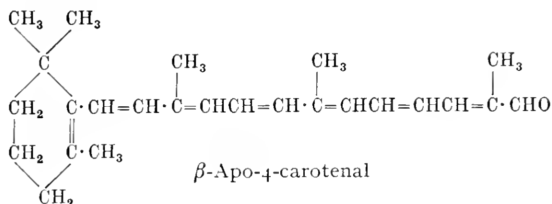
β-Apo-2-carotenol

This compound was obtained by VON EULER, KARRER and SOLMSSEN by the reduction of β-apo-2-carotenal with isopropyl alcohol and aluminium isopropoxide[130]. It crystallises from a mixture of benzene and petroleum ether in yellow plates, m.p. 145°.

Solvent:	*Absorption maxima:*	
Carbon disulphide	486	456 mμ
Petroleum ether	453	423 mμ
Ethanol	456	426 mμ

(cf. Fig. 29, p. 360)

β-Apo-4-carotenal $C_{25}H_{34}O$:



This compound is obtained together with β-apo-2-carotenal by the oxidation of β-carotene with potassium permanganate[131]. It has not been obtained in the crystalline state, but forms a crystalline oxime and semicarbazone.

Solvent:	*Absorption maxima:*
Carbon disulphide	about 460 mμ (diffuse)
Petroleum ether	about 442 mμ (diffuse)

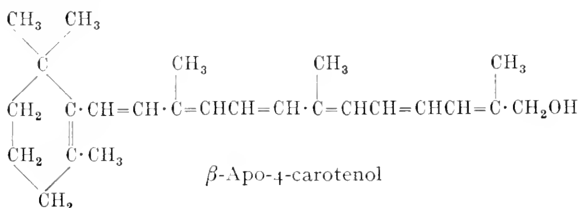
β-Apo-4-carotenal oxime crystallises from methanol in rhombic plates and clusters, m.p. 165°.

Solvent:	*Absorption maxima:*
Petroleum ether	408 mμ
Ethanol	409 mμ
Carbon disulphide	456 mμ (slightly diffuse)

β-Apo-4-carotenal semicarbazone separates from ethanol as a scarlet-red powder, m.p. 217° (with decomposition), sintering above 214°.

Solvent:	*Absorption maxima:*
Carbon disulphide	474 mμ
Ethanol	445 mμ (broad band)

β-Apo-4-carotenol $C_{25}H_{36}O$:



This polyene alcohol is formed by the reduction of β-apo-4-carotenal with isopropyl alcohol and aluminium isopropoxide[132]. β-Apo-4-carotenal has so far only been obtained as a viscous oil.

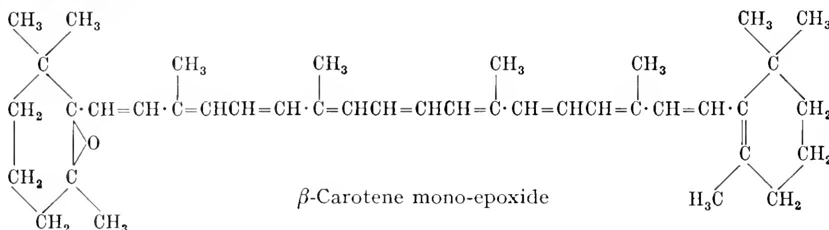
Oxides of β-Carotene

By the oxidation of β-carotene with monoperphthalic acid, KARRER and JUCKER[133] obtained a number of oxidation products which are epoxides or

References p. 165–170.

furanoid oxides. (Cf. p. 61 concerning the constitution of these compounds). The oxides of β-carotene are very similar to the corresponding derivatives of cryptoxanthin (p. 178) and zeaxanthin (p. 189), from which they differ only by the absence of the hydroxyl groups.

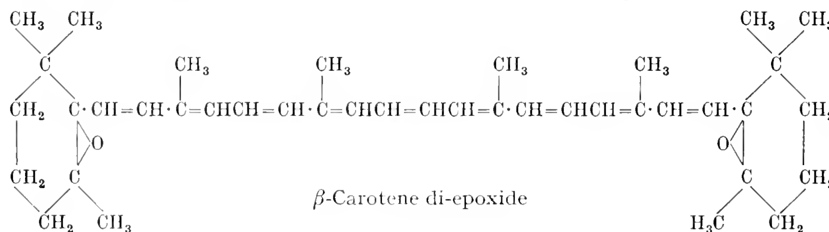
β-Carotene mono-epoxide $C_{40}H_{56}O$:



This compound crystallises from a mixture of benzene and methanol, or ether and methanol, in lustrous orange leaflets, m.p. 160° (uncorr., in vacuum). On shaking an ethereal solution of the pigment with concentrated aqueous hydrochloric acid, the acid layer slowly develops a pale blue colouration which is not very stable.

Solvent:	*Absorption maxima:*	
Carbon disulphide	511	479 mμ
Benzene	492	460 mμ
Petroleum ether	478	447 mμ
Chloroform	492	459 mμ

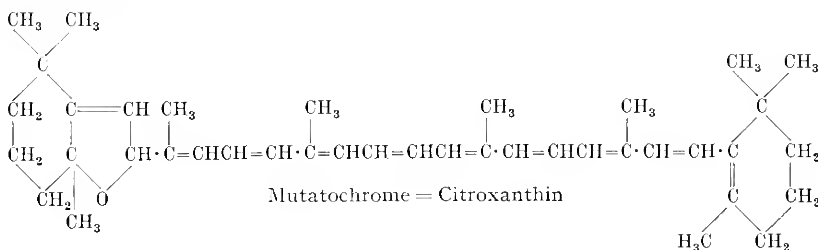
β-Carotene di-epoxide $C_{40}H_{56}O_2$:



β-Carotene di-epoxide crystallises from a mixture of benzene and methanol in yellow-orange leaflets, m.p. 184° (uncorr., evacuated capillary). On shaking an ethereal solution of the pigment with concentrated aqueous hydrochloric acid, the acid layer is coloured dark blue. The colouration is stable for several days. β-Carotene di-epoxide exhibits entirely epiphasic properties.

Solvent:	*Absorption maxima:*	
Carbon disulphide	502	472 mμ
Benzene	485	456 mμ
Petroleum ether	470.5	443 mμ
Chloroform	484	456 mμ

Mutatochrome $C_{40}H_{56}O$*:

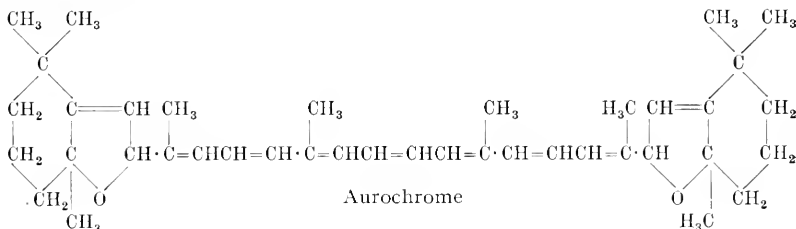


Mutatochrome = Citroxanthin

By the action of hydrogen chloride on β-carotene mono-epoxide, the corresponding furanoid oxide, mutatochrome is formed, together with a small proportion of β-carotene. Mutatochrome crystallises from a mixture of benzene and methanol in yellow-orange leaflets, m.p. 163–164° (uncorr., in vacuum). Mutatochrome behaves in the same way as β-carotene mono-epoxide in the hydrochloric reaction and partition test.

Solvent:	*Absorption maxima:*	
Carbon disulphide	489.5	459 mμ
Benzene	470	440 mμ
Petroleum ether	456	427 mμ
Chloroform	469	438 mμ

Aurochrome $C_{40}H_{56}O_2$:



Aurochrome

Aurochrome is formed by the action of dilute hydrochloric acid on β-carotene di-epoxide. It crystallises from a mixture of benzene and methanol in beautiful yellow leaflets, m.p. 185° (uncorr., in vacuum). On adding concentrated aqueous hydrochloric acid to an ethereal solution of this pigment, a very stable dark blue colouration is formed. On partitioning aurochrome

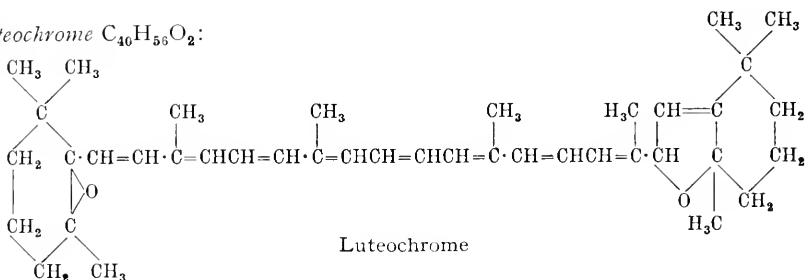
* By the action of perbenzoic acid on β-carotene, H. v. EULER, P. KARRER and O. WALKER, (*Helv. chim. Acta 15* (1932) 1507) obtained an oxide which they formulated as β-carotene mono-epoxide. These authors were unaware of the great sensitivy of this compound to acids and later comparison has shown that they had in fact obtained the furanoid oxide, mutatochrome, and not the mono-epoxide formed primarily. P. KARRER and E. JUCKER, *Helv. chim. Acta 30* (1947) 536 established the identity of mutatochrome with the pigment citroxanthin which they had isolated from orange peel (*Helv. chim. Acta 27* (1944) 1695). Mutatochrome is thus a naturally occurring pigment.

References p. 165–170.

between methanol and petroleum ether, the pigment is found almost quantitatively in the upper layer.

Solvent:	*Absorption maxima:*	
Carbon disulphide	457	428 mμ
Benzene	440	mμ
Petroleum ether	428	mμ
Chloroform	437	mμ

Luteochrome $C_{40}H_{56}O_2$:



Luteochrome

Luteochrome is formed together with β-carotene mono-epoxide and β-carotene di-epoxide during the oxidation of β-carotene with monoperphthalic acid[134]. The pigment crystallises from a mixture of benzene and methanol in thin yellow-orange leaflets, m.p. 176° (uncorr., in vacuum). Concentrated aqueous hydrochloric acid has the same effect as with β-carotene di-epoxide and aurochrome. On partitioning between methanol and petroleum ether, luteochrome is found mainly in the upper layer.

Solvent:	*Absorption maxima:*	
Carbon disulphide	482	451 mμ

Properties of the β-Carotene Oxides

β-Carotene epoxides and furanoid oxides can only be separated with difficulty by chromatographic analysis, using petroleum ether as solvent and calcium hydroxide as adsorbent. The solubilities of these compounds do not differ appreciably from those of β-carotene. They are easily soluble in carbon disulphide, chloroform, benzene and ether, somewhat less easily in petroleum ether, and only very sparingly soluble in methanol and ethanol.

Physiological Properties

The vitamin A activity of the various β-carotene oxides has been examined by VON EULER[135]. It has been found that 17γ-doses of β-carotene di-epoxide and 18γ-doses of luteochrome produce full vitamin A activity in rats. It may

References p. 165–170.

be concluded that they are partly deoxygenated to β-carotene or mutatochrome* in the animal organism, since according to all previous experience, vitamin A activity requires the presence of an unsubstituted β-ionone ring in the carotenoid**.

Cis-trans-Isomers of β-Carotene[136, 137]

In 1935, GILLAM and EL RIDI[136] observed that several zones are developed during the chromatographic adsorption of pure β-carotene. They ascribed this phenomenon to a change of the pigment during adsorption, and were able to isolate a transformation product which they termed pseudo-α-carotene. This compound exhibited the characteristic properties of a carotenoid, melted at 166°, and showed epiphasic behaviour, no optical activity and vitamin A potency.

Solvent:	*Absorption maxima:*	
Carbon disulphide	507	477 mμ
Chloroform	486	456 mμ
Petroleum ether	477	446 mμ
Ethanol	478	447 mμ

More recently, ZECHMEISTER and co-workers[137] have investigated these changes and have shown that they are not produced by adsorption, but that polyene pigments generally can be isomerised by dissolution, melting of the crystals, or certain other operations (cf. p. 39). From a number of considerations, including the fact that these transformations are reversible, ZECHMEISTER concluded that *cis-trans*-isomerism is involved. About 10 zones are observed in the transformation chromatogram and the individual pigments are regarded as stereoisomers of β-carotene. Only *one* of these pigments has so far been obtained in the crystalline state, and has been termed neo-β-carotene U. It is rather more strongly adsorbed chromatographically than β-carotene, is somewhat more soluble in organic solvents, and exhibits epiphasic behaviour on partition between petroleum ether and methanol. Thus 250 mg of β-carotene yielded 41 mg of neo-β-carotene U, m.p. 122–123° (corr., block). According to ZECHMEISTER and collaborators[138], neo-β-carotene U contains *one* cis-double bond whereas the pseudo-α-carotene obtained by GILLAM and EL RIDI[136] may contain two *cis*-double bonds.

Solvent:	*Absorption maxima of neo-β-carotene U:*	
Carbon disulphide	512.5	478.5 mμ
Benzene	494	461 mμ
Petroleum ether	481	450 mμ
Chloroform	493.5	461 mμ
Ethanol	482	450.5 mμ

* Early investigations showed that mutatochrome exhibits vitamin A activity.

** See, however, footnote p. 14.

Neo-β-carotene U shows vitamin A activity (cf. p. 15), but of a lower order than that of natural β-carotene.

The other isomers could not be prepared in the crystalline state. They were characterised by their absorption maxima in petroleum ether solution, and by their positions in the chromatogram. In the following table, the compounds are enumerated in the sequence in which they occur in the chromatogram.

Designation	Absorption maxima in petroleum ether	
Neo-β-carotene U	481	450 mμ
Neo-β-carotene V	472.5	441.5 mμ
Neo-β-carotene A	469	437.5 mμ
Neo-β-carotene B	475.5	444.5 mμ
Neo-β-carotene C	465.5	433 mμ
Neo-β-carotene D	474.5	441.5 mμ
Neo-β-carotene E	477.5	445 mμ

These are followed by a few other transformation products to which no names have been assigned. Natural β-carotene with complete *trans*-configuration occurs between neo-β-carotene V and neo-β-carotene A in the chromatogram. According to POLGÁR and ZECHMEISTER[140], neo-β-carotene B is identical with pseudo-α-carotene.

4. α-CAROTENE $C_{40}H_{56}$

History

1931 α-Carotene is discovered simultaneously by KUHN and LEDERER[141] and KARRER and co-workers[142]. The new pigment is an isomer of β-carotene and generally accompanies the latter in vegetable and animal materials.

1933 KARRER and WALKER[143] introduce calcium hydroxide and calcium oxide as new adsorbents for the chromatographic separation of epiphasic polyene pigments. Pure α-carotene is thus prepared for the first time.

1933 KARRER, MORF and WALKER[144] elucidate the constitution of α-carotene.

Occurrence

α-Carotene is almost as widely distributed in the vegetable kingdom as the β-isomer. It is present in varying amounts in most carotene preparations and is concentrated in the mother liquors during recrystallisation.

MACKINNEY[145] examined the presence of α-carotene in green parts of plants, especially leaves, and found that the following plants contain α-carotene:

References p. 165–170.

Coprosma baueri Endlicher; *Daucus Carota* L., *Petroselinum hortense, Hedera helix* L., *Quercus agrifolia, Aesculus californica* Nuttall; *Parthenocissus quinquefolia, Amsinckia douglasiana* De Candolle, *Cuscuta salina* Engelman, *Solanum tuberosum, Lycopersicum esculentem* Miller, *Citrus maxima, Malva parviflora* L., *Urtica urens* L., *Ficus carica* L., *Thea sp.*, *Camellia sp.*, *Sedum acre* L., *Dracaena draco* L., *Washingtonia filifera* Wendland; *Pinus radiata* Don; *Libocedrus decurrens* Torrey; *Moss sp.*, *Chlorella vulgaris, Ranunculus californicus* Bentham, *Magnolia grandiflora L.*, *Phoenix, Sequoia sempervirens* Engelmann.

Further information regarding the occurrence of α-carotene in plant leaves is given by STRAIN[146]. (Data regarding the α-carotene content of carotene preparations are given on p. 130).

TABLE 36

OCCURRENCE OF α-CAROTENE *

Source	References
Arbutus (fruit)	K. SCHÖN, *Biochem. J. 29* (1935) 1779.
Cantharellus species	H. WILLSTAEDT, *Chem. Centr. 1938*, II, 2272.
Citrullus vulgaris	L. ZECHMEISTER and A. POLGÁR, *J. biol. Chem. 139* (1941) 193.
Cladophora Sauteri	I. M. HEILBRON, E. G. PARRY and R. F. PHIPERS, *Biochem. J. 29* (1935) 1376.
Coleosporium senecionis	E. LEDERER, *Chem. Centr. 1936*, I, 3852.
Convallaria majalis	A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem. 207* (1932) 25.
Cow fat	L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 154.
Cucurbita maxima (fruit)	L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 824.
Cuscuta subinclusa and *Cuscuta salina*	G. MACKINNEY, *J. biol. Chem. 112* (1935) 421.
Oil from *Cyclopterus*	N. A. SØRENSEN, *Chem. Centr. 1934*, I, 3817.
Dendrodoa grossularia (Styclopsis)	E. LEDERER, *Chem. Centr. 1936*, I, 3853.
Diospyros costata (fruit)	K. SCHÖN, *Biochem. J. 29* (1935) 1779.
Formosa tea-leaves	R. YAMAMOTO and T. MURAOKA, *Chem. Centr. 1933*, I, 441.
Genista tridentata	K. SCHÖN and G. MESQUITA, *Biochem. J. 30* (1936) 1966.
Gonocaryum obovatum (skin)	A. WINTERSTEIN, *Z. physiol. Chem. 215* (1933) 51.
Gonocaryum pyriforme (skin)	do.
Gorse	P. KARRER and E. JUCKER, *Helv. chim. Acta 27* (1944) 1585.
Haematococcus pluvialis	J. TISCHER, Z. *Physiol. Chem. 252* (1938) 225.
Hymeniacidon sanguineum	P. J. DRUMM and W. F. O'CONNOR, *Nature 145* (1940) 425.

* Only references to investigations resulting in the isolation and unambiguous identification of the pigment are included.

References p. 165–170.

Source	References
Ipomoea Batatas	J. C. LANZING and A. G. VAN VEEN, *Chem. Centr. 1938*, I, 2081.
Mangifera indica (fruit)	R. YAMAMOTO, Y. OSIMA and T. GOMA, *Chem. Centr. 1933*, I, 441.
Marine and deep-sea soil	D. L. FOX, *Chem. Centr. 1937*, II, 3899.
Oedogonium	I. M. HEILBRON, E. G. PARRY and R. F. PHIPERS, *Biochem. J. 29* (1935) 1376.
Oil from *Orthagoriscus mola*	N. A. SØRENSEN, *Chem. Centr. 1934*, I, 3817.
Palm oil	P. KARRER, H. v. EULER and H. HELLSTRÖM, *Chem. Centr. 1932*, I, 1800. — R. KUHN and H. BROCKMANN *Z. physiol. Chem. 200* (1931) 255. — P. KARRER and O. WALKER, *Helv. chim. Acta 16* (1933) 641. — R. F. HUNTER and A. D. SCOTT, *Biochem. J. 35* (1941) 31.
Paprica	L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 509* (1934) 269.
Oil from *Regalecus*	N. A. SØRENSEN, *Chem. Centr. 1934*, I, 3817.
Red Euglene	H. TISCHER, *Z. physiol. Chem. 259* (1939) 163.
Rhodymenia palmata	I. M. HEILBRON, E. G. PARRY and R. F. PHIPERS, *Biochem. J. 29* (1935) 1376.
Rye-germ oil	H. A. SCHUETTE and R. C. PALMER, *Chem. Centr. 1938*, I, 1898.
Saffron	R. KUHN and A. WINTERSTEIN, *Ber. 67* (1934) 344.
Sorbus aucuparia (fruit)	R. KUHN and E. LEDERER, *Ber. 64* (1931) 1349.
Soya beans	W. C. SCHERMAN, *Chem. Centr. 1941*, I, 2673.
Ulex Gallii	K. SCHÖN, *Biochem. J. 30* (1936) 1960.
Yellow maize	G. S. FRAPS and A. R. KEMMERER, *Chem. Centr. 1942*, II, 1641.

Preparation

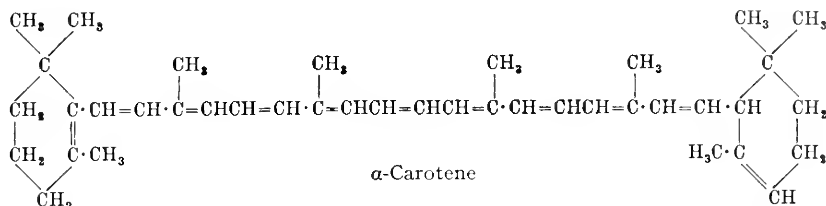
α-Carotene is widely distributed in plants but never occurs in large concentrations. According to KARRER and WALKER[147] it is best prepared from commercial carotene (carrot carotene). The pigment can be separated in good yield from β-carotene by chromatographic adsorption on calcium hydroxide.

Glass tubes about 70 cm in length and 5 cm in diameter are filled with air-dry calcium hydroxide (cf. p. 27) and the columns are wetted with a little ligroin (b.p. 60–70° C). 200 mg of carotene (a mixture of β, α, and a little γ-carotene) are dissolved in about 100 ml of ligroin and the solution is poured on the calcium hydroxide column. The chromatogram is developed with petroleum ether, b.p. 70–80°. As soon as the yellow, α-carotene-containing zone has reached the lower end of the tube, the development is interrupted and the pigment is eluted with a mixture of ether and methanol (10:1). After evaporating the solvent, the pigment remains as a dark-red crystalline mass. For further purification, the α-carotene is crystallised 2–3 times from a mixture of benzene and methanol or from petroleum ether. From 1 g of carotene an average of 80 mg of pure α-carotene are obtained in this way.

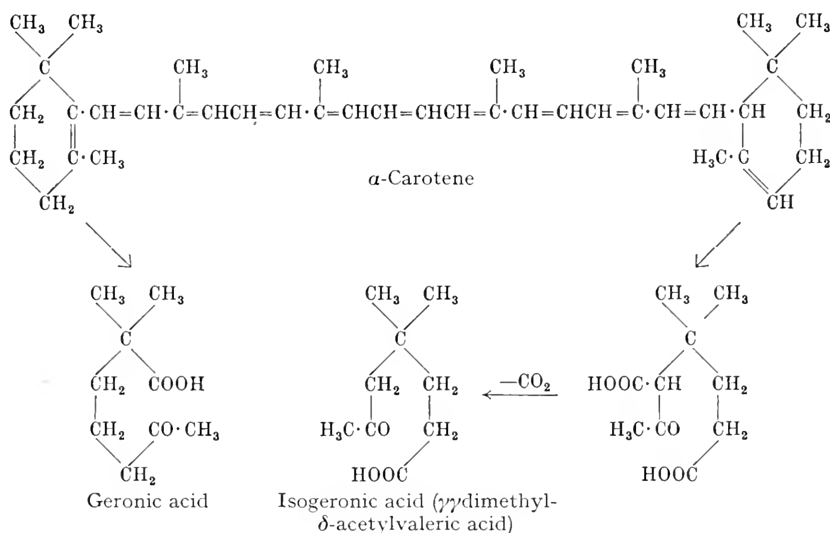
Other methods[148] of preparation of α-carotene are hardly used, as they are relatively cumbersome and do not furnish pure products.

References p. 165–170.

Chemical Constitution



The elucidation of the constitution of α-carotene has been carried out mainly by KARRER and co-workers[149]. The hydrocarbon contains 11 double bonds[150]. The absorption spectrum, the maxima of which are displaced by 12 mμ towards shorter wavelengths as compared with β-carotene, suggests that not all double bonds are in conjugation. A definite proof for the formula of α-carotene was given by KARRER, MORF and WALKER[151] who showed that ozonisation of the pure pigment (chromatographed on calcium hydroxide) furnishes small quantities of isogeronic acid, besides geronic acid which is also formed in the oxidation of β-carotene.



This formula is in agreement with the fact that α-carotene is optically active. Further confirmation of this constitution is provided by the complex oxidation products which have recently been prepared by KARRER and co-workers (cf. p. 155).

Properties

Crystalline form: α-Carotene separates from a mixture of benzene and methanol in violet prisms and clusters and from petroleum ether in dark violet prisms or polygons.

Melting point: 187–188° (corr.)[152].

Solubility: α-Carotene is considerably more soluble than the β-isomer. It is very soluble in carbon disulphide and chloroform, easily soluble in benzene and ether, sparingly soluble in petroleum ether and almost insoluble in alcohols. 100 Ml of hexane at O° dissolve 294 mg of α-carotene[153].

Spectral properties:

Solvent:	*Absorption maxima:*			
Carbon disulphide . .	509	477 mμ		
Petrol	478	447.5 mμ		
Chloroform	485	454 mμ		
Hexane	475	445	420	395 mμ

(cf. Fig. 7, p. 350 and Fig. 31, p. 361)

For quantitative extinction measurements, see HAUSSER and SMAKULA[154].
For Raman spectra, see VON EULER and HELLSTRÖM[155].

Colour reactions: α-Carotene in chloroform solution gives a blue colouration with concentrated sulphuric acid. On adding antimony trichloride to a chloroform solution, a deep blue colouration is produced with an absorption maximum near 542 mμ (KARRER and WALKER[152]).

Optical activity: The specific rotation in benzene is +385° (643.85 mμ cadmium line) (KUHN and LEDERER[156]). The rotatory dispersion was determined by KARRER and WALKER[152]:

$$[\alpha]_{C}^{18} = 315° \ (\pm 7°) \qquad [\alpha]_{643.5}^{18} = +385° \ (\pm 5°)$$

Partition test: α-Carotene exhibits entirely epiphasic properties on partition between petroleum ether and 90 % methanol.

Chromatographic properties: α-Carotene is adsorbed less strongly than β-carotene on calcium hydroxide from petroleum ether solution, and is found below β-carotene on the column. (KARRER and WALKER[152]). It can be eluted by means of ether containing about 5 % methanol.

Behaviour towards oxygen: The oxidation of α-carotene in light is autocatalytic (BAUR[157]).

Detection and estimation: The separation of α-carotene from other carotenoid hydrocarbons is effected by chromatographic adsorption on calcium hydroxide. Its presence can be established by the determination of the absorption maxima. For the colorimetric estimation of the pigment, KUHN and BROCKMANN[158] recommend an alcoholic solution of azobenzene as a standard.

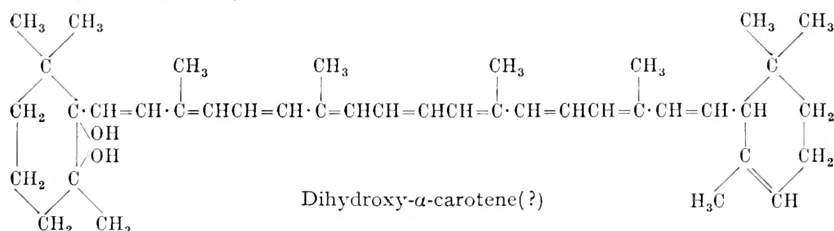
Physiological behaviour: α-Carotene exhibits strong vitamin A activity[159].

Derivatives

"Dihydro-α-carotene":

By the reduction of α-carotene with aluminium amalgam, KARRER and MORF[160] obtained a dihydro-α-carotene as a light yellow oil. The homogeneity and constitution of this product is still uncertain. Degradation by means of ozone yields geronic acid while on oxidation with potassium permanganate α:α-dimethylglutaric acid is formed.

Dihydroxy-α-carotene $C_{40}H_{58}O_2$:

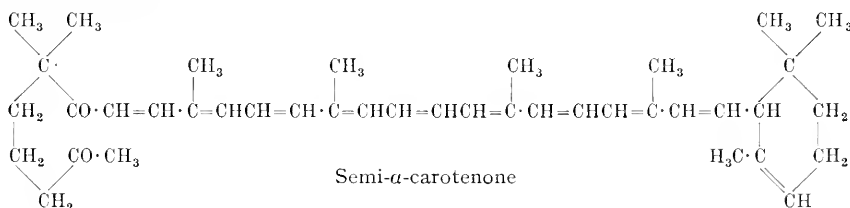


Dihydroxy-α-carotene(?)

Dihydroxy-α-carotene was obtained by KARRER and collaborators together with α-semicarotenone and α-carotone during the oxidation of α-carotene with chromic acid[161]. With regard to the constitution of this derivative, cf. KARRER, VON EULER and SOLMSSEN[162]. The compound crystallises from a mixture of methanol and petroleum ether in needles, m.p. 183° (uncorr.). Dihydroxy-α-carotene is sparingly soluble in petroleum ether. It is dextrorotatory. According to KARRER, VON EULER and SOLMSSEN it has no vitamin A activity.

Solvent:	*Absorption maxima:*		
Carbon disulphide	502	471	440 mμ

Semi-α-carotenone $C_{40}H_{56}O_2$:



Semi-α-carotenone

Semi-α-carotenone is formed by the oxidation of α-carotene with chromic acid[163]. Semi-α-carotenone crystallises from methanol in needles, m.p. 135° (uncorr.). The question of its constitution is dealt with in detail in the original communication[164]. The fact that semi-α-carotenone has no vitamin A activity[165] is in agreement with the formula shown.

Solvent:	*Absorption maxima:*	
Carbon disulphide	533	499 mμ

Semi-α-carotenone mono-oxime $C_{40}H_{57}O_2N$[166] forms red crystals, m.p. 132°.

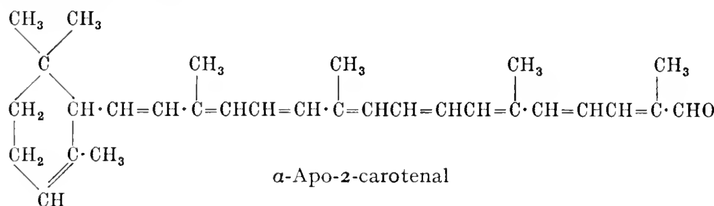
References p. 165–170.

α-Carotone $C_{40}H_{56}O_5$:

α-Carotone is formed by the oxidation of α-carotene with chromic oxide[167]. It crystallises from methanol in glittering steel-blue prisms, m.p. 148° $[\alpha]_{644}$ = +341° (± 15°). α-Carotone has no vitamin A activity.

Solvent:	*Absorption maxima:*		
Carbon disulphide	(535)	502	471 mμ
Chloroform	484	454	mμ

α-Apo-2-carotenal $C_{30}H_{40}O$:



α-Apo-2-carotenal

This aldehyde is formed by the potassium permanganate oxidation of α-carotene. α-Apo-2-carotenal crystallises from petroleum ether in clustered light red prisms, m.p. 158°. It is less easily soluble in the common solvents than the β-isomer.

Solvent:	*Absorption maxima:*		
Carbon disulphide	519	484	454 mμ
Petroleum ether	479	450	mμ

(cf. Fig. 28, p. 359)

α-Apo-2-carotenal oxime separates from absolute methanol in clustered red leaflets, m.p. 178°.

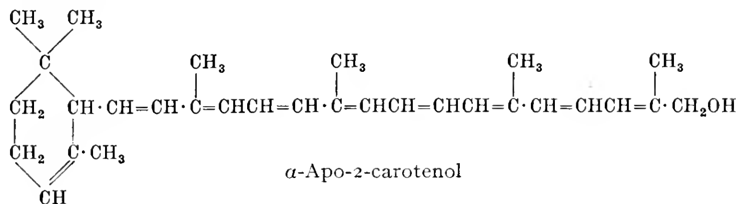
$[\alpha]_D$ = +692° (± 35°) (for further data compare the original communication).

Solvent:	*Absorption maxima:*	
Carbon disulphide	499	469 mμ
Petroleum ether	466	438 mμ (diffuse)
Ethanol	469	439 mμ

By the oxidation of chromatographically unpurified carotene, VON EULER, KARRER and SOLMSSEN obtained a compound which, was spectroscopically indistinguishable from α-apo-2-carotenal. The analytical figures for this compound as well as those for the oxime agreed with the calculated values for α-apo-2-carotenal. This new degradation product had m.p. 174°, however, and the oxime had m.p. 185°. The melting points are considerably higher than for α-apo-2-carotenal. For details, compare the original communication[168].

References p. 165–170.

α-Apo-2-carotenol $C_{30}H_{42}O$:



This alcohol is obtained by the reduction of α-apo-2-carotenal (lower melting form) with isopropyl alcohol and aluminium isopropoxide. It crystallises from a mixture of benzene and petroleum ether in spherical golden-yellow clusters, m.p. 157° (sinters at 150°).

Solvent:	*Absorption maxima:*	
Carbon disulphide	478	448 mμ
Petroleum ether	446	420 mμ
Ethanol	448	423 mμ

On treating a solution of α-apo-2-carotenol in chloroform with antimony trichloride, a fairly stable blue colouration with an absorption maximum at 562 mμ, is observed.

α-Carotene iodide:

α-Carotene adds two atoms of iodine with the formation of a crystalline di-iodide $C_{40}H_{56}I_2$ (KARRER, SOLMSSEN and WALKER)[169]. This iodide exhibits vitamin A activity. With regard to another iodide of β-carotene, cf. KUHN and BROCKMANN[170].

Neo-α-carotene U and neo-α-carotene W:

The question of stereoisomerism in α-carotene was examined several years ago by GILLAM, EL RIDI and KON[171]. These investigations have been renewed by ZECHMEISTER and POLGÁR who succeeded in isolating two *cis-trans* isomers of α-carotene in the crystalline state* [172]. Neo-α-carotene U is formed from α-carotene under the influence of heat, or illumination, or treatment with iodine or acids, or by melting the crystals. It is found above α-carotene in the chromatogram on calcium hydroxide. The pigment crystallises from a mixture of benzene and methanol in orange prisms, m.p. 65° (corr.). Neo-α-carotene U is more soluble in the usual solvents than α-carotene. On treatment with iodine it is partly transformed into α-carotene.

Solvent:	*Absorption maxima:*	
Carbon disulphide	503	470.5 mμ
Benzene	485.5	453.5 mμ
Chloroform	485	453 mμ
Petroleum ether (B.p. 60–70° C)	471.5	441.5 mμ

* The work of L. ZECHMEISTER has recently been confirmed in detail by F. P. ZSCHEILE and co-workers, *Arch. Biochem.* 5 (1944) 77, 211.

References p. 165–170.

Further information can be found in the original communication[172]. Data regarding vitamin A activity are given by ZECHMEISTER and co-workers[173]. Neo-α-carotene U possesses weaker growth-promoting properties than α-carotene.

Neo-α-carotene W is formed together with neo-α-carotene U, as well as several other stereoisomers not obtained in the crystalline state[173]. Neo-α-carotene W is found below neo-α-carotene U but above α-carotene in the chromatogram on calcium hydroxide and crystallises from a mixture of benzene and methanol in small prisms, m.p. 97° (corr.). Its solubility is similar to that of the U-isomer.

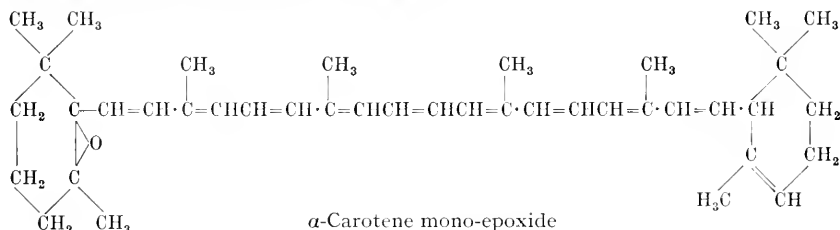
Solvent:	*Absorption maxima:*	
Carbon disulphide	502	469.5 mμ
Benzene	484	453.5 mμ
Chloroform	484	453 mμ
Petroleum ether (B.p. 60–70° C)	470.5	441 mμ

The absorption maxima of the crystalline and non-crystalline transformation products in ligroin are shown below:

Neo-α-carotene U (crystallised)	471.5	441.5 mμ
Neo-α-carotene V	465.5	437 mμ
Neo-α-carotene W (crystallised)	470.5	441 mμ
Neo-α-carotene X	463.5	435 mμ
Neo-α-carotene Y	467.5	437 mμ
α-Carotene (natural)	477	446.5 mμ
Neo-α-carotene A	468.5	439 mμ
Neo-α-carotene B	466.5	437 mμ
Neo-α-carotene C	472.5	442.5 mμ
Neo-α-carotene D	460	432 mμ
Neo-α-carotene E	461.5	433.5 mμ

(The sequence of isomers given is that in which they occur on the chromatogram).

α-Carotene mono-epoxide and flavochrome $C_{40}H_{56}O$:



α-Carotene mono-epoxide

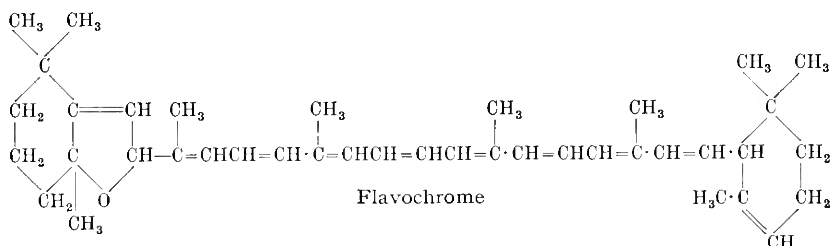
α-Carotene mono-epoxide is formed by the oxidation of α-carotene with monoperphthalic acid (KARRER and JUCKER[174]). It crystallises from a mixture of benzene and methanol in thin, reddish-yellow plates, m.p. 175° (uncorr., in vacuum). On treating an ethereal solution of the pigment with concentrated

References p. 165–170.

aqueous hydrochloric acid, the acid layer assumes a very weak, unstable blue colouration.

Solvent:	*Absorption maxima:*	
Carbon disulphide	503	471 mμ
Benzene	484	455 mμ
Petroleum ether	471	442 mμ
Chloroform	483	454 mμ

α-Carotene mono-epoxide occurs in the blossoms of various plants (*Tragopogon pratensis, Ranunculus acer*)[175]. According to VON EULER, α-carotene mono-epoxide possesses vitamin A potency[176].



Flavochrome is formed by the action of dilute acids (e.g. hydrogen chloride in chloroform) on α-carotene mono-epoxide. It crystallises from a mixture of benzene and methanol in thin lustruous yellow plates, m.p. 189° (uncorr., in vacuum). It exhibits a colour reaction with hydrochloric acid, similar to that of α-carotene mono-epoxide.

Solvent:	*Absorption maxima:*	
Carbon disulphide	482	451 mμ
Benzene	462	434 mμ
Petroleum ether	450	422 mμ
Chloroform	461	433 mμ

Flavochrome does not exhibit growth-promoting properties. Both α-carotene mono-epoxide and flavochrome are epiphasic in the partition test. Flavochrome occurs in *Ranunculus acer* and *Tragopogon pratensis.*

5:6-Dihydro-α-carotene and 5:6-dihydro-β-carotene $C_{40}H_{58}$:

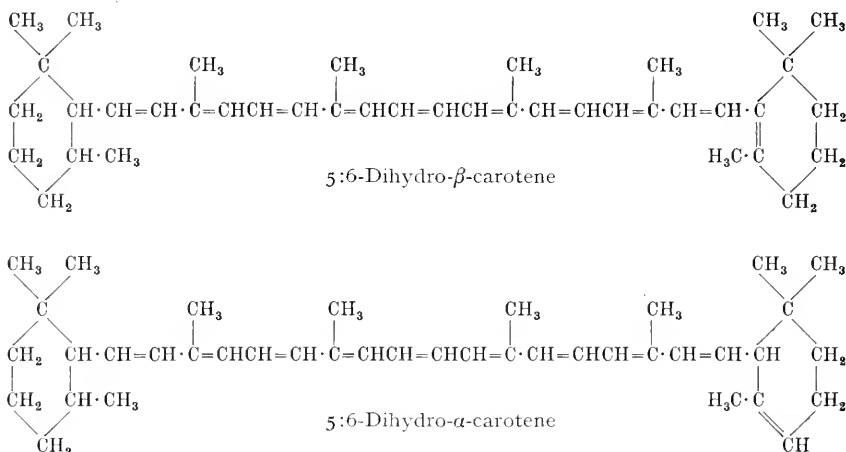
POLGÁR and ZECHMEISTER[177] treated solutions of α-carotene or β-carotene in petroleum ether with cold concentrated hydrogen iodide, and in both cases obtained several chromatographically separable reduction products, from which two pigments could be isolated in the crystalline state.

From the analytical data, the results of catalytic hydrogenation, the absorption maxima, and the absence of isopropylidene groupings, these compounds

References p. 165–170.

are formulated as 5:6-dihydro-α-carotene and 5:6-dihydro-β-carotene, respectively. It is of interest that both these hydrogenation products are obtained from α-carotene as well as β-carotene.

Under the usual conditions of isomerisation (cf. p. 39) both dihydrocarotenes undergo reversible *cis-trans* isomerisation. The absorption spectra exhibit the characteristic "*cis*-peak".



5:6-Dihydro-β-carotene crystallises from mixtures of carbon disulphide and ethanol, benzene and methanol, or chloroform and methanol, in characteristic plates or wedges. The highest m.p. which has been recorded is 164°, but in a number of cases it was considerably lower, e.g. 155 and 160°. The solubility of dihydro-β-carotene and its behaviour in the partition test corresponds to that of β-carotene. In the chromatogram the pigment is adsorbed below β-carotene, but above 5:6-dihydro-α-carotene.

Solvent:	*Absorption maxima:*		
Carbon disulphide	509.5	476	mμ
Benzene	489	458	mμ
Chloroform	489	457	mμ
Petroleum ether	477.5	447.5	mμ
Ethanol	477.5	448	mμ

5:6-Dihydro-α-carotene crystallises from a mixture of carbon disulphide and ethanol in microscopic rectangular yellow leaflets (cf. the original communication). From a mixture of benzene and methanol it is obtained in crystals which resemble those of natural α-carotene, and melt at 202–203° (with previous sintering). 5:6-Dihydro-α-carotene is somewhat more soluble than 5:6-dihydro-β-carotene.

References p. 165–170.

Solvent:	*Absorption maxima:*	
Carbon disulphide	501	486.5 mμ
Benzene	483.5	453.5 mμ
Chloroform	482.5	452.5 mμ
Petroleum ether	470.5	442.5 mμ
Ethanol	471	443 mμ

A 0.1 % solution of the pigment in benzene shows no optical rotation in a 10 cm tube. Dihydro-α-carotene is entirely epiphasic in the partition test.

5. γ-CAROTENE $C_{40}H_{56}$

History

1933 KUHN and BROCKMANN discover a third carotene-isomer, γ-carotene, by means of chromatographic adsorption analysis*, [178]. They elucidate the constitution of the new pigment.

Occurrence

γ-Carotene is one of the rarest carotenoids. In carotene from carrots it occurs only to the extent of about 0.1 % of β-carotene.

TABLE 37

OCCURRENCE OF γ-CAROTENE

Source	References
Aleuria aurantiaca	E. LEDERER, *Chem. Centr. 1939*, I, 2991. *Bl. Soc. Chim. biol. 20* (1938) 611.
Allomyces	R. EMERSON and D. L. FOX, *Proc. Roy. Soc.* (London) (B) *128* (1940) 275.
Bacillus Lombardo Pellegrini, Bacillus Grasberger	E. CHARGAFF and E. LEDERER, *Chem. Centr. 1936*, I, 3159.
Butia capitata	L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc. 64* (1942) 1173.
Carotene from carrots	R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 407.
Chara ceratophylla Wallr.	P. KARRER, W. FATZER, M. FAVARGER and E. JUCKER, *Helv. chim. Acta 26* (1943) 2121.
Chrysemis scripta elegans (Japanese tortoise)	E. LEDERER, *Chem. Centr. 1939*, I, 2990. — *Bl. Soc. Chim. biol. 20* (1938) 554.

* V. N. LUBIMENKO observed a pigment with properties intermediate to those of lycopene and β-carotene in fruit of the *Gonocaryum* species. This was probably γ-carotene (*Rev. gén. bot. 25* (1914) 474). A. WINTERSTEIN isolated impure γ-carotene from *Gonocaryum pyriforme* (*Z. physiol. Chem. 215* (1933) 51), and shortly afterwards established the identity of this pigment with the γ-carotene of R. KUHN and H. BROCKMANN (*Z. physiol. Chem. 219* (1933) 249).

References p. 165–170.

Source	References
Citrullus vulgaris Schrad.	L. ZECHMEISTER and A. POLGÁR, *J. biol. Chem. 139* (1941) 193.
Crocus sativus	R. KUHN and A. WINTERSTEIN, *Ber. 67* (1934) 344.
Cuscuta subinclusa and *Cuscuta salina*	G. MACKINNEY, *J. biol. Chem. 112* (1935) 421.
Gazania rigens	K. SCHÖN, *Biochem. J. 32* (1938) 1566. — L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc. 65* (1943) 1535.
Gonocaryum pyriforme	A. WINTERSTEIN, *Z. physiol. Chem. 215* (1933) 51; *219* (1933) 249.
Mimulus longiflorus	W. A. SCHROEDER, *J. Am. Chem. Soc. 64* (1942) 2510.
Mycobacterium phlei	E. CHARGAFF, *Chem. Centr. 1934*, I, 1662. — Y. TAKEDA and T. OHTA, *Z. physiol. Chem. 265* (1940) 233.
Nitella syncarpa (Thuill.)	P. KARRER, W. FATZER, M. FAVARGER and E. JUCKER, *Helv. chim. Acta 26* (1943) 2121.
Palm oil	R. F. HUNTER and A. D. SCOTT, *Biochem. J. 35* (1941) 31.
Prunus armeniaca	H. BROCKMANN, *Z. physiol. Chem. 216* (1933) 45.
Pyracantha coccinia	P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 28* (1945) 1528.
Red Sponge *(Hymeniacedon Sanguineum)*	P. J. DRUMM and W. O'CONNOR, *Nature* (London) *145* (1940) 425.
Rhodotorula Sanniei	C. FROMAGEOT and J. LÉON TCHANG, *Chem. Centr. 1930*, I, 1580; *Arch. Mikrobiol. 9* (1938) 424.
Rosa rubiginosa L.	R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 342.
Rosa rugosa Thumb.	H. WILLSTAEDT, *Chem. Centr. 1935*, II, 707; *Svensk Kem. Tidskr. 47* (1935) 112.
Rubus Chamaemorus L.	H. WILLSTAEDT, *Chem. Centr. 1937*, I, 2620.

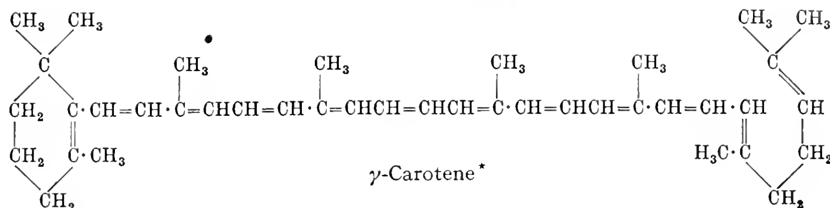
Preparation

According to KUHN and BROCKMANN[179] crude carotene is used for the preparation of γ-carotene. The crude carotene is crystallised three times from a mixture of benzene and methanol, the pigment being extracted with pure boiling methanol after each crystallisation. 300 Mg of pigment purified in this way are dissolved in 300 ml of benzene, the solution is diluted with 900 ml of petroleum ether and poured on a column of alumina (17 × 5 cm). The chromatogram is washed with a benzene-petrol mixture (1:4) until the uppermost zone containing γ-carotene is separated from the next lower zone by a colourless strip. After elution of the pigment with methanolic petroleum ether, the methanol is removed by washing and the carmine-red solution is dried and the solvent distilled off. The residue is repeatedly extracted with boiling pure methanol and recrystallised several times from a mixture of benzene and methanol (2:1). The yield of analytically pure material is about 1%, based on carotene.

WINTERSTEIN[180] prepared γ-carotene from *Gonocaryum pyriforme*. 300 Fruit skins yield 3 mg of pigment.

References p. 165–170.

Chemical Constitution



The elucidation of the constitution of this pigment was especially difficult in view of the small amount of material available. After establishing the carbon and hydrogen content of γ-carotene, KUHN and BROCKMANN[181] proved the presence of 12 double bonds by means of catalytic hydrogenation. γ-Carotene therefore contains one isocyclic ring. The absorption spectrum indicates that only 11 of the 12 double bonds are conjugated. By ozonisation of the pigment KUHN and BROCKMANN obtained 0.85 mol of acetone, and concluded that one end of the molecule must have an open-chain structure. No geronic acid could be isolated, so that the presence of a β-ionone ring has not been finally proved. However, the vitamin A activity (cf. p. 15) of γ-carotene supports the proposed formula since it is known that only compounds containing an unsubstituted β-ionone ring show growth-promoting properties.

Properties

Crystalline form: γ-Carotene crystallises from a mixture of benzene and methanol in microscopic dark red prisms with a blue lustre. On rapid crystallisation, the pigment is obtained in more lightly coloured needles.

Melting point[182]: 178° (corr., in vacuum)[183]; 176.5° (corr.)[184].

Solubility: γ-Carotene is less soluble in the usual solvents than the β-isomer.

Spectral properties:

Solvent:	*Absorption maxima:*		
Carbon disulphide	533.5	496	463 mμ
Chloroform	508.5	475	446 mμ
Benzene	510	477	447 mμ
Petrol	495	462	431 mμ
Hexane	494	462	431 mμ

(cf. Fig. 5, p. 349)

Quantitative extinction measurements: KUHN and BROCKMANN[183].

* A. WINTERSTEIN and U. EHRENBERG, (*Z. physiol. Chem.* *207* (1932) 25) ascribed the above formula to a pigment which they had isolated from *Convallaria majalis*. Subsequent investigations showed, however, that this pigment was a mixture and not identical with γ-carotene.

Optical activity: γ-Carotene is optically inactive.

Partition test: On partition between petroleum ether and 90% methanol, γ-carotene is entirely epiphasic.

Chromatographic behaviour: γ-Carotene is more strongly adsorbed from petroleum ether solution than β-carotene. In the chromatogram on calcium hydroxide or alumina it is found above β-carotene and below lycopene.

Detection and estimation: The separation of γ-carotene from other carotenoid hydrocarbons is achieved by chromatographic adsorption on alumina or calcium hydroxide. Its presence can be established by the determination of the absorption maxima.

Physiological properties: γ-Carotene exhibits strong vitamin A potency[185]. Several investigators have reported on the supposed function of γ-carotene in the sexual metabolism of various plants[186].

Stereoisomers of γ-carotene: By the treatment of γ-carotene with heat, light, or iodine, or fusion of the crystals, ZECHMEISTER and POLGÁR[187] partly converted γ-carotene into various *cis-trans* isomers. None of these compounds has so far been obtained in the crystalline state. The different isomers are separated by means of chromatographic adsorption on calcium hydroxide and can be distinguished by their absorption spectra.

	Absorption maxima (in petroleum ether)		
Neo-γ-carotene U	489	457	mμ
(Natural γ-Carotene)	494	461.5	mμ
Neo-γ-carotene A	486	455.5	mμ
Neo-γ-carotene B	486	455.5	mμ
Neo-γ-carotene H	489	457.5	mμ
Neo-γ-carotene A′	485.5	455	mμ
Neo-γ-carotene B′	486	455	mμ
Neo-γ-carotene G′	483	452	mμ
(γ-Carotene with all-*cis* configuration)	456		mμ

The vitamin A activities of γ-carotene and pro-γ-carotene have recently been investigated by ZECHMEISTER and co-workers[188].

6. PRO-γ-CAROTENE $C_{40}H_{56}$

In 1941, ZECHMEISTER and SCHROEDER[189] found a new polyene pigment in the fruit of *Butia capitata* which they termed pro-γ-carotene. The new pigment has also been observed in the following plants: *Pyracantha angustifolia* Schneid[190], *Evonymus fortunei* L.[191] and *Mimulus longiflorus* Grant[192].

References p. 165–170.

Pro-γ-carotene is a naturally occurring stereoisomer of γ-carotene. According to ZECHMEISTER and SCHROEDER[193], 6 or 7 of the double bonds have a *trans*, and 4 or 5 a *cis* configuration. By the fusion of pro-γ-carotene crystals, by heating solutions of the pigment, or by treatment with concentrated hydrochloric acid or iodine, a mixture of stereoisomers is obtained which contains γ-carotene.

Pro-γ-carotene crystallises from a mixture of benzene and methanol in glistening red plates, m.p. 118–119° (corr.) (cf. ZECHMEISTER and SCHROEDER)[193]. It is easily soluble in benzene, petroleum ether and other organic solvents, with the exception of alcohols.

Solvent:	*Absorption maxima*	
Carbon disulphide	493.5	460.5 mμ
Benzene	477	447.5 mμ
Chloroform	473	(444) mμ
Ethanol	(465)	(437) mμ
Petroleum ether	464	(435) mμ

Pro-γ-carotene is adsorbed a little more weakly than γ-carotene on calcium hydroxide from petroleum ether solution.

REFERENCES

1. HARTSEN, *Chem. Centr.* 1873, 204; *Compt. rend 76* (1873) 385; COURCHET, *Ann. Sci. nat.* (7) *7* (1888) 320, 356.
2. MILLARDET, *Bull. Soc. Sci. Nancy* (2) *1* (1875) p. 21.
3. C. A. SCHUNCK, *Proc. Roy. Soc.* (London) *72* (1903) 165 (cf. ZOPF, *Biol. Zentralbl. 15* (1895) 417.
4. R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 47.
5. P. KARRER and co-workers, *Helv. chim. Acta 11* (1928) 751; *12* (1929) 285; *11* (1928) 1201; *13* (1930) 1084; *14* (1931) 435.
6. R. KUHN and C. GRUNDMANN, *Ber. 65* (1932) 898, 1880.
7. R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 47.
8. R. KUHN and C. GRUNDMANN, *Ber. 65* (1932) 1880.
9. L. ZECHMEISTER and L. v. CHOLNOKY, *Ber. 63* (1930) 422.
10. R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 47.
11. P. KARRER and R. WIDMER, *Helv. Chim. Acta 11* (1928) 751.
12. R. PUMMERER, L. REBMANN and W. REINDEL, *Ber. 62* (1929) 1411.
13. P. KARRER and W. E. BACHMANN, *Helv. Chim. Acta 12* (1929) 285.
14. P. KARRER, A. HELFENSTEIN, B. PIEPER and A. WETTSTEIN, *Helv. Chim. Acta 14* (1931) 435.
15. P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. Chim. Acta 13* (1930) 1084.
16. P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. Chim. Acta 13* (1930) 1084 (cf. *Helv. Chim. Acta 12* (1929) 64).
17. P. KARRER, A. HELFENSTEIN and R. WIDMER, *Helv. Chim. Acta 11* (1928) 1201.
18. P. KARRER, A. HELFENSTEIN, B. PIEPER and A. WETTSTEIN, *Helv. Chim. Acta 14* (1931) 435.
19. R. KUHN and A. WINTERSTEIN, *Ber. 65* (1932) 1873.

20. R. Kuhn and C. Grundmann, *Ber. 65* (1932) 898, 1880.
21. H. H. Strain, *J. Biol. Chem. 102* (1933) 137.
22. B. M. Duggar, *Washington Univ. Stud. 1* (1913) 22.
23. e.g. K. H. Coward, *Biochem. J. 17* (1923) 134.
24. H. v. Euler, P. Karrer, E. v. Krauss and O. Walker, *Helv. Chim. Acta 14* (1931) 154.
25. R. Kuhn and C. Grundmann, *Ber. 65* (1932) 1880.
26. G. Mackinney, *J. Am. Chem. Soc. 56* (1934) 488.
27. C. Montanari, *Chem. Centr. 1905*, I, 544.
28. P. Karrer and R. Widmer, *Helv. chim. Acta 11* (1928) 751.
29. H. Brockmann, *Z. physiol. Chem. 216* (1933) 47.
30. L. Zechmeister and P. Tuzson, *Ber. 63* (1930) 1882.
31. R. Willstätter and H. H. Escher, *Z. physiol. Chem. 64* (1910) 52.
32. R. Willstätter and H. H. Escher, *Z. physiol. Chem. 64* (1910) 52.
33. B. v. Euler, H. v. Euler and P. Karrer, *Helv. chim. Acta 12* (1929) 279. — H. v. Euler, P. Karrer and M. Rydbom, *Ber. 62* (1929) 2446. — R. Kuhn and E. Lederer, *Ber. 65* (1932) 638. — B. v. Euler and P. Karrer, *Helv. chim. Acta 15* (1932) 496. — H. v. Euler, P. Karrer, E. Klussmann and R. Morf, *Helv. chim. Acta 15* (1932) 502.
34. A. Winterstein, *Z. physiol. Chem. 215* (1933) 52.
35. A. Winterstein, and G. Stein, *Z. physiol. Chem. 220* (1933) 250.
36. S. J. B. Connell, *Biochem. J. 18* (1924) 1127.
37. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 45.
38. P. Karrer and R. Widmer, *Helv. chim. Acta 11* (1928) 751.
39. P. Karrer, A. Helfenstein and R. Widmer, *Helv. chim. Acta 11* (1928) 1201.
40. P. Karrer and co-workers, *Helv. chim. Acta 14* (1931) 436.
41. P. Karrer and J. Rutschmann, *Helv. chim. Acta 28* (1945) 793.
42. R. Kuhn and C. Grundmann, *Ber. 65* (1932) 900.
43. R. Kuhn and C. Grundmann, *Ber. 65* (1932) 1887.
44. P. Karrer and W. Jaffé, *Helv. chim. Acta 22* (1939) 69.
45. R. Kuhn and C. Grundmann, *Ber. 65* (1932) 1882 (cf. P. Karrer and W. Jaffé, *Helv. 22* (1939) 69.
46. R. Kuhn and C. Grundmann, *Ber. 65* (1932) 1888.
47. P. Karrer and W. Jaffé, *Helv. chim. Acta 22* (1939) 69.
48. L. Zechmeister and P. Tuzson, *Nature 141* (1938) 249; *Biochem. J. 32* (1938) 1305; *Ber. 72* (1939) 1340.
49. P. Karrer and H. Kramer, *Helv. chim. Acta 27* (1944) 1301.
50. P. Karrer and A. Helfenstein, *Helv. chim. Acta 14* (1931) 78.
51. L. Zechmeister and co-workers, *Proc. Nat. Acad. Sci. 27* (1941) 468.
52. L. Zechmeister and co-workers, *Science 94* (1941) 609.
53. L. Zechmeister and co-workers, *J. biol. Chem. 144* (1942) 315.
54. L. Zechmeister and co-workers, *J. biol. Chem. 144* (1942) 321.
55. L. Zechmeister and co-workers, *Arch. Biochem. 1* (1943) 231.
56. A. L. le Rosen and L. Zechmeister, *J. Am. Chem. Soc. 64* (1942)1075.
57. L. Zechmeister and J. H. Pinckard, *J. Am. Chem. Soc. 69* (1947) 1930.
58. Zeise, *Ann. 62* (1947) 380; *J. prakt. Chem. 40* (1847) 297.
59. A. Arnaud, *Compt. rend. 102* (1886) 1119.
60. R. Willstätter and W. Mieg, *Ann. 355* (1907) 1.
61. L. Zechmeister, L. v. Cholnoky and V. Vrabély, *Ber. 61* (1928) 566; *Ber. 66* (1933) 123.
62. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 1142; *13* (1930) 1084; *14* (1931) 1033.
63. R. Kuhn and H. Brockmann, *Ber. 65* (1932) 894; *66* (1933) 1319; *67* (1934) 1408; *Ann. 516* (1935) 95.
64. cf. Strain, *J. biol. Chem. 111* (1935) 85 for the occurrence of carotene in green leaves.

65. R. WILLSTÄTTER and A. STOLL, *Untersuchungen über Chlorophyll*, Berlin 1913. — R. WILLSTÄTTER and A. STOLL, *Untersuchungen über die Assimilation der Kohlensäure*, Berlin 1918.
66. L. S. PALMER, *Carotenoids and related Pigments*, New York, 1922. — P. KARRER and O. WALKER, *Helv. chim. Acta 17* (1934) 43. — R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 41.
67. R. KUHN and E. LEDERER, *Z. physiol. Chem. 200* (1931) 246.
68. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 200* (1931) 255.
69. L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1935) 824.
70. P. KARRER and W. SCHLIENTZ, *Helv. chim. Acta 17* (1934) 7.
71. R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 47.
72. P. KARRER and O. WALKER, *Helv. chim. Acta 16* (1933) 641. — Cf. also H. H. STRAIN, *J. biol. Chem. 105* (1934) 524; *111* (1935) 86.
73. R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 47.
74. R. KUHN and E. LEDERER, *Ber. 64* (1931) 1349.
75. R. WILLSTÄTTER and W. MIEG, *Ann. 355* (1907) 1.
76. L. ZECHMEISTER, L. v. CHOLNOKY and V. VRABÉLY, *Ber. 61* (1928) 566; *66* (1933) 123.
77. R. PUMMERER and L. REBMANN, *Ber. 61* (1928) 1099.
78. P. KARRER and A. HELFENSTEIN, *Helv. chim. Acta 12* (1929) 1142. — P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. chim. Acta 13* (1930) 1084. — P. KARRER and R. MORF, *Helv. chim. Acta 14* (1931) 1033.
79. P. KARRER and A. HELFENSTEIN, *Helv. chim. Acta 12* (1929) 1142. — cf. R. KUHN and A. WINTERSTEIN and L. KARLOVITZ, *Helv. chim. Acta 12* (1929) 64.
80. R. KUHN and L. EHMANN, *Helv. chim. Acta 12* (1929) 904.
81. R. KUHN and F. L'ORSA, *Ber. 64* (1931) 1732.
82. P. KARRER, A. HELFENSTEIN, H. WEHRLI and A. WETTSTEIN, *Helv. chim. Acta 13* (1930) 1084.
83. P. KARRER and R. MORF, *Helv. chim. Acta 14* (1931) 1033.
84. R. PUMMERER, L. REBMANN and W. REINDEL, *Ber. 64* (1931) 492.
85. H. H. STRAIN, *J. biol. Chem. 102* (1933) 137.
86. R. KUHN and A. WINTERSTEIN, *Ber. 66* (1933) 429.
87. R. KUHN and H. BROCKMANN, *Ann. 516* (1935) 95; *Ber. 65* (1932) 894; *Z. physiol. Chem. 213* (1932) 1; *Ber. 66* (1933) 1319; *67* (1934) 1408.
88. P. KARRER and E. JUCKER, *Helv. chim. Acta 30* (1947) 266.
89. R. KUHN and E. LEDERER, *Ber. 64* (1931) 1352; *Z. physiol. Chem. 200* (1931) 247.
90. P. KARRER and co-workers, *Helv. chim. Acta 14* (1931) 615.
91. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 408.
92. E. S. MILLER, *Chem. Centr. 1935*, I, 3545.
93. K. W. HAUSSER and A. SMAKULA, *Z. angew. Chem. 47* (1934) 663; *48* (1935) 152.
94. H. v. EULER and H. HELLSTRÖM, *Z. physik. Chem.* (B) *15* (1932) 343.
95. H. v. EULER, P. KARRER and M. RYDBOM, *Ber. 62* (1929) 2445; *Helv. chim. Acta 14* (1931) 1428.
96. L. ZECHMEISTER, *Carotinoide*, Berlin 1934, p. 127.
97. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 46.
98. P. KARRER and O. WALKER, *Helv. chim. Acta 16* (1933) 641.
99. R. WILLSTÄTTER and W. MIEG, *Ann. 355* (1907) 19. — R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 64* (1910) 57.
100. H. v. EULER, P. KARRER and M. RYDBOM, *Ber. 62* (1929) 2445.
101. C. H. WARNER, *Proc. Roy. Soc.* (London) (B) *87* (1914) 384; *Chem. Centr. 1914*, I, 2110.
102. R. PUMMERER, L. REBMANN and W. REINDEL, *Ber. 64* (1931) 496, 500.
103. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 43.
104. B. v. EULER, H. v. EULER and H. HELLSTRÖM, *Biochem. Z. 203* (1928) 370. — B. v. EULER, H. v. EULER and P. KARRER, *Helv. chim. Acta 12* (1929) 278. — H. v. EULER, P. KARRER and M. RYDBOM, *Ber. 62* (1929) 2445.
105. For a review of the literature see *Helv. chim. Acta 23* (1940) 955.

106. P. KARRER and A. RÜEGGER, *Helv. chim. Acta 23* (1940) 955.
107. L. ZECHMEISTER, L. v. CHOLNOKY and V. VRABÉLY, *Ber. 61* (1928) 566; *66* (1933) 123. — cf. J. H. C. SMITH, *J. biol. Chem. 90* (1931) 597; *96* (1932) 35.
108. H. v. EULER, V. DEMOLE, P. KARRER and O. WALKER, *Helv. chim. Acta 13* (1930) 1078.
109. R. KUHN and E. LEDERER, *Naturwissenschaften* 19, (1931) 306; *Ber. 65* (1932) 639. — cf. A. E. GILLAM, I. M. HEILBRON, R. A. MORTON and J. C. DRUMMOND, *Biochem. J. 26* (1932) 1174. — P. KARRER, K. SCHÖPP and R. MORF, *Helv. chim. Acta 15* (1932) 1158.
110. P. KARRER and G. SCHWAB, *Helv. chim. Acta 23* (1940) 578.
111. P. KARRER, K. SCHÖPP and R. MORF, *Helv. chim. Acta 15* (1932) 1158. — cf. R. KUHN and E. LEDERER, *Ber. 65* (1932) 639. — A. E. GILLAM, I. M. HEILBRON, R. A. MORTON and J. C. DRUMMOND, *Biochem. J. 26* (1932) 1174.
112. K. W. HAUSSER and A. SMAKULA, *Angew. Chem. 47* (1934) 663; *48* (1935) 152.
113. H. v. EULER, P. KARRER and O. WALKER, *Helv. chim. Acta 15* (1932) 1507.
114. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 427.
115. R. KUHN and H. BROCKMANN, *Ber. 65* (1932) 896; *Ber. 67* (1934) 1408; cf. *Ann. 516* (1935) 95.
116. R. KUHN and H. BROCKMANN, *Ann. 516* (1935) 98, 120.
117. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 1319.
118. R. KUHN and H. BROCKMANN, *Ann. 516* (1935) 120.
119. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 18* (1935) 25.
120. R. KUHN and H. BROCKMANN, *Ann. 516* (1935) 113, 122.
121. R. KUHN and H. BROCKMANN, *Ber. 65* (1932) 894; *67* (1934) 885; *Ann. 516* (1935) 123.
122. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 1324.
123. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 213* (1932) 3.
124. K. W. HAUSSER and A. SMAKULA, *Angew. Chem. 47* (1934) 663; *48* (1935) 152.
125. R. KUHN and H. BROCKMANN, *Ann. 516* (1935) 113; *516* (1935) 123.
126. R. KUHN and H. BROCKMANN, *Ann. 516* (1935) 113, 124.
127. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 1324.
128. R. KUHN and H. BROCKMANN, *Ber. 67* (1934) 886; *Ann. 516* (1935) 98, 125, 127; cf. *Z. physiol. Chem. 215* (1932) 5.
129. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 20* (1937) 682. — P. KARRER, U. SOLMSSEN and W. GUGELMANN, *Helv. chim. Acta 20* (1937) 1020.
130. H. v. EULER, P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 21* (1938) 211.
131. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 20* (1937) 688. — P. KARRER, U. SOLMSSEN and W. GUGELMANN, *Helv. chim. Acta 20* (1937) 1023.
132. H. v. EULER, P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 21* (1938) 211.
133. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 427.
134. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 427.
135. P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLEIN, *Helv. chim. Acta 28* (1945) 1150.
136. A. E. GILLAM and M. S. EL RIDI, *Nature* (London) *136* (1935) 914; *Biochem. J. 30* (1936) 1735; *31* (1937) 251.
137. L. ZECHMEISTER and co-workers, *Nature* (London) *141* (1938) 249; *Biochem. J. 32* (1938) 1305; *Ber. 72* (1939) 1340; *J. Am. Chem. Soc. 64* (1942) 1856; *65* (1943) 1528; *66* (1944) 137; *Arch. Biochem. 5* (1944) 107; *7* (1945) 247. — cf. also F. P. ZSCHEILE and co-workers, *Arch. Biochem. 5* (1944) 211, and H. H. STRAIN, *J. Am. Chem. Soc. 63* (1941) 3448.
138. L. ZECHMEISTER and co-workers, *Nature* (London) *141* (1938) 249; *Biochem. J. 32* (1938) 1305; *Ber. 72* (1939) 1340; *J. Am. Chem. Soc. 64* (1942) 1856; *65* (1943) 1528; *66* (1944) 137; *Arch. Biochem. 5* (1944) 107; *7* (1945) 247. — cf. also F. P. ZSCHEILE and co-workers, *Arch. Biochem. 5* (1944) 211; H. H. STRAIN, *J. Am. Chem. Soc. 63* (1941) 3448.

139. A. E. GILLAM and M. S. EL RIDI, *Nature* (London) *136* (1935) 914; *Biochem. J. 30* (1936) 1735; *31* (1937) 251.
140. For further data see *J. Am. Chem. Soc. 64* (1942) 1860.
141. R. KUHN and E. LEDERER, *Ber. 64* (1931) 1349; *Naturwissenschaften 19* (1931) 306.
142. P. KARRER and co-workers, *Helv. chim. Acta 14* (1931) 614; *Ark. Kemi. B. 10* (1931) No. 15.
143. P. KARRER and O. WALKER, *Helv. chim. Acta 16* (1933) 641.
144. P. KARRER, R. MORF and O. WALKER, *Helv. chim. Acta 16* (1933) 975.
145. G. MACKINNEY, *J. biol. Chem. 111* (1935) 75.
146. H. H. STRAIN, *J. biol. Chem. 111* (1935) 85.
147. P. KARRER and O. WALKER, *Helv. chim. Acta 16* (1933) 641.
148. R. KUHN and E. LEDERER, *Ber. 64* (1931) 1349; *Z. physiol. Chem. 200* (1931) 246. — R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 200* (1931) 255. — H. H. STRAIN, *J. biol. Chem. 105* (1934) 523; *111* (1935) 86.
149. P. KARRER, A. HELFENSTEIN, H. WEHRLI, B. PIEPER and R. MORF, *Helv. chim. Acta 14* (1931) 614. — P. KARRER and R. MORF, *Helv. chim. Acta 14* (1931) 833. *14* (1931) 1033. — P. KARRER, R. MORF, E. v. KRAUSS and A. ZUBRYS, *Helv. chim. Acta 15* (1932) 490. — P. KARRER, K. SCHÖPP and R. MORF, *Helv. chim. Acta 15* (1932) 1158. R. KUHN and E. LEDERER, *Ber. 64* (1931) 1349.
150. J. H. C. SMITH, *J. biol. Chem. 102* (1932) 159. — R. KUHN and E. F. MÖLLER, *Z. angew. Chem. 47* (1934) 145.
151. P. KARRER, R. MORF and O. WALKER, *Helv. Chim. Acta 16* (1933) 975.
152. P. KARRER and O. WALKER, *Hev. chim. Acta 16* (1933) 642.
153. R. KUHN and E. LEDERER, *Z. physiol. Chem. 200* (1931) 254.
154. K. W. HAUSSER and A. SMAKULA, *Angew. Chem. 47* (1934) 663; *48* (1935) 152.
155. H. v. EULER and H. HELLSTRÖM, *Z. physik. Chem.* (B) *15* (1932) 343.
156. R. KUHN and E. LEDERER, *Naturwissenschaften 19* (1931) 306; *Ber. 64* (1931) 1349.
157. E. BAUR, *Helv. chim. Acta 19* (1936) 1210.
158. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1942) 43.
159. H. v. EULER, P. KARRER, H. HELLSTRÖM and M. RYDBOM, *Helv. chim. Acta 14* (1931) 839. — R. KUHN and H. BROCKMANN, *Ber. 64* (1931) 1859; *Z. physiol. Chem. 221* (1933) 130. — H. BROCKMANN and M. L. TECKLENBURG, *Z. physiol. Chem. 221* (1933) 117. — H. BROCKMANN, *Angew. Chem. 47* (1934) 523.
160. P. KARRER and R. MORF, *Helv. chim. Acta 14* (1931) 836. — cf. U. SOLMSSEN, *Dissertation*, Zürich 1936.
161. P. KARRER, U. SOLMSSEN and O. WALKER, *Helv. chim. Acta 17* (1934) 417. — P. KARRER, H. v. EULER and U. SOLMSSEN, *Helv. chim. Acta 17* (1934) 1171.
162. P. KARRER, H. v. EULER and U. SOLMSSEN, *Helv. chim. Acta 17* (1934) 1171.
163. P. KARRER, U. SOLMSSEN and O. WALKER, *Helv. chim. Acta 17* (1934) 417. — P. KARRER, H. v. EULER and U. SOLMSSEN, *Helv. chim. Acta 17* (1934) 1171.
164. P. KARRER, H. v. EULER and U. SOLMSSEN, *Helv. chim. 17* (1934) 1171.
165. P. KARRER, H. v. EULER and U. SOLMSSEN, *Helv. chim. Acta 17* (1934) 1169.
166. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 18* (1935) 25.
167. P. KARRER, U. SOLMSSEN and O. WALKER, *Helv. chim. Acta 17* (1934) 417. — P. KARRER, H. v. EULER and U. SOLMSSEN, *Helv. chim. Acta 17* (1934) 1169.
168. H. v. EULER, P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 21* (1938) 211.
169. P. KARRER, U. SOLMSSEN and O. WALKER, *Helv. chim. Acta 17* (1934) 418.
170. R. KUHN and E. LEDERER, *Ber. 65* (1932) 639.
171. A. E. GILLAM, M. S. EL RIDI and S. K. KON, *Biochem. J. 31* (1937) 1605.
172. L. ZECHMEISTER and A. POLGÁR, *J. Am. Chem. Soc. 66* (1944) 137.
173. L. ZECHMEISTER and co-workers, *Arch. Biochem. 6* (1945) 157.
174. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 471.
175. P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta, 28* (1945) 1146.

176. P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta 28* (1945) 1150.
177. A. POLGÁR and L. ZECHMEISTER, *J. Am. Chem. Soc. 65* (1943) 1528.
178. R. KUHN and H. BROCKMANN, *Naturwissenschaften 21* (1933) 44; *Ber. 66* (1933) 407.
179. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 407.
180. A. WINTERSTEIN, *Z. physiol. Chem. 219* (1933) 249.
181. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 407.
182. Cf. communication of L. ZECHMEISTER and W. A. SCHROEDER, *Arch. Biochem. 1* (1942) 231.
183. R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 407.
184. H. WILLSTAEDT, *Chem. Centr. 1935*, II, 707.
185. A. WINTERSTEIN, *Z. physiol. Chem. 215* (1933) 55. — R. KUHN and H. BROCKMANN, *Chem. Centr. 1933*, II, 1205; *Z. physiol. Chem. 221* (1933) 131. — H. BROCKMANN and M. L. TECKLENBURG, *Z. physiol. Chem. 221* (1933) 117.
186. R. EMERSON and D. L. FOX, *Proc. Roy. Soc.* (London) (B) *128* (1940) 275. — P. KARRER and co-workers, *Helv. chim. Acta 26* (1943) 2121.
187. L. ZECHMEISTER and A. POLGÁR, *J. Am. Chem. Soc. 67* (1945) 108.
188. L. ZECHMEISTER and co-workers, *Arch. Biochem. 5* (1944) 365.
189. L. ZECHMEISTER and W. A. SCHROEDER, *Science 94* (1941) 609. — Cf. *J. Am. Chem. Soc. 64* (1942) 1173.
190. L. ZECHMEISTER and W. A. SCHROEDER, *J. biol. Chem. 144* (1942) 315.
191. L. ZECHMEISTER and R. B. ESCUE, *J. biol. Chem. 144* (1942) 321.
192. L. ZECHMEISTER and W. A. SCHROEDER, *Arch. Biochem. 1* (1942) 231.
193. L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc. 64* (1942) 1173.

CHAPTER XI

Carotenoids of known structure containing hydroxyl groups

I. LYCOXANTHIN $C_{40}H_{56}O$

History and Occurrence

1936 In the course of investigations on lycopene from *Solanum Dulcamara*, ZECHMEISTER and VON CHOLNOKY[1] isolate two new phytoxanthins, lycoxanthin and lycophyll. Lycoxanthin also occurs in *Solanum esculentum* and *Tamus communis**' [1].

Preparation[1]

17 Kg of fresh berries of *Solanum Dulcamara* are dehydrated with ethanol and extracted with ether at room temperature. After evaporation of the solvent, the pigment mixture is dissolved in benzene and chromatographed on calcium hydroxide. This procedure is repeated several times and the pigment is finally recrystallised from a mixture of benzene and methanol. The yield was 125 mg lycoxanthin, 920 mg lycopene and 9 mg lycophyll.

Chemical Constitution

```
 CH3  CH3                                                                   CH3  CH3
   \  /                                                                        \  /
    C            CH3           CH3             CH3           CH3                C
   //            |             |               |             |                   \\
  CH  HC·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CH=CHCH    CH
  |    ||                                                          ||    |
HOCH   C·CH3                                                    H3C·C    CH2
   \  /                        Lycoxanthin                            \  /
    CH2                                                                CH2
```

Owing to the small amount of material available, the elucidation of the constitution of lycoxanthin presented difficulties and could not be carried through completely. From the similarity of the absorption maxima of lycoxanthin and lycopene, ZECHMEISTER and VON CHOLNOKY[1] concluded that the

* According to E. LEDERER, *Bull. Soc. chem. Biol.* 20 (1938) 613, *Polystigma rubrum* also contains lycoxanthin besides an acidic pigment.

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two pigments contain identical chromophoric systems. The oxygen is present in the form of a hydroxyl group which can be acetylated. The position of the hydroxyl group has not been established, but it is probably at carbon atom 3 by analogy with other phytoxanthins (cf. KARRER and co-workers[2]).

Properties

Lycoxanthin crystallises from a mixture of benzene and petroleum ether in jagged or circular reddish-brown plates. From carbon disulphide, the pigment is obtained in violet needles, m.p. 168° (corr.). Lycoxanthin is easily soluble in carbon disulphide and benzene, somewhat less easily in petroleum ether, and only very sparingly in ethanol. On partition between methanol and petroleum ether it behaves in the same way as cryptoxanthin and rubixanthin. It can only be adsorbed on calcium carbonate from petroleum ether solution, whereas it can be adsorbed on calcium hydroxide and aluminium oxide from benzene solution.

Solvent:	*Absorption maxima:*		
Carbon disulphide	546	506	472 mμ
Petroleum ether	504	473	444 mμ
Benzene	521	487	456 mμ
Ethanol	505	474	444 mμ

On shaking an ethereal solution of lycoxanthin with concentrated hydrochloric acid, no blue colouration is observed.

The monoacetate of lycoxanthin is formed by treating lycoxanthin with acetyl chloride in pyridine. The monoacetate crystallised from a mixture of benzene and methanol in violet-red needles, m.p. 137° (corr.).

The acetate is easily soluble in carbon disulphide but only sparingly soluble in ethanol and petroleum ether. Its absorption spectrum is identical with that of lycoxanthin.

2. RUBIXANTHIN $C_{40}H_{56}O$

History

1934 KUHN and GRUNDMANN[3] discover a pigment isomeric with cryptoxanthin amongst the pigments of *Rosa rubiginosa*. They propose the name rubixanthin and propose a constitutional formula.

Occurrence

Rubixanthin is one of the polyene pigments which are not widely distributed in nature. It occurs mainly in different species of roses.

References p. 214–217.

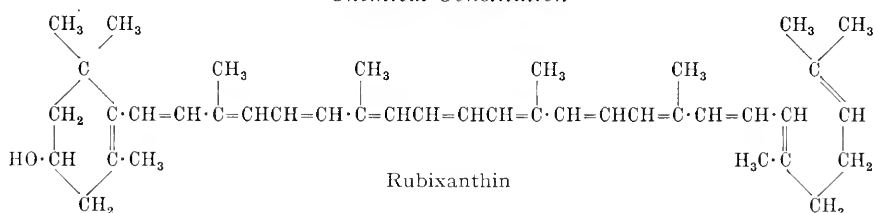
Source	References
Cuscuta salina	G. MACKINNEY, *J. biol. Chem.* *112* (1935) 421.
Cuscuta subinclusa,	do.
Gazania rigens	K. SCHÖN, *Biochem. J.* *32* (1938) 1566. — L. ZECHMEISTER and W. A. SCHROEDER, *J. Am. Chem. Soc.* *65* (1943) 1535.
Rosa canina, Rosa rubiginosa, Rosa damascena	R. KUHN and H. GRUNDMANN, *Ber.* *67* (1934) 341, 1133.
Rosa rugosa	H. WILLSTAEDT, *Svensk Kemisk Tidskr.* *47* (1935) 113.
Rubus Chamaemorus	H. WILLSTAEDT, *Scand. Arch. Physiol.* *75* (1936) 155.

Preparation

27 Kg of fresh, ripe hips *(Rosa rubiginosa)* are mashed, dehydrated with pure methanol and dried at 37°. The kernels are separated from the skins by grinding in a mill and the skins are extracted at room temperature with a mixture of benzene, absolute methanol and petroleum ether. The dark red extract is concentrated (finally in vacuum) to a small volume and saponified with ethanolic potassium hydroxide for two hours at 40°, and for a further two hours at room temperature. After this period, the pigments are extracted with a benzene-petrol (1:4) mixture, the solution is washed free from alkali, dried and adsorbed on alumina. The chromatogram is developed with the same solvent mixture and the rubixanthin is eluted with petroleum ether containing a little ethanol. The pigment is crystallised from a benzene-petroleum ether (1:5) mixture. The yield is about 400 mg.

For further purification the crude pigment is again saponified by allowing a benzene solution to stand with about 50 ml of 10% ethanolic potassium hydroxide for 4 hours at 40°. The pigment is extracted with petroleum ether and the solvent is removed by distillation. The rubixanthin is recrystallised from a mixture of benzene and methanol. The yield of pure pigment amounted to 36 mg.

Chemical Constitution



Rubixanthin

The formula for rubixanthin was proposed by KUHN and GRUNDMANN[4]. Like γ-carotene, the pigment takes up 12 mols of hydrogen on catalytic hydrogenation and therefore contains 1 isocyclic ring. On ozonisation the pigment yields 0.94 mol of acetone, evidently derived from the open end of the molecule. Rubixanthin exhibits absorption bands of the same wavelength location as γ-carotene and the two polyenes may therefore be assumed to have the same

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chromophoric systems. ZEREWITINOFF determinations show that the oxygen atom is present as a hydroxyl group. Since rubixanthin exhibits no vitamin A activity, the hydroxyl group must be substituted in the β-ionone ring, since compounds containing an unsubstituted β-ionone ring generally possess growth promoting properties. For reasons of analogy, KUHN and GRUNDMANN assume that the hydroxyl group is present in position 3. Although the formula of rubixanthin has thus not been finally proved, it appears the most probable according to present knowledge, and is in complete agreement with all the properties of the pigment.

Properties

Crystalline form: Rubixanthin crystallises from a mixture of benzene and petroleum ether in orange-red needles, and from a mixture of benzene and methanol in dark red needles with a copper-like lustre.

Melting point: 160°.

Solubility: The pigment is easily soluble in benzene and chloroform, but, only sparingly soluble in alcohols and petroleum ether.

Spectral properties:

Solvent:	*Absorption maxima:*		
Carbon disulphide	533	494	461 mμ
Chloroform	509	474	439 mμ
Ethanol	496	463	433 mμ
Petroleum ether	495.5	463	432 mμ
Hexane	494	462	432 mμ

Optical activity: Rubixanthin is optically inactive.

Partition test: On partition between petroleum ether and 90 % methanol, the pigment is found in the upper layer. If 95 % methanol is used, however, the pigment is hypophasic.

Chromatographic behaviour: Owing to the presence of one hydroxyl group, rubixanthin is very much more strongly adsorbed on calcium hydroxide than carotenoid hydrocarbons. On the other hand, rubixanthin is more weakly adsorbed on zinc carbonate or alumina than phytoxanthins containing 2 hydroxyl groups (zeaxanthin, xanthophyll, etc.). The separation of rubixanthin from cryptoxanthin is very tedious as these two compounds show almost identical adsorption properties.

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Detection and estimation: After separation from other phytoxanthins by chromatographic analysis, rubixanthin can be identified by its spectral properties.

Physiological properties: Rubixanthin exhibits no vitamin A activity.

3. CRYPTOXANTHIN $C_{40}H_{56}O$

History

1932 YAMAMOTO and TIN[5] isolate a phytoxanthin from *Carica papaya* and propose the name caricaxanthin. The formula $C_{40}H_{56}O_2$ is assigned to the new pigment.

1933 KUHN and GRUNDMANN[6] discover a new polyene pigment in the red berries of *Physalis* Alkekengi and *Physalis* Franchetii. They term the new pigment cryptoxanthin, determine the correct molecular formula, and derive a constitution formula.

1933 KARRER and SCHLIENTZ[7] establish the identity of caricaxanthin and cryptoxanthin, and correct the formula given by YAMAMOTO and TIN.

TABLE 38

OCCURRENCE OF CRYPTOXANTHIN

Source	References
Arbutus Unedo	K. SCHÖN, *Biochem. J. 29* (1935) 1779, 1782.
Blood serum of cattle	A. E. GILLAM and M. S. EL RIDI, *Biochem. J. 29* (1935) 2465.
Butter	A. E. GILLAM and I. M. HEILBRON, *Biochem. J. 29* (1935) 834.
Capsicum annuum	L. ZECHMEISTER and L. V. CHOLNOKY, *Ann. 509* (1934) 269.
Carica papaya	R. YAMAMOTO and S. TIN, *L. Sci. Pap. Inst. phys. chem. Res. 20* (1933) 411. — *Chem. Centr. 1933*, I, 3090.
Celastrus scandens L.	A. L. LE ROSEN and L. ZECHMEISTER, *Arch. of Biochem. 1* (1943) 17.
Citrus poonensis (fruit)	R. YAMAMOTO and S. TIN, *L. Sci. Pap. Inst. phys. chem. Res. 21* (1933) 422/425. — *Chem. Centr. 1934*, I, 1660.
Cucurbita Pepo	L. ZECHMEISTER, T. BÉRES and E. UJHELYI, *Ber. 68* (1935) 1322.
Diospyros costata (fruit)	K. SCHÖN, *Biochem. J. 29* (1935) 1779, 1782.
Egg yolk	A. E. GILLAM and I. M. HEILBRON, *Biochem. J. 29* (1935) 1064.
Grevillae robusta, Cunningham	L. ZECHMEISTER and A. POLGÁR, *J. biol. Chem. 140* (1941) 1.
Helianthus annuus	L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 170.

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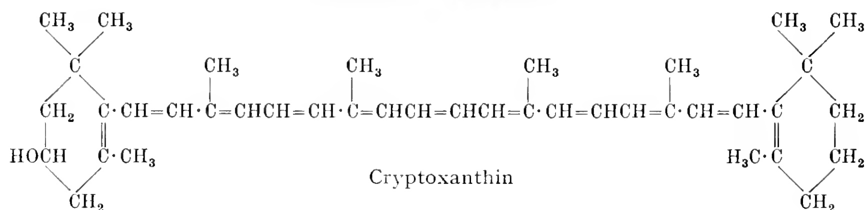
Source	References
Iris of chicks	L. BUSCH and H. J. NEUMANN, *Naturwissenschaften* *29* (1941) 782.
Mycobacterium phlei	M. A. INGRAHAM and H. STEENBOCK, *Biochem. J.* *29* (1935) 2553.
Nitzschia closterium	NELLO PACE, *J. biol. Chem.* *140* (1941) 483.
Orange peels	L. ZECHMEISTER and P. TUZSON, *Ber.* *69* (1936) 1878. — P. KARRER and E. JUCKER, *Helv. chim. Acta* *27* (1944) 1695.
Physalis Alkekengi and *Physalis Franchetii*	R. KUHN and C. GRUNDMANN, *Ber.* *66* (1933) 1746.
Tangerines	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem.* *240* (1936) 191.
Zea Mays	R. KUHN and C. GRUNDMANN, *Ber.* *67* (1934) 593.

Preparation[8]

Dried physalis cups are finely ground and extracted with methanol to remove resinous materials. The cup meal is then continuously extracted with benzene at room temperature. Most of the solvent is removed in vacuum and the residue which contains zeaxanthin and cryptoxanthin esters is saponified at room temperature with alcoholic potassium hydroxide. After about 15 hours, petroleum ether is added to the solution, followed by distilled water until the zeaxanthin precipitate begins to become resinous. The petroleum ether-benzene layer contains cryptoxanthin which is isolated by adsorption on alumina. The pigment is purified by crystallisation from a mixture of benzene and methanol. The yield amounts to about 100 mg pure cryptoxanthin from 1600 cups.

Chemical Constitution



Cryptoxanthin

The constitution of cryptoxanthin was elucidated mainly by KUHN and GRUNDMANN[9]. On hydrogenation, the pigment takes up 11 mols of hydrogen. It therefore contains 11 double bonds and 2 isocyclic rings. The spectral properties correspond to those of β-carotene and indicate that all the 11 double bonds are conjugated. Cryptoxanthin gives exactly 1 mol of methane with methyl magnesium iodide, indicating the presence of 1 hydroxyl group. This is confirmed by the formation of a monoacetate. The position of the hydroxyl group could not be determined with certainty, but by analogy the 3-position

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is the most probable. By oxidation with chromic acid, KUHN and GRUNDMANN obtained 4.85 mols of acetic acid.

The formula for cryptoxanthin is in agreement with the fact that this phytoxanthin possesses vitamin A activity[9, 10, 11].

Properties

Crystalline form: Cryptoxanthin crystallises from a mixture of benzene and methanol in lustrous prisms which tenaciously retain some methanol.

Melting point: 169° (corr., evacuated capillary).

Solubility: As would be expected from its constitution, cryptoxanthin has properties intermediate between those of β-carotene and zeaxanthin. The pigment is easily soluble in chloroform, benzene and pyridine, and less soluble in ligroin, petroleum ether, methanol and ethanol.

Spectral properties:

Solvent:	*Absorption maxima:*		
Carbon disulphide	519	483	452 mμ
Chloroform	497	463	433 mμ
Ethanol, absolute	486	452	424 mμ
Petrol	485.5	452	424 mμ
Hexane	484	451	423 mμ

Optical activity: Cryptoxanthin exhibits no optical activity.

Partition test: On partition between petroleum ether and 90 % methanol, cryptoxanthin is found in the upper layer, and thus behaves like a hydrocarbon. With 95 % methanol, however, it is hypophasic.

Chromatographic behaviour: Cryptoxanthin is more strongly adsorbed on calcium hydroxide than the carotenes and can readily be separated from the latter in this way. On zinc carbonate or calcium carbonate, it is held less strongly than phytoxanthins with two hydroxyl groups. The separation of cryptoxanthin from rubixanthin presents some difficulty.

Colour reactions: Cryptoxanthin gives a dark blue colouration with antimony trichloride in chloroform solution. The solution exhibits a maximum at 590 mμ (cf. β-carotene).

Detection and estimation: The separation of cryptoxanthin from other carotenoids is achieved by means of chromatographic adsorption. The pigment can be identified by the determination of absorption maxima and by means of the partition test. For the colourimetric estimation, a standard solution of azobenzene in ethanol can be used[12].

Physiological properties: Cryptoxanthin exhibits vitamin A activity (cf. p. 14).

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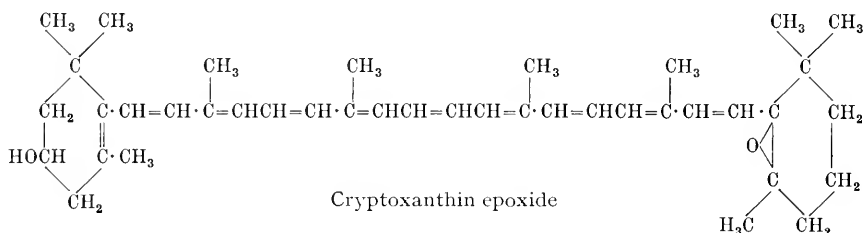
Derivatives

Cryptoxanthin monoacetate $C_{42}H_{58}O_2$: This derivative is formed by treating cryptoxanthin with acetic anhydride in pyridine[12]. It crystallises in red needles, m.p. 117–118° (corr.). The absorption maxima are identical with those of cryptoxanthin. The monoacetate is entirely epiphasic.

Cryptoxanthin mono-epoxide $C_{40}H_{56}O_2$: This compound was obtained by KARRER and JUCKER[13] by the action of monoperphthalic acid on cryptoxanthin acetate. The biological assay[14] of cryptoflavin derived from cryptoxanthin epoxide showed that it is vitamin A-inactive even in high doses. It thus contains no unsubstituted β-ionone ring. For this reason the following formula is ascribed to cryptoxanthin epoxide.

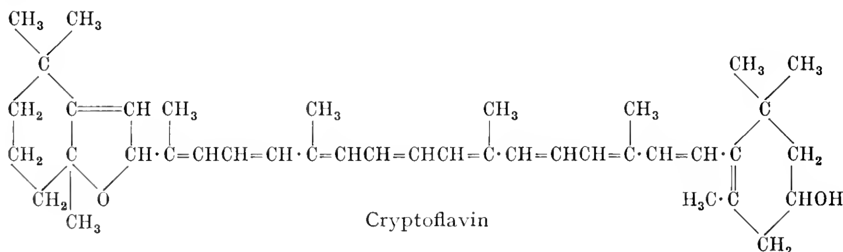


The solubility of cryptoxanthin epoxide is similar to that of cryptoxanthin. It crystallises from a mixture of benzene and methanol in beautiful needles or plates, m.p. 154° (uncorr. in vacuum).

Solvent:	*Absorption maxima:*	
Carbon disulphide	512	479 mμ
Benzene	494	461 mμ
Chloroform	488	456 mμ
Ethanol	481	449 mμ

On shaking an ethereal solution of the pigment with concentrated hydrochlorid acid, the latter assumes a somewhat unstable blue colouration.

Cryptoflavin[15]: By the action of mineral acids on cryptoxanthin monoepoxide, the latter is transformed into the furanoid oxide, cryptoflavin, which is biologically inactive[16].

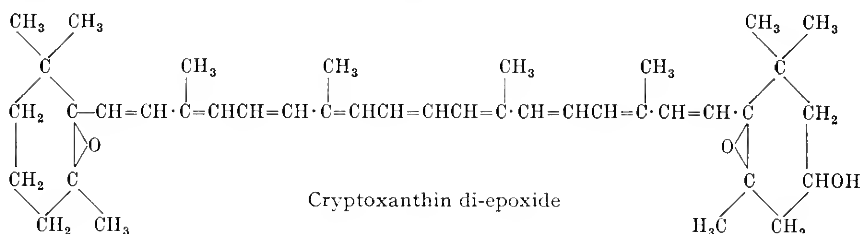


References p. 214–217.

Cryptoflavin crystallises from a mixture of benzene and petroleum ether in beautiful lustrous plates, m.p. 171° (uncorr., in vacuum). The pigment exhibits the same behaviour towards aqueous hydrochloric acid as cryptoxanthin mono-epoxide.

Solvent:	*Absorption maxima:*	
Carbon disulphide	490	459 mμ
Benzene	470	439 mμ
Chloroform	468	438 mμ
Ethanol	460	430 mμ

Cryptoxanthin di-epoxide $C_{40}H_{56}O_3$[15]: This compound is formed by the oxidation of cryptoxanthin acetate with monoperphthalic acid.



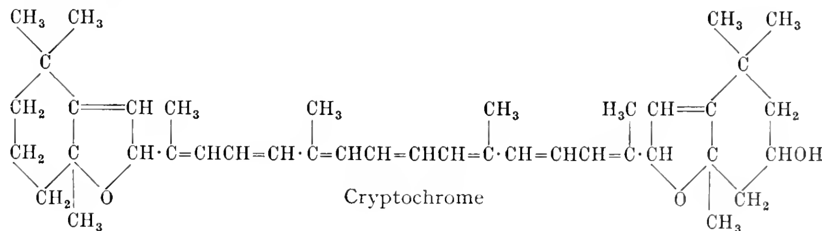
Cryptoxanthin di-epoxide

Cryptoxanthin di-epoxide crystallises from a mixture of benzene and petroleum ether. M.p. 194° (uncorr., in vacuum).

Solvent:	*Absorption maxima:*	
Carbon disulphide	503	473 mμ
Benzene	486	455 mμ
Chloroform	482	453 mμ
Ethanol	473	442 mμ

With concentrated aqueous hydrochloric acid the di-epoxide gives a dark blue colouration which is stable for several days.

Cryptochrome $C_{40}H_{56}O_3$:



Cryptochrome

Cryptochrome is formed by the action of hydrogen chloride in chloroform on cryptoxanthin di-epoxide, besides cryptoflavin and cryptoxanthin. Because

References p. 214–217.

of the small amount of material available it has not yet been obtained in a crystalline state.

Solvent:	*Absorption maxima:*	
Carbon disulphide	456	424 mμ

Cis-trans Isomers

A number of *cis-trans* isomers of cryptoxanthin have been prepared by ZECHMEISTER and LEMMON[17]. According to these authors, natural cryptoxanthin has an all-*trans* configuration (cf. p. 38). By standing or boiling a solution of the pigment, by fusion of the crystals, by treatment with iodine or illumination with sunlight, various isomers are formed which are believed to contain a number of *cis*-double bonds.

The sequence of isomers in the table below is that in which they are observed in the chromatogram.

	Absorption maxima (in petroleum ether)	
Neo-Cryptoxanthin U	478.5	448 mμ
(Cryptoxanthin)	(483.5	452.5 mμ)
Neo-Cryptoxanthin A	477	446 mμ
Neo-Cryptoxanthin B	479.5	449.5 mμ

Apart from natural cryptoxanthin, none of these compounds has been obtained in a crystalline state. The absorption curves of the pigments can be found in the original communication.

4. ZEAXANTHIN $C_{40}H_{56}O_2$

History

1929 KARRER, SALOMON and WEHRLI[18] isolate a new phytoxanthin, for which they propose the name zeaxanthin, from maize.

1931–32 KARRER and co-workers elucidate the constitution of zeaxanthin[19].

Occurrence

Zeaxanthin is widely distributed in plants in the free state as well as esterified (physalien). Some plants contain zeaxanthin as the main pigment, so that its isolation in appreciable amounts is relatively easy to achieve.

TABLE 39

OCCURRENCE OF ZEAXANTHIN

Source	References
Capsicum annuum	L. Zechmeister and L. v. Cholnoky, *Ann.* *509* (1934) 269.
Capsicum frutescens japonicum	L. Zechmeister and L. v. Cholnoky, *Ann.* *489* (1931) 1.
Celastrus scandens	A. L. Le Rosen and L. Zechmeister, *Arch. Biochem.* 1 (1943) 17.
Citrus aurantium	L. Zechmeister and P. Tuzson, *Ber.* *69* (1936) 1878.
Crocus sativus	R. Kuhn and A. Winterstein, *Ber.* *67* (1934) 344.
Cucurbita Pepo L.	L. Zechmeister and co-workers, *Ber.* *68* (1935) 1322.
Diospyros costata	K. Schön, *Biochem. J.* *29* (1935) 1779.
Diospyros Kaki	P. Karrer, R. Morf, E. v. Krauss and A. Zubrys, *Helv. chim. Acta* *15* (1932) 490.
Egg yolk	R. Kuhn, A. Winterstein and E. Lederer, *Z. physiol. Chem.* *197* (1931) 141.
Evonymus europaeus	L. Zechmeister and co-workers, *Z. physiol. Chem.* *190* (1930) 67; *Z. physiol. Chem.* *196* (1931) 199.
Feathers of *Serinus canaria*	H. Brockmann and O. Voelker, *Z. physiol. Chem.* *224* (1934) 193.
Fucus vesiculosus	I. M. Heilbron and R. F. Phipers, *Biochem. J.* *29* (1935) 1369; (with H. R. Wright) *J. Chem. Soc.* *1934* 1572.
Halyseris polypodioides	P. Karrer, F. Rübel and F. M. Strong, *Helv. chim. Acta* *19* (1935) 28.
Hippophae rhamnoides	P. Karrer and H. Wehrli, *Helv. chim. Acta* *13* (1930) 1104.
Human fat	L. Zechmeister and P. Tuzson, *Z. physiol. Chem.* *225* (1934) 189; *Z. physiol. Chem.* *231* (1935) 259.
Human liver	H. Willstaedt and T. Lindqvist, *Z. physiol. Chem.* *240* (1936) 10.
Lycium barbarum	A. Winterstein and U. Ehrenberg, *Z. physiol. Chem.* *207* (1932) 25.
Lycium halimifolium	L. Zechmeister and L. v. Cholnoky, *Ann.* *481* (1930) 42.
Physalis Alkekengi, *Physalis Franchetii*	R. Kuhn and W. Wiegand, *Helv. chim. Acta.* *12*, (1929) 499; R. Kuhn, A. Winterstein and W. Kaufmann, *Ber.* *63* (1930) 1489.
Prunus persica	G. Mackinney, *Plant. Physiol.* *12* (1937) 216.
Rana esculenta (liver)	L. Zechmeister and P. Tuzson, *Z. physiol. Chem.* *238* (1936) 197.
Rosa canina, *Rosa rubiginosa*, *Rosa damascena*	R. Kuhn and C. Grundmann, *Ber.* *67* (1934) 339, 1133.
Rubus Chamaemorus	H. Willstaedt, *Skand. Arch. Physiol.* *75* (1936) 155.

Source	References
Rudbeckia Neumannii	P. Karrer and A. Notthafft, *Helv. chim. Acta 15* (1932) 1195.
Sarcina aurantiaca	E. Chargaff, *Compt. rend. 197* (1933) 946.
Senecio Doronicum	P. Karrer and A. Notthafft, *Helv. chim. Acta 15* (1932) 1195.
Solanum Hendersonii	A. Winterstein and U. Ehrenberg, *Z. physiol. Chem. 207* (1932) 25.
Solanum Lycopersicum L.	R. Kuhn and C. Grundmann, *Ber. 65* (1932) 1880.
Staphylococcus aureus	E. Chargaff, *Compt. rend. 197* (1933) 946.
Vaccinium vitis idaea	H. Willstaedt, *Svensk Kemisk Tidskr. 48* (1936 (212).
Viola tricolor	P. Karrer and J. Rutschmann, *Helv. chim. Acta 27* (1944) 1684.
Zea Mays	P. Karrer, H. Salomon and H. Wehrli, *Helv. chim. Acta 12* (1929) 790.

TABLE 40

ZEAXANTHIN CONTENT OF VARIOUS PLANTS

Source	Quantity	Yield of Zeaxanthin	References
Maize meal	100 kg	100–200 mg	20
Physalis leaves	1 kg (dry)	4 g	21
Evonymus europaeus	1 kg seeds	200 mg	22
Lycium halimifolium berries	1 kg (fresh)	400–500 mg	23
Hippophaes rhamnoides berries	1 kg (fresh)	25 mg	24

Preparation

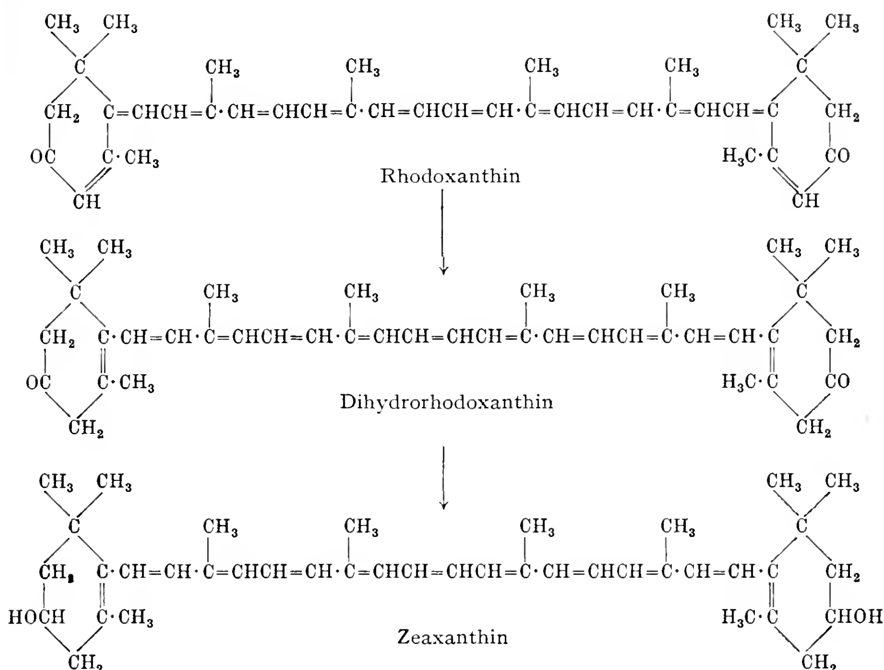
Zeaxanthin can be prepared either from maize[20] or from leaves of *physalis* cups[25]. In *physalis*, the pigment occurs in the form of the palmitic acid ester, physalien, the preparation of which is described on p. 187. Zeaxanthin is obtained from physalien by saponification.

3 g of physalien are dissolved in ether and saponified by shaking with 10% methanolic potassium hydroxide at room temperature. By dilution with water, the zeaxanthin is transferred to the ether layer, which is concentrated slightly until the pigment crystallises. For further purification, the zeaxanthin is crystallised once from a mixture of chloroform and ether. The yield is just under 1 g.

Formation

Karrer and Solmssen[26] succeeded in converting a carotenoid with 40 carbon atoms into another natural pigment with the same number of carbon atoms by converting rhodoxanthin (p. 221) into zeaxanthin by reduction of the dihydroderivative with aluminium *iso*propoxide.

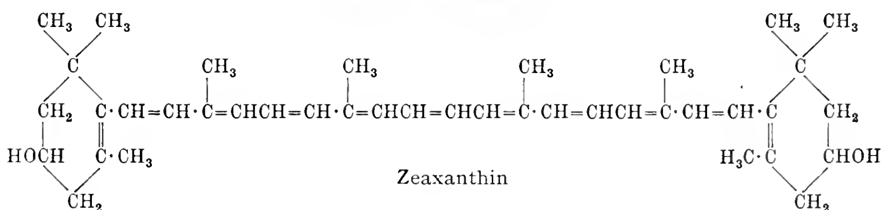
References p. 214–217.



This transformation of rhodoxanthin into zeaxanthin represents the first partial synthesis of a C_{40} carotenoid and also confirms the constitution of both pigments.

KARRER and JUCKER[27] also carried out another partial synthesis of zeaxanthin by treating xanthophyll with sodium ethoxide. This resulted in the displacement of the isolated double bond into conjugation and the zeaxanthin thus obtained was identical in spectral properties and melting point with the natural pigment. These experiments show that polyenes containing an isolated double bond readily undergo prototropic rearrangement to the fully conjugated isomer.

Chemical Constitution[28,29]



The elucidation of the constitution of zeaxanthin is mainly due to KARRER and co-workers, and is similar to the corresponding investigations on xanthophyll (cf. p. 201). The empirical formula and the number of hydroxyl groups

present indicate that zeaxanthin is an isomer of xanthophyll. The nature of the isomeric relationship was established by the investigations of KARRER and co-workers[28,29] who showed that xanthophyll is derived from α-carotene, whereas zeaxanthin is the corresponding derivative of β-carotene. By the dry distillation of the pigment, KUHN and WINTERSTEIN[30] obtained toluene, *m*-xylene and 2:6-dimethylnaphthalene. By ozonisation and potassium permanganate oxidation of zeaxanthin, KARRER and co-workers[31] obtained α:α-dimethylsuccinic acid. The arguments applied in the case of xanthophyll regarding the position of the hydroxyl groups are therefore also valid for zeaxanthin (cf. p. 201). The number of side-chain methyl groups and double bonds was determined by KUHN and co-workers[32].

In 1938, KARRER and co-workers provided further confirmation for the constitution of zeaxanthin by preparing β-citraurin from zeaxanthin by partial oxidative degradation with potassium permanganate[33].

```
   CH3  CH3
     \  /
      C              CH3          CH3              CH3          CH3
     / \              |            |                |            |
  CH2   C·CH=CH·C=CHCH=CH·C=CHCH=CHCH=C·CH=CHCH=C·CHO
   |    ||
 HOCH   C·CH3
     \  /                   β-Citraurin
      CH2
```

β-Citraurin

Properties and Physical Constants

Crystalline form: Zeaxanthin crystallises from methanol in long, yellow plates and clusters which, in contrast to xanthophyll, do not occlude solvent. The pigment crystallises especially well from ethanol in short thick rombic prisms. From a mixture of carbon disulphide, petroleum ether and ether, zeaxanthin separates in clustered needles.

Melting point: 205° (uncorr.)[34], 215.5° (corr.)[35].

Solubility: 1 g zeaxanthin dissolves in about 1.5 l of boiling methanol. The pigment is almost insoluble in petroleum ether and hexane. Its solubility in ether, chloroform, carbon disulphide and pyridine is somewhat greater. A suspension of the pigment in glacial acetic acid becomes homogeneous on addition of hexane.

Spectral properties (cf. Fig. 8, p. 350):

Solvent:	*Absorption maxima:*			
Carbon disulphide	517	482	450	mμ
Chloroform	495	462	429	mμ
Ethanol	483	451	423.5	mμ
Petrol	483.5	451.5	423	mμ
Methanol	480.5	449.5	421.5	mμ

Quantitative extinction measurements: In carbon disulphide, KUHN and SMAKULA[36]; in ethanol and carbon disulphide, HAUSSER and SMAKULA[37] and HAUSSER[38].

Optical activity: According to several workers, zeaxanthin, like β-carotene, is optically inactive. Recently, however, ZECHMEISTER and co-workers[39] reported that their zeaxanthin preparations have a rotation of $[\alpha]_C = -40-50°$ in chloroform. Perhydrocarotene obtained by the reduction of perhydrozeaxanthin dibromide, is optically inactive in contrast to the corresponding products from xanthophyll.

Partition test: Zeaxanthin exhibits entirely hypophasic character on partition between methanol and petroleum ether.

Chromatographic behaviour: Zeaxanthin is easily adsorbed on calcium carbonate or zinc carbonate from benzene solution.

Detection and estimations: Zeaxanthin can be separated from other phytoxanthins by adsorption on zinc carbonate. It can be identified by its absorption maxima, in conjunction with the partition test. According to KUHN and BROCKMANN the pigment can be determined colorimetrically, using a solution of azobenzene in ethanol as a standard[40].

Physiological behaviour: Zeaxanthin exhibits no vitamin A activity, but it yields an active product on treatment with phosphorous tribromide[41].

Colour reactions: Zeaxanthin dissolves in concentrated sulphuric acid with a fairly stable deep blue colouration. On treating a solution of the pigment in chloroform with antimony trichloride a blue colouration is produced which has been examined spectroscopically[42].

Derivatives

Perhydrozeaxanthin $C_{40}H_{78}O_2$: Colourless, viscous oil[13,*] which is leavorotatory in contrast to perhydroxanthophyll. $[\alpha]_D^{20} = -24.5°$[43,*]

Zeaxanthinhalogenides: KARRER and co-workers[44] replaced the two hydroxyl groups in perhydrozeaxanthin by bromine, thus obtaining 3:3′-dibromoperhydrozeaxanthin. On treating a solution of zeaxanthin with bromine, 8 mols of the halogen are absorbed.

Zeaxanthin monomethyl ether $C_{41}H_{58}O_2$: This compound is formed on treating zeaxanthin with the potassium derivatives of tertiary amyl alcohol and methyl iodide[45]. It crystallises from methanol in needles, m.p. 153°.

Zeaxanthin dimethyl ether $C_{42}H_{60}O_2$: This compound is obtained as by-product in the preparation of the monomethyl ether[45]. It crystallises from petroleum ether in dark red needles, m.p. 176°. It is very sparingly soluble in methanol and ethanol.

Zeaxanthin diacetate $C_{44}H_{60}O_4$: KARRER and SOLMSSEN obtained this ester by treating a solution of zeaxanthin in pyridine with acetic anhydride[46]. The diacetate crystallises from a mixture of benzene and methanol. M.p. 154–155°.

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* According to P. KARRER and co-workers, *Helv. chim. Acta 15* (1932) 492, perhydrozeaxanthin is optically inactive.

References p 214–217.

Zeaxanthin dipropionate $C_{46}H_{64}O_4$[47]: Crystallises from a mixture of benzene and methanol. M.p. 142°.

Zeaxanthin dibutyrate $C_{48}H_{68}O_4$[47]. Crystallises from a mixture of benzene and methanol. M.p. 132°.

Zeaxanthin di-n-valerate $C_{50}H_{72}O_4$[47]: Crystallises from a mixture of benzene and methanol. M.p. 125°.

Zeaxanthin di-n-capronate $C_{52}H_{76}O_4$[47]: M.p. 117–118°.

Zeaxanthin di-n-caprylate $C_{56}H_{84}O_4$[47]: This compound crystallises from benzene. M.p. 107°.

Zeaxanthin dilaurate $C_{64}H_{100}O_4$[48]. M.p. 104°.

Zeaxanthin monopalmitate $C_{56}H_{86}O_3$: This compound was obtained by KARRER and SCHLIENTZ[49] by partial saponification of physalien. The half-ester crystallises from a mixture of benzene and ethanol in plates, m.p. 148°.

Zeaxanthin distearate $C_{76}H_{124}O_4$: Prepared by treating a solution of zeaxanthin in pyridine with stearic acid chloride. M.p. 95°.

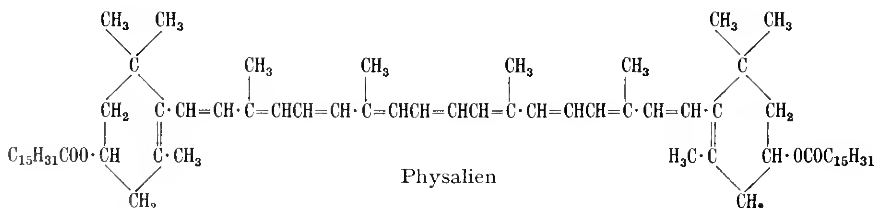
Physalien (zeaxanthin dipalmitate) $C_{72}H_{116}O_4$: KUHN and WIEGAND found a carotenoid in *Physalis Alkekengi* and *Physalis Franchetii*[50] which they termed physalien. This pigment was later obtained from numerous other plants.

TABLE 41

OCCURRENCE OF PHYSALIEN

Source	References
Asparagus officinalis	A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem. 207* (1932) 26.
Hippophae rhamnoides (berries)	P. KARRER and H. WEHRLI, *Helv. chim. Acta 13* (1930) 1104.
Lycium barbarum (skin) and *Solanum Hendersonii*	A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem. 207* (1932) 26.
Lycium halimifolium (skins)	L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 481*, (1930) 42.

Investigations by ZECHMEISTER and VON CHOLNOKY[51] and KUHN and co-workers[52] showed that physalien is an ester of zeaxanthin, namely zeaxanthin dipalmitate.



Physalien

References p. 214–217.

Physalien has been partially synthesized from zeaxanthin and palmitic acid chloride, thus confirming its constitution.

Physalien is best prepared from physalis cups[53], the pigment content of which is exceptionally large, amounting to 0.9–1.8% of dry weight. A third of the pigment consists of cryptoxanthin (cf. p. 176).

Physalis cups are dried at 40–50°, coarsely ground and exhaustively extracted with benzene at room temperature. The combined extracts are concentrated in vacuum to a small volume and the polyene wax is precipitated with acetone. The precipitation is not carried out all at once, but at intervals of several hours, each precipitate being filtered separately. The mother liquors are finally diluted with much ethanol and set aside in the cold. In this way additional amounts of pigment are obtained. The purification of physalien is carried out as follows: the pigment wax is dissolved in hot benzene and fractionally precipitated in the hot with methanol. The first fractions sometimes contain a waxy colourless substance, which must be separated from the solution by filtration through a steam-jacketed filter. The subsequent fractions yield the physalien. It is purified by re-crystallisation from a mixture of benzene and methanol.

The purification of crude physalien can also be achieved more simply by dissolution in about 60 parts of hot benzene, followed by addition of 160 parts of hot ethanol. On cooling, the pigment wax separates and can be purified by crystallisation from a mixture of benzene and methanol. 1 g of the crude products yield about 0.5 to 0.7 g of pure physalien.

Physalien can also be isolated from *Physalis* berries[54]. 2 kg of berries yield about 1 g of polyene wax. L. ZECHMEISTER and VON CHOLNOKY[55] also describe the isolation of the pigment from fresh *Lycium* berries, 5 kg of which yielded 5 g physalien.

Properties

Physalien crystallises from a mixture of benzene and methanol in long, flat rods, or in fine needles. It can also be obtained in the form of stout needles. From cyclohexane and ethanol, the pigment separates in flat, dark red prisms, several millimeters long. In larger quantities, physalien has the appearance of a fiery brilliant red powder, with the consistency of a hard wax. It melts at 98.5–99.5°. It is very easily soluble in carbon disulphide, benzene, chloroform, and carbon tetrachloride, and readily soluble in petroleum ether, hexane, tetralin, decalin, ether and pyridine. Cyclohexane, glacial acetic acid and acetic anhydride readily dissolve the pigment in the hot, but only sparingly in the cold. The compound is almost insoluble in ethanol and acetone.

According to KUHN and BROCKMANN[56], physalien is optically inactive. The positions of the absorption bands are indistinguishable from those of zeaxanthin. Quantitative extinction measurements are reported by HAUSSER and SMAKULA[57]. On standing in air, physalien slowly absorbs oxygen, which results in a lightening of the colour, lowering of the m.p. and increase in the solubility in ethanol. Physalien dissolves in concentrated sulphuric acid to give a dark blue solution[58]. Further colour reactions are described by KUHN and WIEGAND[59].

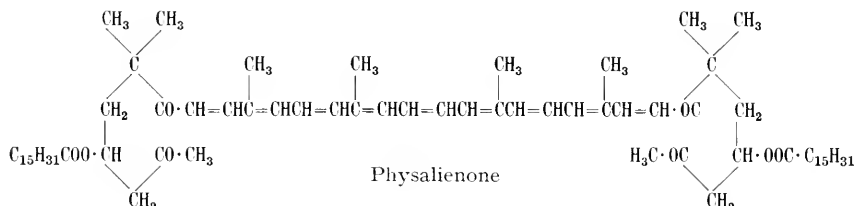
References p. 214–217.

Derivatives of Physalien

a) *Perhydrophysalien* $C_{72}H_{138}O_4$: A colourless oil, easily soluble in ether, sparingly in ethanol.

b) *Physalien iodide:* This compound is formed by treating an ethereal solution of the pigment with an ethereal solution of iodine. On treating the addition product with thiosulphate, unchanged physalien is regenerated.

c) *Physalienone:* By the treatment of physalien with chromium trioxide, KARRER, SOLMSSEN and WALKER[60] obtained a tetraketone of the following constitution (cf. KARRER and GUGELMANN[61]):



Physalienone

The tetraketone crystallises in clustered needles, m.p. 144–145°. Its optical properties are very similar to those of β-carotenone.

Solvent:	*Absorption maxima:*					
	Physalienone			*β-Carotenone*		
Carbon disulphide	536	500	463	538	499	466 mμ
Petroleum ether	497	464	436	502	468	440 mμ
Chloroform	525	488	452	527	489	454 mμ

With regard to the action of bromine on physalien, cf. ZECHMEISTER and VON CHOLNOKY[62]. With regard to the reaction with iodine, cf. KUHN and co-workers[63]. The assimilation of physalien by rats and chicks has been investigated by KUHN and BROCKMANN[64].

Cis-trans Isomers of Zeaxanthin

By a variety of treatments, such as heating in solution, dissolution of the crystals, catalysis with iodine and illumination with sunlight, ZECHMEISTER and co-workers converted zeaxanthin into mixtures of different pigments which they regard as *cis-trans* isomers[65]. Three compounds could be obtained in the crystalline state, namely neo-zeaxanthin A, neo-zeaxanthin B and neo-zeaxanthin C. Another pigment different in character from the other isomers is also formed[66]. Treatment of this compound with iodine (except in alcohol solution) results in a displacement of the absorption maxima towards *shorter* wavelength by 2–4 mμ.

The three crystalline neo-zeaxanthins have the following properties:

References p. 214–217.

Neo-zeaxanthin A: Small plates from methanol, m.p. about 106° (not sharp, corr.).

Solvent	*Absorption maxima:*	
Carbon disulphide	508	475.5 mμ
Benzene	489	457.5 mμ
Petrol	477	447 mμ
Ethanol	478.5	448.5 mμ

$[\alpha]_c$ about +120° (in chloroform).

Neo-zeaxanthin B: Flat, obliquely cut plates, from dilute methanol, m.p. 92° (not sharp, corr.). Absorption maxima in carbon disulphide, benzene, petrol and ethanol are the same as for neo-zeaxanthin A. The optical rotation shows varying values.

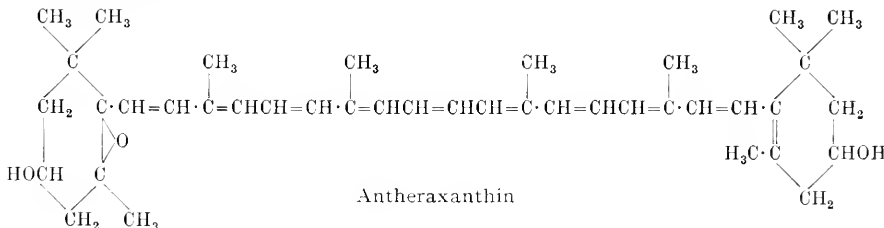
Neo-zeaxanthin C: Small crystals from a mixture of carbon disulphide and benzene. M.p. 154° (corr.).

Solvent:	*Absorption maxima:*	
Carbon disulphide	502	470 mμ
Benzene	488.5	455 mμ
Petrol	473.5	444 mμ
Ethanol	473	443.5 mμ

Epoxides of Zeaxanthin and their Transformation Products

By the oxidation of zeaxanthin acetate with monoperphthalic acid, KARRER and JUCKER[67] obtained various epoxides and furanoid transformation products some of which proved to be identical with natural carotenoids, the structure of which had previously been unknown. As the natural oxides of zeaxanthin are dealt with in detail in the following chapter only a brief description of these compounds will be given here.

a) *Zeaxanthin mono-epoxide, Antheraxanthin*:*

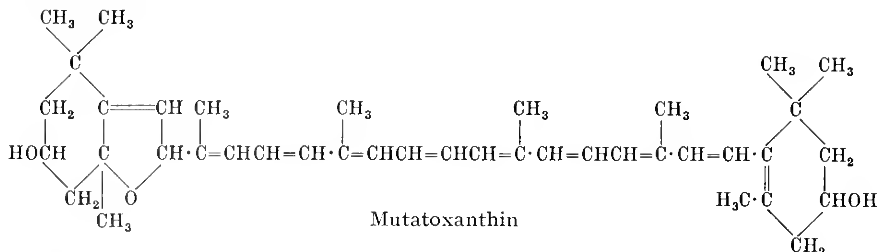


Antheraxanthin

A detailed description of this pigment will be found on p. 191.

* Antheraxanthin was first isolated by P. KARRER and A. OSWALD from anthers of *Lilium tigrinum, Helv. chim. Acta 18* (1935) 1303.

b) *Mutatoxanthin:*

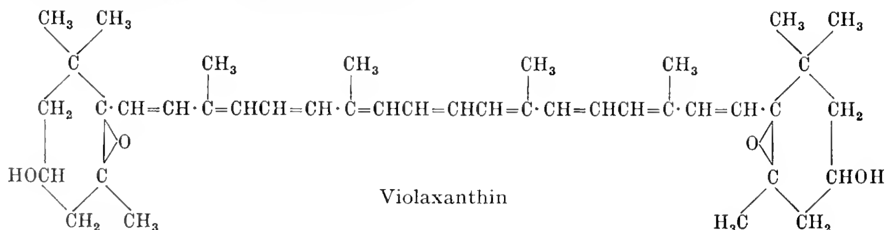


Mutatoxanthin was first obtained by KARRER and RUTSCHMANN[68] by the action of dilute hydrochloric acid on natural violaxanthin (cf. p. 195). It has the formula $C_{40}H_{56}O_3$, and contains 10 double bonds and 2 hydroxyl groups[68]. The nature of the third oxygen atom was first established by the partial synthesis[69] of the pigment, in which mutatoxanthin was obtained by the action of acidic chloroform on zeaxanthin mono-epoxide (antheraxanthin) (cf. p. 62). The formula given above for mutatoxanthin is in accord with all the properties of the pigment. This formulation also explains the formation of mutatoxanthin from violaxanthin. By the action of dilute hydrochloric acid on the latter, one of the epoxide groups is converted into a more stable furanoid system, while the oxygen of the other epoxide group is split off. (With regard to the formula of violaxanthin, see below).

Mutatoxanthin crystallises from methanol or from a mixture of benzene and methanol. M.p. 177° (uncorr., evacuated capillary). On partition between methanol and petroleum ether, the pigment is found in the lower layer. On treating an ethereal solution of mutatoxanthin with concentrated aqueous hydrochloric acid, a blue colouration is produced which is weaker than that given by auroxanthin (cf. p. 197) and not very stable.

Solvent:	*Absorption maxima:*	
Carbon disulphide	488	459 mμ
Ethanol	457	427 mμ
Benzene	468	439 mμ
Petroleum ether	456	426 mμ
Chloroform	468	437 mμ
Pyridine	473	443 mμ

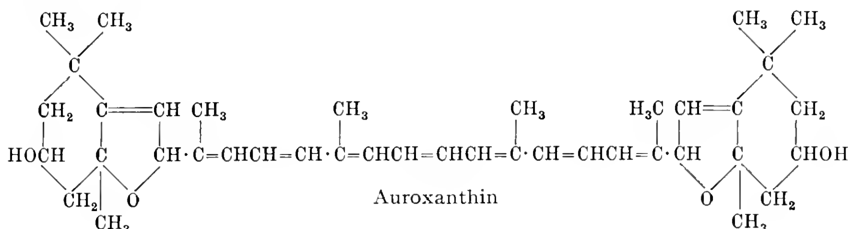
c) *Violaxanthin, Zeaxanthin di-epoxide:*



References p. 214–217.

Violaxanthin is formed together with antheraxanthin, though in somewhat poorer yield. It is a natural pigment of wide occurrence and has been repeatedly investigated within recent years. It will be described in detail on p. 193.

d) *Auroxanthin:*



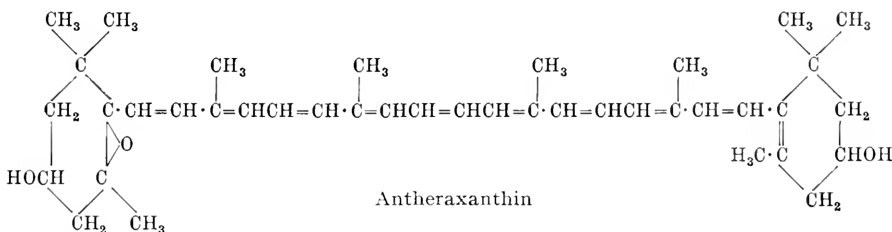
Auroxanthin

The furanoid dioxide of zeaxanthin, auroxanthin, is a carotenoid occurring in blossoms. It can be obtained by the isomerisation of violaxanthin[70] in the presence of acids, a reaction which proves its constitution. A detailed description of auroxanthin will be found on p. 196.

5. ANTHERAXANTHIN $C_{40}H_{56}O_3$

In the course of investigations on the carotenoids of the anthers of *Lilium tigrinum*, KARRER and OSWALD[71] discovered a previously unknown phytoxanthin which they termed antheraxanthin. Antheraxanthin occurs together with capsanthin and is found below the latter in the chromatogram. It shows an entirely hypophasic character in the partition test and its absorption spectrum differs very little from that of zeaxanthin. KARRER and OSWALD determined the molecular formula of the pigment, but were unable to establish the nature of the third oxygen atom.

The constitution of antheraxanthin was elucidated later[72], when the pigment was identified with zeaxanthin mono-epoxide obtained by the oxidation of zeaxanthin with monoperphthalic acid. The two compounds could not be separated by chromatography on zinc carbonate and gave no mixed melting point depression. This partial synthesis established the constitution of antheraxanthin (cf. p. 189). The formula is in agreement with the fact that the pigment absorbs 11 mols of hydrogen on catalytic hydrogenation[72].



Antheraxanthin

Antheraxanthin crystallises from methanol or a mixture of benzene and methanol in needles or thin plates, m.p. 205°. By the action of acidic chloroform, mutatoxanthin and a little zeaxanthin are formed from antheraxanthin. On shaking an ethereal solution of the pigment with concentrated aqueous hydrochloric acid, a blue colouration is produced after a period of time.

Solvent:	*Absorption maxima:*	
Carbon disulphide	510	478 mμ
Chloroform	490.5	460.5 mμ

Recently, TAPPI and KARRER[72a] have isolated a *cis* isomer of antheraxanthin from *Lilium candidum*. The formulation of the pigment is based on the following facts. *Cis* antheraxanthin has a lower melting point than *trans* antheraxanthin and its absorption maxima are displaced towards shorter wavelengths by 4–6 mμ. Spectral measurements show that on irradiation with ultraviolet light and on treatment with iodine the new isomer is partly isomerised to *trans* antheraxanthin, though the amount of available material was insufficient to isolate the latter in a cystalline state. On treatment with chloroform containing traces of hydrochloric acid, *cis* antheraxanthin is converted into mutatoxanthin which is also obtained from *trans* antheraxanthin under these conditions.

Cis antheraxanthin is the first natural carotenoid epoxide with a partial *cis* configuration. The small difference in the location of the absorption maxima of *cis* and *trans* antheraxanthin suggests that only one double bond in the former has a *cis*-configuration. The large difference in melting points, on the other hand, suggests that there is a considerable difference in molecular shape and that the double bond involved in the geometrical isomerism may perhaps be situated in the centre of the polyene chain.

Cis antheraxanthin crystallises from methanol in long, yellow-red needles, m.p. 110° (uncorr., evacuated capillary). It is easily soluble in benzene, carbon disulphide and ether, fairly soluble in warm methanol and very sparingly soluble in petroleum ether.

Solvent:	*Absorption maxima:*	
Carbon disulphide	506	476 mμ
Benzene	487	457 mμ
Ethanol	472	445 mμ

On shaking an ethereal solution with concentrated hydrochloric acid, the latter assumes a fairly stable blue colouration.

References p. 214–217.

6. VIOLAXANTHIN $C_{40}H_{56}O_4$

History

1931 KUHN and WINTERSTEIN[73] isolate a pigment of the formula $C_{40}H_{56}O_4$ from the yellow blossoms of pansies (*viola tricolor*). The pigment is named violaxanthin.

1931–44 KARRER and co-workers[74] carry out detailed investigations on the constitution of violaxanthin.

1945 KARRER and JUCKER achieve a partial synthesis of violaxanthin by the oxidation of zeaxanthin and thus establish the constitution of the pigment[75].

Occurrence

Recent investigations have shown that violaxanthin is fairly widely distributed in nature. In view of the formation of violaxanthin from zeaxanthin[75] it was to be expected that plants containing the former would also contain the latter pigment. As the investigations regarding the genetic relationship of the two compounds are very recent, this question has so far received comparatively little attention.

TABLE 42

OCCURRENCE OF VIOLAXANTHIN

a) Blossoms:	References
Calendula officinalis	L. ZECHMEISTER and L. v. CHOLNOKY, *Z. physiol. Chem.* *208* (1932) 27.
Cytisus laburnum	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta* *15* (1932) 1195.
Laburnum anagyroides	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta* *15* (1932) 1195.
Ranunculus acer	R. KUHN and H. BROCKMANN, *Z. physiol. Chem.* *213* (1932) 192.
Sinapis officinalis	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta* *15* (1932) 1195.
Stinging nettles	R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem.* *197* (1931) 141. — P. KARRER and co-workers, *Helv. chim. Acta* *28* (1945) 1146.
Tagetes grandiflora	R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem.* *197* (1933) 141.
Taraxacum officinale	R. KUHN and E. LEDERER, *Z. physiol. Chem.* *200* (1931) 108.
Tragopogon pratensis	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta* *15* (1932) 1195.
Tulipa (yellow variety)	C. A. SCHUNCK, *Proc. Roy. Soc.* *72* (1903) 165.
Tussilago Farfara	P. KARRER and R. MORF, *Helv. chim. Acta* *15* (1932) 863.

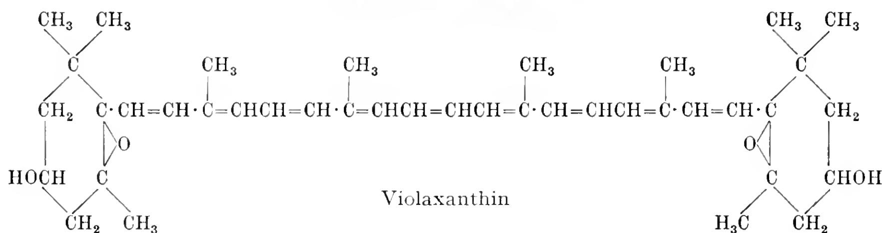
References p. 214–217.

	References
Ulex europaeus	C. A. Schunck, *Proc. Roy. Soc. 72* (1903) 165.
Viola tricolor	R. Kuhn and A. Winterstein, *Ber. 64* (1931) 326. — P. Karrer and J. Rutschmann, *Helv. chim. Acta 25* (1942) 1624; *Helv. chim. Acta 27* (1944) 1684.
b) In fruit:	
Arbutus Unedo L.	K. Schön, *Biochem. J. 29* (1935) 1779.
Carica Papaya	R. Yamamoto and S. Tin, *Chem. Centr. 1933*, I, 3090.
Citrus aurantium	L. Zechmeister and P. Tuzson, *Naturwissenschaften 19* (1931) 307. — P. G. F. Vermast, *Naturwissenschaften 19* (1931) 442.
Citrus poonensis hort.	R. Yamamoto and S. Tin, *Chem. Centr. 1934*, I, 1660.
Cucurbita maxima	L. Zechmeister and P. Tuzson, *Ber. 67* (1934) 824.
Diospyros costata	K. Schön, *Biochem. J. 29* (1935) 1779.
Iris Pseudocorus	P. J. Drumm, F. O'Connor, *Biochem. J. 39* (1945) 211.
c) Human liver(?)	H. Willstaedt and T. Lindqvist, *Z. physiol. Chem. 240* (1936) 10.

Preparation[76]

Yellow blossoms of *Viola tricolor*, containing as few deeply pigmented patches as possible, are dried and extracted at room temperature with petroleum ether. The combined extracts are concentrated in vacuum to a small volume and the pigment esters are saponified with a solution of sodium ethoxide in ethanol. The free phytoxanthins are dissolved in methanol, petroleum ether is added, and the violaxanthin is precipitated by very careful addition of water. The crude pigment is filtered and recrystallised from a mixture of methanol and ether. The yield amounts to 0.05 to 0.07% of the dry blossom powder.

Chemical Constitution[77]



Violaxanthin

Since 1931, numerous experiments have been made with the object of elucidating the constitution of violaxanthin. There is some disagreement between the results of different workers and a complete proof of the constitution was only obtained by the partial synthesis of the pigment[78].

Kuhn and Winterstein determined the correct molecular formula ($C_{40}H_{56}O_4$) of violaxanthin[79]. Karrer and Morf[80] subjected the pigment to permanganate degradation and obtained α:α-dimethylsuccinic acid, but no α:α-dimethyl-

References p. 214–217.

glutaric acid. These results established the relationship of violaxanthin to the xanthophyll and zeaxanthin series (cf. p. 201). KARRER and co-workers[81] showed that violaxanthin absorbs 10 mols of hydrogen on catalytic hydrogenation. The position of the absorption maxima indicated that all 10 double bonds must be conjugated*.

According to KARRER and RUTSCHMANN[82] only two of the four oxygen atoms in the violaxanthin molecule are present as hydroxyl groups; the nature of the other two oxygen atoms could not be established**. Eventually KARRER and JUCKER were able to identify violaxanthin with their synthetic zeaxanthin di-epoxide. The constitution of violaxanthin was thus proved (cf. p. 190).

Properties

Violaxanthin crystallises from methanol in yellow-orange prisms, or from carbon disulphide in reddish-brown spears, m.p. 200°. It is easily soluble in ethanol, methanol, carbon disulphide and ether, but almost insoluble in petroleum ether.

Solvent:	*Absorption maxima:*			
Carbon disulphide	501	470	440	mμ
Chloroform	482	451.5	424	mμ
Petrol	472	443	417.5	mμ
Ethanol	471.5	442.5	417.5	mμ
Methanol	469	440	415	mμ
		(cf. Fig. 9, p. 351)		

For quantitative extinction measurements, cf. HAUSSER and SMAKULA[83]. Violaxanthin gives a very characteristic, stable deep-blue colouration on shaking an ethereal solution with 20% hydrochloric acid***. The pigment is separated from other phytoxanthins by chromatographic adsorption from benzene solution on zinc carbonate or calcium carbonate. The specific rotation in chloroform is $[\alpha]^{20}_{Cd} = +35°$.

Violaxanthin is converted into mutatoxanthin, auroxanthin and zeaxanthin under the influence of dilute acids[84]. These reactions are discussed on p. 62.

Derivatives

Perhydroviolaxanthin: This compound is obtained on catalytic reduction of violaxanthin in ethanol. It is a colourless, weakly leavo-rotatory oil[85]. In view

* With regard to apparent contradictions concerning the number of double bonds, cf. *Helv. chim. Acta 28* (1945) 300.

** With regard to the contradictory data concerning the number of hydroxyl groups, cf. *Helv. chim. Acta 28* (1945) 300.

*** For further colour reactions cf. R. KUHN and A. WINTERSTEIN, *Ber. 64* (1931) 326.

of the fact that the epoxide nature of violaxanthin was not realized at the time, some of the hydrogenations were carried out in glacial acetic acid solution, resulting in a partial isomerisation of the pigment (cf. p. 61).

Violaxanthin di-p-nitrobenzoate $C_{54}H_{62}O_{10}N_2$[86]: The ester is obtained by treating violaxanthin dissolved in pyridine with p-nitrobenzoyl chloride. M.p. 208° (with decomposition, uncorr., evacuated capillary).

Violaxanthin dibenzoate $C_{54}H_{64}O_6$[86]: The dibenzoate is prepared in a manner analogous to that employed for the compound described above. M.p. 217° (uncorr. evacuated capillary). In agreement with the formula of violaxanthin, neither ester contains any active hydrogen atoms.

7. AUROXANTHIN $C_{40}H_{56}O_4$

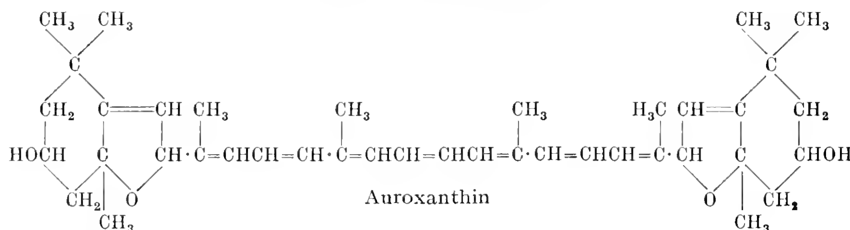
History and Occurrence

During the chromatographic separation of carotenoids from *Viola tricolor*, KARRER and RUTSCHMANN[87] isolated a new pigment which they termed auroxanthin in view of the beautiful golden-yellow colour of its crystals. Up to the present time this phytoxanthin has only been found in the blossoms of *Viola tricolor*, but it is probable that it occurs more widely together with its isomer, violaxanthin*.

Preparation

Dried blossoms of *Viola tricolor* are extracted with petroleum ether, the combined extracts are strongly concentrated in vacuum and the phytoxanthin esters are saponified with methanolic potassium hydroxide. After the saponification has been completed, the free phytoxanthins are dissolved in methanol, petroleum ether is added, and the pigments are precipitated by careful addition of water. The crude carotenoid mixture thus obtained is recrystallised once from methanol. Auroxanthin remains in the mother liquors. The pigments from the mother liquors are extracted with benzene and chromatographed on zinc carbonate. Auroxanthin is eluted with a mixture of ether and methanol from the upper part of the absorption column. After two recrystallisations from methanol, the pigment is obtained analytically pure.

Chemical Constitution[89]



Auroxanthin

* For the relationships between auroxanthin and violaxanthin, cf. below.

References p. 214–217.

The empirical formula of auroxanthin was determined by KARRER and RUTSCHMANN[90]. The number of double bonds was determined by catalytic hydrogenation. The position of the absorption bands (454, 423 mμ in carbon disulphide) indicated the presence of seven conjugated double bonds. ZEREWITINOFF determinations gave values corresponding to two or three active hydrogen atoms. Since the determination of active hydrogen in xanthophyll, however, gave values corresponding to 2.5 active hydrogen atoms, it could be assumed that auroxanthin contains only two hydroxyl groups. The nature of the other two oxygen atoms could not at first be established. However, when auroxanthin, together with mutatoxanthin and a little zeaxanthin had been obtained by the acid treatment of violaxanthin[91], a connection between these pigments was clearly indicated.

The nature of this relationship was elucidated by the investigations of KARRER and JUCKER in the course of which violaxanthin was identified as zeaxanthin di-epoxide (cf. p. 195) and auroxanthin as the corresponding di-furanoid oxide[92]. The proposed formula of auroxanthin is in complete agreement with all the properties of the compound. The configurations of natural and partially synthetic auroxanthin appear to be the same[93].

Properties

Auroxanthin crystallises from methanol in golden-yellow needles m.p. 203° (uncorr., evacuated capillary). The solubility of the pigment is very similar to that of violaxanthin. The pigment shows no optical rotation in benzene solution.

A characteristic test for auroxanthin is the blue colouration which is observed on shaking an ethereal solution with 15 % hydrochloric acid. This blue colouration is very stable. In carbon disulphide, auroxanthin shows absorption maxima at 454 and 423 mμ (cf. Fig. 10, p. 351).

8. XANTHOPHYLL $C_{40}H_{56}O_2$*

History

1837 BERZELIUS[99] coins the term "xanthophyll" for the yellow pigment of autumn leaves.

1907 WILLSTÄTTER and MIEG[95] isolate xanthophyll from green leaves in the crystalline state and determine its empirical formula and its molecular weight.

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* There is no uniformity in the literature regarding the designation of this compound. Some investigators employ the term "lutein" proposed by KUHN, and use the term xanthophyll only as a generic term for hydroxyl-containing carotenoids, for which KARRER proposed the term phytoxanthins. In this monograph the name xanthophyll is retained for the yellow leaf pigment $C_{40}H_{56}O_2$.

1912 WILLSTÄTTER and ESCHER[96] isolate lutein, later found to be a mixture of xanthophyll and zeaxanthin, from egg yolk.

1930–33 KARRER and co-workers[97] elucidate the constitution of xanthophyll.

Occurrence

Xanthophyll is very widely distributed in nature. It is found together with carotene and chlorophyll in all green parts of plants. It also occurs very frequently in red and yellow blossoms, sometimes as an ester (e.g. as the dipalmitic acid ester, helenien[98]).

According to a communication by JUNGE[99], xanthophyll occurs in various green insects combined with protein. These findings, however, require further confirmation.

TABLE 43

OCCURRENCE OF XANTHOPHYLL

a) Blossoms:	References
Acacia decurrens var. mollis	R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem. 197* (1931) 141.
Acacia discolor	J. M. PETRIE, *Biochem. J. 18* (1924) 957.
Acacia linifolia	do.
Acacia longifolia	do.
Calendula officinalis	L. ZECHMEISTER and L. v. CHOLNOKY, *Z. physiol. Chem. 208* (1932) 27.
Caltha palustris	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta 15* (1932) 1195.
Cucurbita Pepo	L. ZECHMEISTER, T. BÉRES and E. UJHELYI, *Ber. 68* (1935) 1321; *69* (1936) 573.
Gazania rigens	K. SCHÖN, *Biochem. J. 32* (1938) 1566. — L. ZECHMEISTER and W. A. SCHRÖDER, *J. Am. Chem. Soc. 65* (1943) 1535.
Genista tridentata	K. SCHÖN and B. MESQUITA, *Biochem. J. 30* (1936) 1966.
Helianthus annuus	R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem. 197* (1931) 141. — L. ZECHMEISTER and P. TUZSON, *Ber. 63* (1930) 3203; *67* (1934) 170.
Helenium autumnale	R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem. 197* (1931) 141.
Kerria japonica	T. ITO, H. SUGINOME, K. UENO and S. WATANABE, *Bul. chem. Soc. Japan 11* (1936) 770.
Leontodon autumnalis	R. KUHN and E. LEDERER, *Z. physiol. Chem. 213* (1932) 188.
Ranunculus acer	C. A. SCHUNCK, *Proc. Roy. Soc. London 27* (1903) 165. — R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 213* (1932) 192. — P. KARRER and co-workers, *Helv. chim. Acta 28* (1945) 1146.

References p. 214–217.

	References
Ranunculus arvensis	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta 15* (1932) 1195.
Ranunculus Steveni	H. H. ESCHER, *Helv. chim. Acta 11* (1928) 752.
Rudbeckia Neumannii	R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem. 197* (1931) 141.
Sarothamnus scoparius	P. KARRER and E. JUCKER, *Helv. chim. Acta 27* (1944) 1586.
Tagetes erecta T.	R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem. 197* (1931) 141.
Tagetes grandiflora	do.
Tagetes nana	do.
Tagetes patula	do.
Taraxacum officinale	P. KARRER and H. SALOMON, *Helv. chim. Acta 13* (1930) 1063. — P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 25* (1942) 1144. — R. KUHN and E. LEDERER, *Z. physiol. Chem. 200* (1931) 108.
Tragopogon pratensis	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta 15* (1932) 1195. — P. KARRER and co-workers, *Helv. chim. Acta 28* (1945) 1146.
Trollius europaeus	P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta 15* (1932) 1195. — P. KARRER and E. JUCKER, *Helv. 29* (1946) 1539.
Viola tricolor	R. KUHN and A. WINTERSTEIN, *Ber. 64* (1931) 326. — P. KARRER and co-workers, *Helv. chim. Acta 25* (1942) 1624; *27* (1944) 1684.
Winter asters	P. KARRER and E. JUCKER, *Helv. chim. Acta 26* (1943) 626.

b) Fruit:

Ananas sativus	O. C. MAGISTAD, *Plant. Physiol. 10* (1935) 187.
Arbutus	K. SCHÖN, *Biochem. J. 29* (1935) 1779.
Capsicum annuum	L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 509* (1934) 269.
Citrus aurantium	L. ZECHMEISTER and P. TUZSON, *Ber. 69* (1936) 1878.
Citrus madurensis	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 221* (1933) 278; *240* (1936) 191.
Convallaria majalis	A. WINTERSTEIN and U. EHRENBERG, *Z. physiol. Chem. 207* (1932) 25.
Cranberries	H. WILLSTAEDT, *Svensk Kemisk. Tidskr. 48* (1936) 212.
Cucurbita maxima	H. SUGINOME and K. UENO, *Chem. Centr. 1931*, II, 2892. — L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 824.
Luffa-species	T. N. GODNEW and S. K. KORSCHENEWSKY, *Chem. Centr. 1931*, I, 1299.
Mangifera indica	R. YAMAMOTO, Y. OSIMA and T. GOMA, *Chem. Centr. 1933*, I, 441.
Momordica Balsamina	G. and F. TOBLER, *Ber. bot. Ges. 28* (1910) 365, 496. — B. M. DUGGAR, *Washington Univ. Stud. 1* (1913) 22.

	References
Musa paradisiaca	H. v. LOESECKE, *J. Am. Chem. Soc. 51* (1929) 2439.
Prunus persica	G. MACKINNEY, *Plant. Physiol. 12* (1937) 216.
Rosa rubiginosa	R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 339.
Wheat germ	B. SULLIVAN and C. H. BAILEY, *J. Am. Chem. Soc. 58* (1936) 383.

c) Other vegetable and animal sources:

Bombix mori	F. G. DIETEL, *Kl. Wschr. 12* (1933) 601. — C. RAND. *Biochem. Z. 281* (1935) 200.
Chicken fat	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 225* (1934) 189.
Cladophora Sauteri	I. M. HEILBRON, E. G. PARRY and R. F. PHIPERS, *Biochem. J. 29* (1935) 1376.
Egg yolk (chicken)	A. E. GILLAM and I. M. HEILBRON, *Biochem. J. 29* (1935) 1064.
Feathers (*Serinus canaria*)	H. BROCKMANN and O. VOELKER, *Z. physiol. Chem. 224* (1934) 193.
Feathers (woodpecker)	do.
Haematococcus pluvialis	J. TISCHER, *Z. physiol. Chem. 250* (1937) 147.
Human fat	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 225* (1934) 189; *231* (1935) 259.
Human liver	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 234* (1935) 241. — H. WILLSTAEDT and T. LINDQVIST, *Z. physiol. Chem. 240* (1936) 10.
Marsh soil	O. BAUDISCH and H. v. EULER, *Arch. Chem. Mineral. Geol. Ser.* A 11, No. 21, *Chem. Centr. 1935*, II, 1390.
Mycobacterium phlei	M. A. INGRAHAM and H. STEENBOCK, *Biochem. J. 29* (1935) 2553.
Nitella opaca	I. M. HEILBRON, E. G. PARRY and R. F. PHIPERS, *Biochem. J. 29* (1935) 1376.
Oedogonium	do.
Peat	R. C. JOHNSON and R. TIESSEN, *Chem. Centr. 1934*, I, 2686.
Rana esculenta	L. ZECHMEISTER and P. TUZSON, *Z. physiol. Chem. 238* (1936) 197.
Rhodymenia palmata	I. M. HEILBRON, E. G. PARRY and A. F. PHIPERS, *Biochem. J. 29* (1935) 1376.
Serum (cattle)	A. E. GILLAM and M. S. EL RIDI, *Biochem. J. 29* (1935) 2465.

This summary of the occurrence of xanthophyll, though incomplete, indicates the extraordinarily wide distribution of this phytoxanthin.

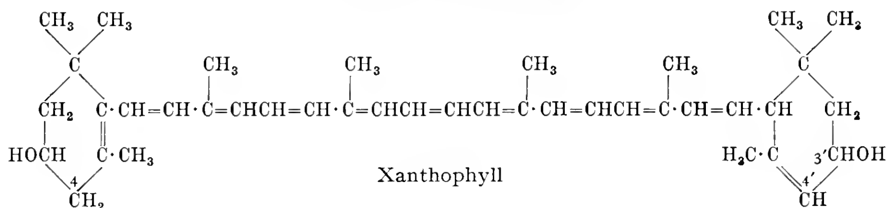
Preparation[100]

6 kg of finely ground, dry stinging nettles are extracted with 80% methanol in completely filled bottles. The extraction is then continued with peroxide-free ether. The combined extracts are shaken with water, saponified with methanolic

References p. 214–217.

potassium hydroxide and washed free from alkali. The solvent is removed by distillation in a stream of carbon dioxide until the volume is reduced to 300 ml; on cooling the solution, part of the xanthophyll crystallises out. Further quantities of pigment can be obtained from the mother liquors after further concentration and addition of petroleum ether. The remaining mother liquors are finally taken up in methanol and diluted with water under petroleum ether, when the remainder of the pigment is precipitated. The total yield amounts to up to 6 g. The preparations obtained in this way differ widely with regard to rotation and m.p. The pigment can be purified by repeated crystallisation from methanol or chromatography on zinc carbonate from benzene solution. For other methods of preparation cf. KUHN, WINTERSTEIN and LEDERER[101], MILLER[102] and ZECHMEISTER and TUZSON[103].

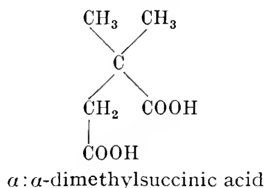
Chemical Constitution

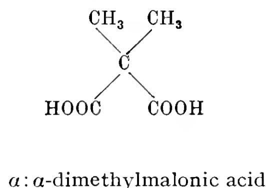


The constitution of xanthophyll was elucidated mainly by KARRER and co-workers[104]. The empirical formula of xanthophyll was determined by WILLSTÄTTER and MIEG[105]. ZECHMEISTER and TUZSON[106] established the presence of 11 double bonds by catalytic hydrogenation. This was confirmed by PUMMERER and REBMANN[107], who showed that the pigment absorbs 11 mols of iodine chloride. The spectral properties of xanthophyll are similar to those of α-carotene, indicating the presence of similar chromophoric systems. According to KARRER, HELFENSTEIN and WEHRLI[108], the two oxygen atoms are present as hydroxyl groups, which can be quantitatively determined by the method of ZEREWITINOFF. These findings were confirmed by the preparation of a number of esters and of a monomethyl ether of xanthophyll (KARRER and co-workers[109,110]). Furthermore, KARRER, ZUBRYS and MORF[111] were able to oxidise perhydroxanthophyll to a diketone, thus proving that the two hydroxyl groups are secondary and do not below to enol groupings. The number of side-chain methyl groups was determined by means of chromic acid and permanganate oxidations[112]. KARRER and co-workers[113] obtained further evidence for the structure of xanthophyll by the potassium permanganate oxidation of the pigment, which yielded α:α-dimethylsuccinic acid and dimethylmalonic acid. No geronic acid or α:α-dimethylglutaric acid were formed and it was concluded from the result that xanthophyll differs from carotene in the structure of the two carbon rings. The two hydroxyl groups are contained in the rings and the two most likely positions are the carbon atoms 3 or 4, and 3′ or

References p. 214–217.

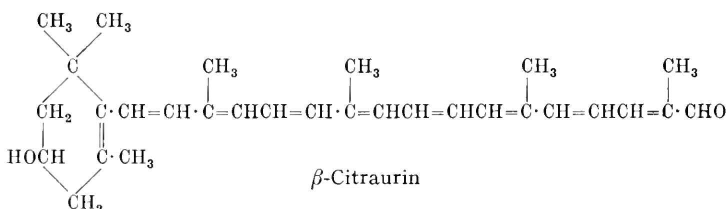
4′, since only these will explain the formation of α:α-dimethylsuccinic acid and the absence of α:α-dimethylglutaric acid on oxidation.

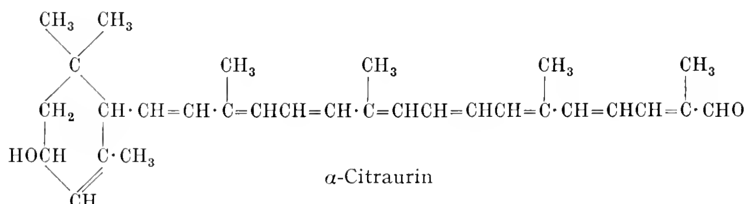

α:α-dimethylsuccinic acid


α:α-dimethylmalonic acid

The spectral properties of xanthophyll indicate a resemblance to α-carotene. NILSSON and KARRER[114] converted perhydroxanthophyll into the dibromide and then removed the two bromine atoms by reductive fission. The perhydrocarotene $C_{40}H_{78}$ formed was optically active and the rotation had the same sign and was of the same magnitude as that of the perhydrocarotene obtained by the reduction of α-carotene. Hence xanthophyll is a dihydroxy-α-carotene. These investigations also proved that the hydroxyl group must occupy position 3′ in the α-ionone ring, since a hydroxyl group on carbon atom 4′ would have enolic character.

Very recently KARRER, KOENIG and SOLMSSEN[115] succeeded in isolating α-citraurin, an isomer of β-citraurin discovered by ZECHMEISTER and TUZSON[116], by the careful potassium permanganate degradation of xanthophyll. The formula of xanthophyll was thus further confirmed.


β-Citraurin


α-Citraurin

Properties and Physical Constants

Crystalline form: The pigment crystallises from methanol in violet prisms which have a metallic lustre and characteristic dovetail shape. The crystals contain one molecule of methanol of crystallisation.

References p. 214–217.

Melting point: 193° corr.

Solubility: Xanthophyll is easily soluble in chloroform, benzene, acetone, ether and carbon disulphide, sparingly soluble in ethanol and methanol, and almost insoluble in petroleum ether. 1 g of xanthophyll dissolves in about 700 g of boiling methanol.

Spectral properties (cf. Fig. 11, p. 352)

Solvent:	*Absorption maxima*[117]		
Carbon disulphide	508	475	445 mμ
Chloroform	487	456	428 mμ
Ethanol	476	446.5	420 mμ
Petrol	477.5	447.5	420 mμ
Methanol	473.5	444	418 mμ

Solutions of xanthophyll in carbon disulphide are red. Dilute solutions in benzene, ethanol, ether and chloroform are golden yellow, while concentrated solutions in these solvents are orange in colour.

Optical activity: Xanthophyll, like α-carotene exhibits strong optical activity. The specific rotation is $[\alpha]_{Cd}^{20} = +145°$ (in ethyl acetate), $[\alpha]_{Cd}^{20} = +160°$ (in chloroform).

Colour reactions:* Xanthophyll dissolves in concentrated sulphuric acid first with a green, then with a blue colouration. Concentrated formic acid gives a bright green, trichloracetic acid a dark blue, and antimony trichloride in chloroform solution an intense dark blue colouration.

Partition test: On partition between petroleum ether and 90% methanol, xanthophyll is found in the lower layer.

Chromatographic behaviour: Xanthophyll is easily adsorbed on calcium carbonate and zinc carbonate from benzene solution. It is eluted with ether containing a few per cent of methanol.

Detection and estimation: Xanthophyll is separated from other phytoxanthins by adsorption on zinc carbonate (or calcium carbonate). It can be identified by a determination of the absorption maxima. According to KUHN and BROCKMANN[118], the colourimetric determination can be carried out using a standard solution of azobenzene in ethanol.

Physiological properties: Xanthophyll possesses no vitamin A activity. According to VON EULER, KARRER and ZUBRYS, however, a vitamin A-active product is formed on treating the phytoxanthin with phosphorous tribromide[119].

* Concerning the colour intensity of the antimony trichloride reaction, cf. H. v. EULER, P. KARRER and M. RYDBOM, *Ber. 62* (1929) 2445.

Derivatives

Perhydroxanthophyll $C_{40}H_{78}O_2$: This compound is obtained by the catalytic reduction of xanthophyll. It is a viscous, colourless oil which is weakly dextrarotatory[120] $[\alpha]_D = +28°$ in chloroform. Perhydroxanthophyll is much more soluble in organic solvents than xanthophyll, and can be esterified to give an oily diacetate[121].

Xanthophyll halogenides: On treating xanthophyll with bromine, an oxygen-free bromide $C_{40}H_{40}Br_{22}$ is formed[122]. The action of bromine dissolved in chloroform is milder and only 8 mols are absorbed[123]. Using iodine chloride, ten double bonds are saturated after 24 hours, but the eleventh double bond is only saturated after 7 days[124].

Xanthophyll diiodide $C_{40}H_{56}O_2I_2$ is a well-defined, beautifully crystalline compound, the analysis of which confirms the molecular weight of xanthophyll[125]. Xanthophyll is liberated unchanged from the iodide by means of sodium thiosulphate[126].

Synthetic esters of xanthophyll[127]: The two hydroxyl groups in xanthophyll can be esterified with acid anhydrides or acid chlorides in pyridine solution. The spectral properties of xanthophyll and its esters are largely identical, but in the partition test the esters show the opposite behaviour from xanthophyll and are found quantitatively in the upper layer. The melting points of the esters generally decrease with increasing length of the acid chain. The esters are easily soluble in benzene and petroleum ether, but very sparingly in alcohols.

a) Xanthophyll diacetate $C_{44}H_{60}O_4$: Crystallises from a mixture of benzene and methanol. M.p. 170°.

b) Dipropionate $C_{46}H_{64}O_4$: The dipropionate crystallises from a mixture of benzene and methanol in reddish-yellow plates, m.p. 138°.

c) Dibutyrate $C_{48}H_{68}O_4$: Reddish-yellow plates from methanol. M.p. 156°.

d) Di-n-valerate $C_{50}H_{72}O_4$: The ester crystallises from a mixture of benzene and methanol in reddish-yellow plates, m.p. 128°.

e. Di-*n*-caproate $C_{52}H_{76}O_4$: Reddish-yellow plates from a mixture of benzene and methanol. M.p. 117°.

f) Dioenanthate $C_{54}H_{80}O_4$: Reddish-yellow plates from a mixture of benzene and methanol. M.p. 111°.

g) Dicaprylate $C_{56}H_{84}O_4$: Reddish-yellow plates from a mixture of benzene and methanol. M.p. 108°.

h) Dipalmitate, *Helenien*, $C_{72}H_{116}O_4$:

This compound was discovered by KUHN and WINTERSTEIN[128] in the blossom leaves of *Helenium autumnale*. Helenien is widely distributed in the vegetable kingdom. A summary of plants containing helenien is given by KUHN and WINTERSTEIN[128].

References p. 214–217.

Helenien can be obtained synthetically from xanthophyll and palmitic acid chloride[129]. It crystallises from ethanol in red needles, m.p. 92°. With regard to the colourimetric estimation, see KUHN and BROCKMANN[130].

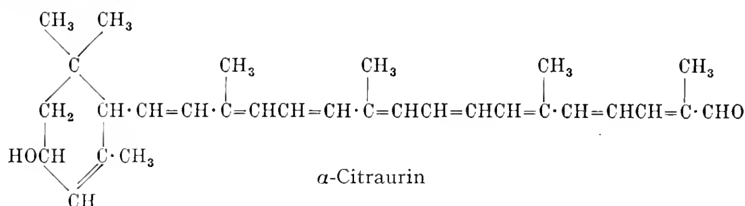
i) Distearate $C_{76}H_{124}O_4$: Red plates which appear yellow under the microscope. M.p. 87°[131].

k) Dibenzoate $C_{54}H_{64}O_4$: Red plates from ethanol. M.p. about 165°[131].

l) Bis-*p*-nitrobenzoate $C_{54}H_{62}O_8N_2$: This compound separates from benzene as a red micro-crystalline powder, m.p. 210°[131].

Xanthophyll monomethyl ether $C_{41}H_{58}O_2$[132]: The ether is obtained from the potassium derivative of xanthophyll (prepared by the interaction of xanthophyll and potassium *tert.*-amylate) and methyl iodide. It crystallises from methanol in needles, m.p. 150°. On partition between petroleum ether and 90% methanol the pigment exhibits predominately epiphasic behaviour, but both layers are coloured.

α-Citraurin $C_{30}H_{40}O_2$:



α-Citraurin

α-Citraurin was obtained by KARRER, KOENIG and SOLMSSEN[133] by the careful potassium permanganate oxidation of xanthophyll. α-Citraurin is related to β-citraurin[134] in the same way as α-apo-2-carotenal to β-apo-2-carotenal.

α-Citraurin crystallises from methanol in brilliant orange plates, m.p. 153°.

Solvent	*Absorption maxima*		
Carbon disulphide	514	480	449 mμ
Petroleum ether	477	438	mμ

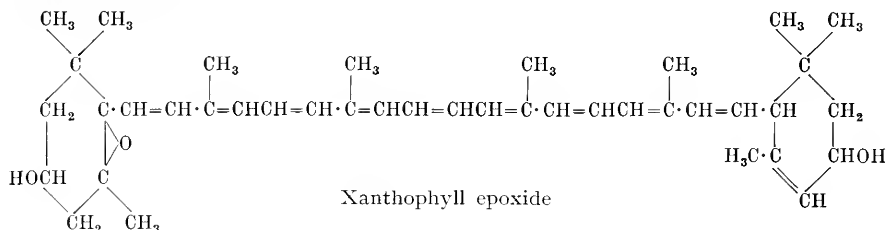
$[\alpha]_D^{18} = +372° (\pm 25°)$. (cf. Fig. 28, p. 359)

Treatment of α-citraurin with hydroxylamine acetate affords the oxime, which crystallises from methanol in clustered needles, m.p. 148°.

Solvent	*Absorption maxima*	
Carbon disulphide	499	468 mμ
Ethanol	470	440 mμ

9. XANTHOPHYLL EPOXIDE AND ITS TRANSFORMATION PRODUCTS

Xanthophyll epoxide $C_{40}H_{56}O_3$:



Xanthophyll epoxide

Xanthophyll mono-epoxide was obtained by KARRER and JUCKER[135] by the oxidation of xanthophyll diacetate with monoperphthalic acid. It crystallises from a mixture of benzene and methanol in reddish-yellow crystals, m.p. 192° (uncorr., evacuated capillary). Recent investigations[135a] have shown that this epoxide is widely distributed in blossoms and also occurs in large quantities (as much as 40% of the xanthophyll content) in leaves of all kinds. Since xanthophyll epoxide is readily isomerised to flavoxanthin (see p. 207) by traces of acids, it is probable that the latter is often an artefact.

TABLE 44

OCCURRENCE OF XANTHOPHYLL EPOXIDE

Sources	References
Asters	P. KARRER and E. JUCKER, *Helv. chim. Acta 26* (1943) 626.
Elodea canadensis	P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 28* (1945) 1526. — D. HEY, *Biochem. J. 31* (1937) 532.
Green or etiolated leaves	P. KARRER, E. KRAUSE-VOITH and K. STEINLIN, *Helv. chim. Acta 31* (1948) 113.
Kerria japonica DC	P. KARRER and E. JUCKER, *Helv. chim. Acta 29* (1946) 1539.
Laburnum anagyroides	do.
Ranunculus acer	P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta 28* (1945) 1146.
Sarothamnus scoparius	P. KARRER and E. JUCKER, *Helv. chim. Acta 27* (1944) 1585.
Stinging nettles	P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta 28* (1945) 1146.
Tragopogon pratensis	do.
Trollius europaeus	P. KARRER and E. JUCKER, *Helv. chim. Acta 29* (1946) 1539.

The absorption spectrum of xanthophyll epoxide is very similar to that of violaxanthin (p. 195) and the two pigments are best distinguished by treatment with chloroform containing traces of hydrochloric acid when they are converted into flavoxanthin (maxima 478, 449 mμ in carbon disulphide) and auroxanthin (maxima 454, 423 mμ in carbon disulphide), respectively.

Solvent	*Absorption maxima*	
Carbon disulphide	501.5	472 mμ
Benzene	482	453 mμ
Petroleum ether	471	442 mμ
Ethanol	473	445 mμ

On treating xanthophyll epoxide with acetic anhydride in pyridine, xanthophyll epoxide diacetate is obtained. This compound exhibits entirely epiphasic behaviour. M.p. 184–185° (uncorr., evacuated capillary).

Xanthophyll epoxide has approximately the same solubility in organic solvents as xanthophyll. It shows entirely hypophasic properties in the partition test. On treating an ethereal solution with concentrated aqueous hydrochloric acid, the latter assumes a blue colour.

Xanthophyll epoxide is extremely unstable towards acids. Even traces of hydrochloric acid, such as occur, for instance, in chloroform which has been stored for some time, convert the pigment into the two isomeric furanoid oxides, flavoxanthin and chrysanthemaxanthin. These pigments are described below (p. 207, 211).

Eloxanthin (Xanthophyll epoxide)

During an investigation of the carotenoids from the leaves of *elodea canadensis*, Hey[136] discovered a previously unknown pigment for which he proposed the term eloxanthin. The properties of eloxanthin are so similar to those of xanthophyll epoxide, that the identity of the two pigments appeared very probable. Karrer and Rutschmann[137] were in fact able to show the presence of xanthophyll epoxide in *elodea canadensis* and as no second pigment showing the properties of eloxanthin was present, the identity of the two compounds is very probable.

Natural xanthophyll epoxide (eloxanthin) is optically active, $[\alpha]^{18}_{Cd} = +225°$ in benzene. The optical activity of the partially synthetic product has not yet been determined.

10. FLAVOXANTHIN $C_{40}H_{56}O_3$

History

1932 Kuhn and Brockmann[138] isolate flavoxanthin from blossoms of *Ranunculus acer*.

1942 Karrer and Rutschmann[139] report the occurrence of this phytoxanthin in other plants and propose a provisional formula.

1945 Karrer and Jucker[140] carry out a partial synthesis of flavoxanthin which establishes the constitution of the pigment with a high degree of certainty.

References p. 214–217.

Occurrence

Flavoxanthin occurs fairly widely in plants, but only in small concentrations and not as the main pigment.

TABLE 45

OCCURRENCE OF FLAVOXANTHIN

Source	References
Dandelion	P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta* *25* (1942) 1144.
Ranunculus acer	R. KUHN and H. BROCKMANN, *Z. physiol. Chem.* *213* (1932) 192. — P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta* *28* (1945) 1146.
Sarothamnus scoparius	P. KARRER and E. JUCKER, *Helv. chim. Acta* *27* (1944) 1585.
Senecio vernalis (?)	R. KUHN and H. BROCKMANN, *Z. physiol. Chem.* *213* (1932) 192.
Ulex europaeus	K. SCHÖN, *Biochem. J.* *30* (1936) 1960.
Viola tricolor	P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta* *27* (1944) 1684.

Preparation

According to KUHN and BROCKMANN[138], the pigment is best extracted from crowfoot blossoms. The yield amounts to approximately 40 mg flavoxanthin from 1 kg of blossoms. KARRER and RUTSCHMANN[139] extracted phytoxanthin from dandelion blossoms by means of petroleum ether and obtained 80 mg of pure flavoxanthin from 1.5 kg of dry blossoms.

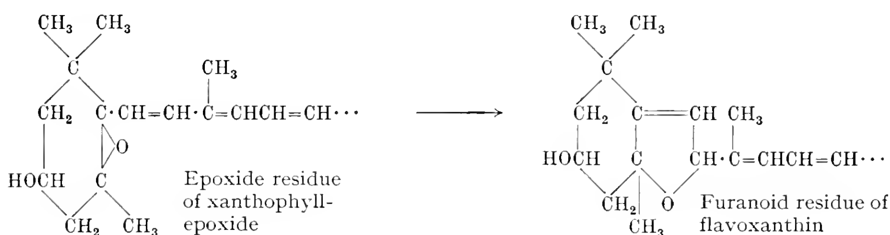
a) Flavoxanthin from *Ranunculus acer:* The dried and finely ground blossoms are extracted with methanol at room temperature and the solution is concentrated to a small volume in vacuum. The pigments are then extracted with ether and saponified with alcoholic potassium hydroxide. After partitioning into an epiphasic and hypophasic fraction the phytoxanthins are chromatographed on calcium carbonate from a mixture of benzene and petrol, and the chromatogram is developed with petrol. The flavoxanthin is eluted with petrol containing 1% of methanol and crystallised repeatedly from methanol.

b) Flavoxanthin from dandelion: The dried and finely ground dandelion blossoms are continuously extracted with petroleum ether and the pigments are saponified with methanolic hydroxide. After a separation into hypophasic and epiphasic constituents, the former are subjected to a preliminary purification by adsorption on alumina. The crystalline mixture of phytoxanthins is adsorbed on zinc carbonate, and eluted with ether containing methanol. Pure flavoxanthin is obtained by crystallisation from methanol.

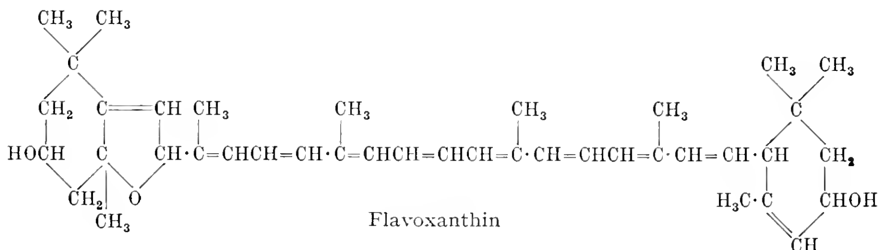
References p. 214–217.

Chemical Constitution

The first attempts to elucidate the constitution of flavoxanthin were made by KUHN and BROCKMANN[138]. These authors determined the empirical formula and the number of double bonds and reported the presence of three hydroxyl groups. According to more recent investigations[140], however, flavoxanthin contains only 2 hydroxyl groups, the third oxygen atom being present in the form of an ether grouping. The nature of this oxygen atom was only established by the investigation of KARRER and JUCKER[140] in the course of which flavoxanthin was partially synthesized. Flavoxanthin was obtained together with the isomeric chrysanthemaxanthin by the action of very dilute hydrochloric acid (in the form of aged chloroform) on xanthophyll epoxide. According to the discussion on p. 62 this transformation proceeds as follow.



Thus flavoxanthin and the isomeric chrysanthemaxanthin have the following constitution:



All the properties of the pigment are in complete agreement with this formulation. The partially synthetic product proved to be identical in all respects with natural flavoxanthin[140,141].

Properties and Physical Constants

Crystalline form: Flavoxanthin crystallises from methanol on rapid cooling in narrow, clustered prisms, which are golden yellow in colour and possess a beautiful surface lustre. M.p. 184° (corr., evacuated capillary).

Solubility: The solubilities of flavoxanthin are similar to those of xanthophyll. Flavoxanthin is easily soluble in chloroform, benzene, and acetone, more sparingly in methanol and ethanol, and almost insoluble in petroleum ether.

Spectral properties (cf. Fig. 12, p. 352):

Solvent	*Absorption maxima*	
Carbon disulphide	479	449 mμ
Chloroform	459	430 mμ
Petroleum ether	450	421 mμ
Ethanol	448	421 mμ

Optical activity: $[\alpha]_C^{20} = +190°$ (in benzene).

Partition test: Flavoxanthin is entirely hypophasic on partition between 90% methanol and petroleum ether.

Chromatographic behaviour: For the separation of flavoxanthin from other phytoxanthins, especially xanthophyll and chrysanthemaxanthin, zinc carbonate is a particularly suitable adsorbent[140]. A good separation of the pigments can also be achieved by adsorption on calcium carbonate. Benzene is employed as solvent, and ether containing a little methanol is used as eluent. Flavoxanthin is found below violaxanthin, but above chrysanthemaxanthin, on the chromatogram column. Xanthophyll is found in the lowest part of the column.

Colour reactions:

Concentrated sulphuric acid	deep blue
Trichloracetic acid	blue
Antimony trichloride in chloroform	blue
Anhydrous formic acid	green
Picric acid in ether	green
Concentrated aqueous hydrochloric acid	blue (not very stable, cf. violaxanthin)

Identification and estimation: Flavoxanthin is separated from other phytoxanthins by adsorption on zinc carbonate. Prolonged washing is required for the separation from chrysanthemaxanthin which may also be present. The pigment is identified by the determination of the absorption maxima and by the colour reaction with hydrochloric acid.

Physiological behaviour: As would be expected from its structure, flavoxanthin has no vitamin A activity.

Derivative

Flavoxanthin diacetate $C_{44}H_{60}O_5$[142]: The diacetate crystallises from methanol in brilliant orange red leaflets, m.p. 157° (uncorr., evacuated capillary).

References p. 214–217.

11. CHRYSANTHEMAXANTHIN $C_{40}H_{56}O_3$

History

1943 KARRER and JUCKER isolate chrysanthemaxanthin from red and yellow blossoms of asters[143].

1944 The new phytoxanthin is found in blossoms of *Sarothamnus scoparius* and investigated in more detail[144].

1945 KARRER and JUCKER prepare chrysanthemaxanthin by a partial synthesis and thus establish its constitution[145].

Occurrence

Up to the present time, chrysanthemaxanthin has not been frequently found in nature. It is always accompanied by xanthophyll and usually by flavoxanthin. This fact allows certain conclusions to be drawn concerning its mode of formation in the plant (cf. the original communication by KARRER and JUCKER[145]).

TABLE 46

OCCURRENCE OF CHRYSANTHEMAXANTHIN IN BLOSSOMS

Asters	P. KARRER and E. JUCKER, *Helv. chim. Acta 26* (1943) 626.
Gorse	P. KARRER and E. JUCKER, *Helv. chim. Acta 27* (1944) 1585.
Ranunculus acer	P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta 28* (1945) 1146.

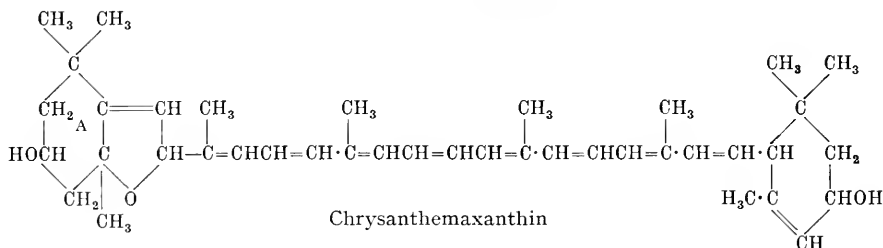
Preparation

The extraction of the pigment meets with some difficulty. The supply of large quantities of asters is costly, and the yield of chrysanthemaxanthin is small[146]. Larger quantities of this phytoxanthin can be obtained from gorse blossoms, but the picking of the blossoms must be done very carefully and their content of chrysanthemaxanthin depends on the season[147]. It is therefore preferable to prepare chrysanthemaxanthin synthetically from xanthophyll[148].

For this purpose xanthophyll is converted into xanthophyll epoxide by the action of a dilute ethereal solution of monoperphthalic acid. By treatment with dilute hydrochloric acid, the epoxide is converted into a mixture of flavoxanthin, chrysanthemaxanthin and a little xanthophyll. The separation and purification of the pigments can be achieved by adsorption on zinc carbonate from benzene solution, followed by crystallisation of the phytoxanthins from a mixture of benzene and methanol.

References p. 214–217.

Chemical Constitution



Chrysanthemaxanthin

Chrysanthemaxanthin has the same empirical formula as flavoxanthin[149]. It is identical with the latter in many respects, with the exception of the melting point, the reaction with concentrated aqueous hydrochloric acid and the strength of adsorption on zinc carbonate. On the basis of these facts and its mode of formation together with flavoxanthin by the action of dilute acids on xanthophyll epoxide, KARRER and JUCKER[148] assigned the above formula to chrysanthemaxanthin. The formula is in complete accord with all the properties of the pigment.

The isomerism of flavoxanthin and chrysanthemaxanthin is presumably steric in origin. Structural isomerism is unlikely as the two pigments have identical absorption spectra. The isomerism may be due to the fact that the hydroxyl and ether groups in ring A are in *cis*-positions in one pigment and in *trans*-positions in the other. The question of this isomerism requires further investigation.

Properties and Physical Constants

Crystalline form: Chrysanthemaxanthin crystallises from a mixture of benzene and methanol in golden yellow leaflets.

Melting point: 184–185° (uncorr., evacuated capillary).

Solubility: Chrysanthemaxanthin exhibits the same solubilities as flavoxanthin. It is readily soluble in chloroform, benzene, acetone and ether, a little more sparingly in ethanol and methanol, and almost insoluble in petroleum ether.

Spectral properties:

Solvent	*Absorption maxima*	
Carbon disulphide	479	449 mμ
Chloroform	459	430 mμ
Petroleum ether	450	421 mμ
Ethanol	448	421 mμ

References p. 214–217.

Optical activity: Natural and partially synthetic chrysanthemaxanthin have the same optical activity[150]. $[\alpha]_C^{20} = +180\text{–}190°$ (in benzene).

Partition test: Chrysanthemaxanthin is entirely hypophasic in character.

Chromatographic behaviour: Chrysanthemaxanthin is easily adsorbed on zinc carbonate (or calcium carbonate) from benzene solution. It is found below flavoxanthin but above xanthophyll epoxide on the chromatogram.

Colour reactions: With concentrated sulphuric acid, chrysanthemaxanthin gives a dark blue colouration. In contrast to flavoxanthin, concentrated hydrochloric acid produces no blue colouration.

Detection and estimation: Chrysanthemaxanthin is separated from other phytoxanthins by adsorption on zinc carbonate and can be identified by the determination of the absorption maxima and by the lack of colour reaction with hydrochloric acid.

Physiological behaviour: Chrysanthemaxanthin exhibits no vitamin A activity.

12. LYCOPHYLL $C_{40}H_{56}O_2$

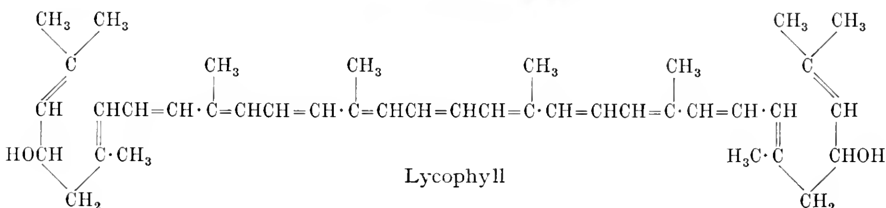
History and Occurrence

1936 ZECHMEISTER and VON CHOLNOKY isolate lycophyll from *Solanum dulcamara* and also establish the occurrence of the new phytoxanthin in *Solanum esculentum*[151].

Preparation

17 Kg of fresh berries of *Solanum dulcamara* were dehydrated with ethanol and extracted at room temperature with peroxide-free ether. After removal of the solvent by distillation, the residue was dissolved in benzene and the mixture of pigments was adsorbed on calcium hydroxide. The adsorption was repeated several times and the pigment was finally crystallised from a mixture of benzene and methanol. The yield was 9 mg of lycophyll.

Chemical Constitution



Lycophyll

References p. 214–217.

ZECHMEISTER and VON CHOLNOKY assigned the above formula to lycophyll, but this formula could not be proved because of lack of material. The molecular formula $C_{40}H_{56}O_2$ and the purely hypophasic behaviour of the pigment suggest that it is a dihydroxy-compound. The location of the absorption maxima, which are identical with those of lycopene, indicate the presence of 13 double bonds. For these reasons lycophyll is probably 4:4'-dihydroxylycopene. The presence of two hydroxyl groups is confirmed by the preparation of lycophyll dipalmitate[15].

Properties

Lycophyll crystallises from a mixture of benzene and methanol in violet leaflets, or from benzene and petroleum ether in violet-red needles, m.p. 179° (corr.). It is readily soluble in carbon disulphide, less soluble in benzene and ethanol, and only very sparingly soluble in petroleum ether. On partition between methanol and petroleum ether, it is found quantitatively in the lower layer. Lycophyll is adsorbed somewhat more strongly than lycoxanthin on calcium hydroxide from benzene solution.

Solvent	*Absorption maxima*		
Carbon disulphide	546	506	472 mμ
Benzene	521	487	456 mμ
Petrol	504	473	444 mμ
Ethanol	505	474	444 mμ

Lycophyll dipalmitate: This compound crystallises from a mixture of benzene and methanol in violet-red needles ,m.p. 76° (corr.). It shows purely epiphasic behaviour and is easily soluble in carbon disulphide and benzene, less easily in petroleum ether and almost insoluble in ethanol. The light absorption properties of the dipalmitate in the visible region are identical with those of lycophyll.

REFERENCES

1. L. ZECHMEISTER and L. v. CHOLNOKY, *Ber. 69* (1936) 422.
2. P. KARRER and co-workers, *Helv. chim. Acta 13* (1930) 268, 1084; *14* (1931) 614, 843.
3. R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 339, 1133.
4. R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 339, 1133.
5. R. YAMAMOTO and S. TIN, *Sci. Pap. Inst. physic. chem. Res. 20* (1933) 411; *Chem. Centr. 1933*, I, 3090.
6. R. KUHN and C. GRUNDMANN, *Ber. 66* (1933) 1746.
7. P. KARRER and W. SCHLIENTZ, *Helv. chim. Acta 17* (1934) 55.
8. R. KUHN and C. GRUNDMANN, *Ber. 66* (1933) 1746.
9. R. KUHN and C. GRUNDMANN, *Ber. 66* (1933) 1746.
10. R. YAMAMOTO and Y. KATO, *Chem. Centr. 1934*, II, 2993.
11. P. KARRER and W. SCHLIENTZ, *Helv. chim. Acta 17* (1934) 55.
12. R. KUHN and C. GRUNDMANN, *Ber. 66* (1933) 1746.
13. P. KARRER and E. JUCKER, *Helv. chim. Acta 29* (1946) 229.
14. H. v. EULER, P. KARRER and E. JUCKER, *Helv. chim. Acta 30* (1947) 1159.
15. P. KARRER and E. JUCKER, *Helv. chim. Acta 29* (1946) 229.

16. H. v. EULER, P. KARRER, E. JUCKER, *Helv. chim. Acta 30* (1947) 1159.
17. L. ZECHMEISTER and R. M. LEMMON, *J. Am. Chem. Soc. 66* (1944) 317.
18. P. KARRER, H. SALOMON and H. WEHRLI, *Helv. chim. Acta 12* (1929) 790. — P. KARRER H. WEHRLI and H. HELFENSTEIN, *Helv. chim. Acta 13* (1930) 268.
19. P. KARRER and co-workers, *Helv. chim. Acta 14* (1931) 619; *15* (1932) 492; *Arch. Sci. biol. 18* (1936) 36.
20. P. KARRER and co-workers, *Helv. chim. Acta 12* (1929) 791; *13* (1930) 268.
21. R. KUHN and co-workers, *Helv. chim. Acta 12* (1929) 499; *Naturwissenschaften 18* (1930) 418; *Ber. 63* (1930) 1489.
22. L. ZECHMEISTER and co-workers, *Z. physiol. Chem. 190* (1930) 67; *196* (1931) 199.
23. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 481* (1930) 42.
24. P. KARRER and H. WEHRLI, *Helv. chim. Acta 13* (1930) 1104.
25. R. KUHN and A. WINTERSTEIN and W. KAUFMANN, *Ber. 63* (1930) 1489.
26. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 18* (1935) 477.
27. P. KARRER and E. JUCKER, *Helv. chim. Acta 30* (1947) 266.
28. P. KARRER and co-workers, *Helv. chim. Acta 13* (1930) 268; *Helv. chim. Acta 14* (1931) 614; *15* (1932) 492.
29. P. KARRER, *Arch. Scienze Biol. 18* (1933) 30.
30. R. KUHN and A. WINTERSTEIN, *Ber. 65* (1932) 1873; *Ber. 66* (1933) 429.
31. P. KARRER and co-workers, *Helv. chim. Acta 13* (1930) 1095.
32. R. KUHN and co-workers, *Ber. 63* (1930) 1496.
33. P. KARRER and co-workers, *Helv. chim. Acta 21* (1938) 448.
34. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 18* (1935) 479.
35. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 21* (1938) 448.
36. R. KUHN and A. SMAKULA, *Z. physiol. Chem. 197* (1931) 161.
37. K. W. HAUSSER and A. SMAKULA, *Z. angew. Chem. 47* (1934) 663; *48* (1935) 152.
38. K. W. HAUSSER, *Z. tech. Phys. 15* (1934) 13.
39. L. ZECHMEISTER and co-workers, *Ber. 72* (1939) 1678.
40. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 43.
41. H. v. EULER, P. KARRER and A. ZUBRYS, *Helv. chim. Acta 17* (1934) 24.
42. H. v. EULER, P. KARRER, E. KLUSSMANN and R. MORF, *Helv. chim. Acta 15* (1930) 502.
43. R. KUHN, A. WINTERSTEIN and W. KAUFMANN, *Ber. 63* (1930) 1496.
44. P. KARRER and co-workers, *Helv. chim. Acta 15* (1932) 490.
45. P. KARRER and T. TAKAHASHI, *Helv. chim. Acta 16* (1933) 1163.
46. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 18* (1935) 479.
47. P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta 15* (1932) 1195.
48. R. KUHN ,A. WINTERSTEIN and L. KAUFMANN, *Ber. 63* (1930) 1497.
49. P. KARRER and W. SCHLIENTZ, *Helc. chim. Acta 17* (1934) 55.
50. R. KUHN and W. WIEGAND, *Helv. chim. Acta 12* (1929) 499.
51. L. ZECHMEISTER and L. v. CHOLNOKY, *Z. physiol. Chem. 189* (1930) 159; *Ann. 481* (1930) 42.
52. R. KUHN and co-workers, *Naturwissenschaften 18* (1930) 418; *Ber. 63* (1930) 1489.
53. R. KUHN, A. WINTERSTEIN and W. KAUFMANN, *Ber. 63* (1930) 1489.
54. R. KUHN and W. WIEGAND, *Helv. chim. Acta 12* (1929) 499.
55. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 481* (1930) 42.
56. R. KUHN and H. BROCKMANN, *Ber. 67* (1934) 596.
57. K. W. HAUSSER and A. SMAKULA, *Z. Angew. Chem. 47* (1934) 663; *48* (1935) 152.
58. R. KUHN and K. MEYER, *Z. physiol. Chem. 185* (1929) 193. — L. ZECHMEISTER, *Carotinoide*, Julius Springer, Berlin 1934.
59. R. KUHN and W. WIEGAND, *Helv. chim. Acta 12* (1929) 499.
60. P. KARRER, U. SOLMSSEN and O. WALKER, *Helv. chim. Acta 17* (1934) 417.
61. P. KARRER and W. GUGELMANN, *Helv. chim. Acta 20* (1937) 405.
62. L. ZECHMEISTER and L. v. CHOLNOKY, *Z. physiol. Chem. 189* (1930) 159; ***Ann. 481*** (1930) 42.
63. R. KUHN and co-workers, *Ber. 63* (1930) 1489.

64. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 61.
65. L. ZECHMEISTER and co-workers, *Biochem. J. 32* (1938) 1305; *Ber. 72* (1939) 1340, 1678; *J. Am. Chem. Soc. 66* (1944) 317.
66. L. ZECHMEISTER and R. M. LEMMON, *J. Am. Chem. Soc. 66* (1944) 317.
67. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
68. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 27* (1944) 1684.
69. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
70. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 27* (1944) 1684. — P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
71. P. KARRER and A. OSWALD, *Helv. chim. Acta 18* (1935) 1303.
72. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
72*a*. G. TAPPI and P. KARRER, *Helv. Chim. Acta 32* (1949) 50.
73. R. KUHN and A. WINTERSTEIN, *Ber. 64* (1931) 326.
74. P. KARRER and co-workers, *Helv. chim. Acta 14* (1931) 1044; *16* (1933) 977; *19* (1936) 1024; *27* (1944) 1684.
75. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
76. R. KUHN and A. WINTERSTEIN, *Ber. 64* (1931) 326.
77. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
78. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
79. R. KUHN and A. WINTERSTEIN, *Ber. 64* (1931) 326.
80. P. KARRER and R. MORF, *Helv. chim. Acta 14* (1931) 1045.
81. P. KARRER and co-workers, *Helv. chim. Acta 19* (1936) 1024; *Helv. chim. Acta 27* (1944) 1684.
82. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 27* (1944) 1684.
83. K. W. HAUSSER and A. SMAKULA, *Z. angew. Chem. 47* (1934) 663; *48* (1935) 152; K. W. HAUSSER, *Z. techn. Phys. 15* (1934) 13.
84. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 27* (1944) 1684. — P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
85. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 19* (1936) 1024. — R. KUHN and A. WINTERSTEIN, *Ber. 64* (1931) 332.
86. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 27* (1944) 1684.
87. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 25* (1942) 1624.
88. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 25* (1942) 1624.
89. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
90. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 25* (1942) 1624; *27* (1944) 320.
91. P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta 27* (1944) 1684.
92. P. KARRER and E. JUCKER, *Helv. chim. Acta 28* (1945) 300.
93. P. KARRER, E. JUCKER and J. RUTSCHMANN, *Helv. chim. Acta 28* (1945) 1156.
94. J. J. BERZELIUS, *Ann. 21* (1837) 261.
95. R. WILLSTÄTTER and W. MIEG, *Ann. 355* (1907) 1.
96. R. WILLSTÄTTER and H. H. ESCHER, *Z. physiol. Chem. 76* (1912) 214.
97. P. KARRER and co-workers, *Helv. chim. Acta 13* (1930) 268, 1094; *14* (1931) 614, 843; *16* (1933) 977.
98. R. WILLSTÄTTER and A. STOLL, *Untersuchungen über Chlorophyll*, Julius Springer, Berlin 1913; P. KARRER and co-workers, *Helv. chim. Acta 14* (1931) 614; R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 41; R. KUHN and A. WINTERSTEIN, *Naturwissenschaften 18* (1930) 754.
99. H. JUNGE, *Z. physiol. Chem. 268* (1941) 179.
100. R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem. 197* (1931) 153. — P. KARRER and co-workers, *Helv. chim. Acta 14* (1931) 625.
101. R. KUHN, A. WINTERSTEIN and E. LEDERER, *Z. physiol. Chem. 197* (1931) 153.
102. E. S. MILLER, *Chem. Centr. 1935*, I, 3545.
103. L. ZECHMEISTER and P. TUZSON, *Ber. 63* (1930) 3203; *Ber. 67* (1934) 170.
104. P. KARRER and co-workers, *Helv. chim. Acta 13* (1930) 268, 1084; *14* (1931) 614, 843; *16* (1933) 977.

105. R. Willstätter and W. Mieg, *Ann. 355* (1907) 1.
106. L. Zechmeister and P. Tuzson, *Ber. 61* (1928) 2003.
107. R. Pummerer and L. Rebmann, *Ber. 61* (1928) 1099.
108. P. Karrer, A. Helfenstein and H. Wehrli, *Helv. chim. Acta 13* (1930) 87.
109. P. Karrer and co-workers, *Helv. chim. Acta 13* (1930) 709, 1099, 1102.
110. P. Karrer and co-workers, *Helv. chim. Acta 13* (1930) 1103.
111. P. Karrer, A. Zubrys and R. Morf, *Helv. chim. Acta 16* (1933) 977.
112. P. Karrer and co-workers, *Helv. chim. Acta 14* (1931) 631.
113. P. Karrer and co-workers, *Helv. chim. Acta 13* (1930) 270, 1094.
114. R. Nilsson, and P. Karrer, *Helv. chim. Acta 14* (1931) 843.
115. P. Karrer, H. Koenig and U. Solmssen, *Helv. chim. Acta 21* (1938) 445.
116. L. Zechmeister and P. Tuzson, *Ber. 69* (1936) 1878.
117. For quantitative extinctions measurements see R. Kuhn and A. Smakula, *Z. physiol. Chem. 197* (1931) 163; K. W. Hausser and A. Smakula, *Z. angew. Chem. 47* (1934) 663; *48* (1935) 152; K. W. Hausser, *Z. Techn. Phys. 15* (1934) 13. For X-ray diffration pattern see G. Mackinney, *J. Am. Chem. Soc. 56* (1934) 488.
118. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 43.
119. H. v. Euler, P. Karrer and A. Zubrys, *Helv. chim. Acta 17* (1934) 24.
120. L. Zechmeister and P. Tuzson, *Ber. 61* (1928) 2003; *Ber. 62* (1929) 2226.
121. P. Karrer and S. Ishikawa, *Helv. chim. Acta 13* (1930) 709, 1099.
122. R. Willstätter and H. H. Escher, *Z. physiol. Chem. 64* (1910) 47. — H. Escher, *Dissertation*, 1909 Zürich.
123. L. Zechmeister and P. Tuzson, *Ber. 62* (1929) 2226.
124. R. Pummerer, L. Rebmann and W. Reindel, *Ber. 62* (1929) 1411.
125. R. Willstätter and W. Mieg, *Ann. 355* (1907) 1.
126. P. Karrer and O. Walker, *Helv. chim. Acta 17* (1934) 43.
127. P. Karrer and S. Ishikawa, *Helv. chim. Acta 13* (1930) 709, 1099.
128. R. Kuhn and A. Winterstein, *Naturwissenschaften 18* (1930) 754. — R. Kuhn, E. Lederer, A. Winterstein, *Z. physiol. Chem. 197* (1931) 147, 150.
129. P. Karrer and S. Ishikawa, *Helv. chim. Acta 13* (1930) 1099.
130. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 43.
131. P. Karrer and S. Ishikawa, *Helv. chim. Acta 13* (1930) 713.
132. P. Karrer and B. Jirgensons, *Helv. chim. Acta 13* (1930) 1103.
133. P. Karrer, H. Koenig and U. Solmssen, *Helv. chim. Acta 21* (1938) 445.
134. L. Zechmeister and P. Tuzson, *Ber. 69* (1936) 1878. — P. Karrer and co-workers, *Helv. chim. Acta 20* (1937) 682, 1020.
135. P. Karrer and E. Jucker, *Helv. chim. Acta 28* (1945) 300.
135*a*. P. Karrer, E. Krause-Voith and K. Steinlin, *Helv. chim. Acta 31* (1948) 113.
136. D. Hey, *Biochem. J. 31* (1937) 532.
137. P. Karrer and J. Rutschmann, *Helv. chim. Acta 28* (1945) 1526.
138. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 213* (1932) 192.
139. P. Karrer and J. Rutschmann, *Helv. chim. Acta 25* (1942) 1144.
140. P. Karrer and E. Jucker, *Helv. chim. Acta 28* (1945) 300.
141. P. Karrer, E. Jucker and J. Rutschmann, *Helv. chim. Acta 28* (1945) 1156.
142. P. Karrer and J. Rutschmann, *Helv. chim. Acta 25* (1942) 1144.
143. P. Karrer and E. Jucker, *Helv. chim. Acta 26* (1943) 626.
144. P. Karrer and E. Jucker, *Helv. chim. Acta 27* (1944) 1585.
145. P. Karrer and E. Jucker, *Helv. chim. Acta 28* (1945) 300.
146. P. Karrer and E. Jucker, *Helv. chim. Acta 26* (1943) 626.
147. P. Karrer and E. Jucker, *Helv. chim. Acta 27* (1944) 1585.
148. P. Karrer and E. Jucker, *Helv. chim. Acta 28* (1945) 300.
149. P. Karrer and E. Jucker, *Helv. chim. Acta 27* (1944) 1585.
150. P. Karrer, E. Jucker and J. Rutschmann, *Helv. chim. Acta 28* (1945) 1156.
151. L. Zechmeister and L. v. Cholnoky, *Ber. 69* (1936) 422.

CHAPTER XII

Carotenoids of known or largely known structure containing one or more carbonyl groups

1. β-CITRAURIN $C_{30}H_{40}O_2$

History and Occurrence

In the course of investigations of carotenoids from orange peel (*Citrus aurantium*), ZECHMEISTER and TUZSON[1] discovered a previously unknown pigment for which they proposed the term citraurin*. Citraurin occurs in oranges together with carotene, cryptoxanthin, zeaxanthin, xanthophyll and violaxanthin. It has not, so far, been isolated from any other source.

Preparation[1]

Orange peels are dehydrated with ethanol and extracted with peroxide-free ether. After removal of the solvent in a partial vacuum, a red oily residue remains. In order to separate the pigments from the oily constituents, this residue is dissolved in petrol and chromatographed on calcium carbonate. The oily components, together with carotene and cryptoxanthin, are removed by prolonged washing. The violet-red zone which contains β-citraurin is eluted with a mixture of ether and methanol, and the pigment esters are saponified with methanolic potassium hydroxide. After saponification is complete, the free phytoxanthins are dissolved in ether, the solution is evaporated to dryness and the residue is dissolved in warm carbon disulphide. On cooling this solution, much pigment material crystallises (violaxanthin etc.) while β-citraurin remains in solution. It is adsorbed on a calcium carbonate column which is washed with carbon disulphide. β-Citraurin forms a red zone in the chromatogram and is eluted with a mixture of ether and methanol. After removal of the solvent, the residue is dissolved in a little hot methanol, from which β-citraurin crystallises in round, reddish aggregates on addition of a little water. 35 mg of pigment were obtained from 100 kg of oranges.

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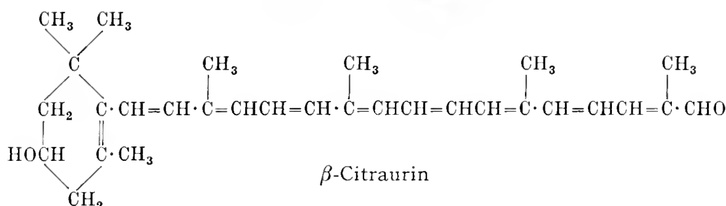
* P. KARRER and co-workers, *Helv. chim. Acta 20* (1937) 682, 1020 proposed the term β-citraurin for the pigment discovered by L. ZECHMEISTER and P. TUZSON[1] in order to avoid confusion with the isomeric α-citraurin (cf. p. 205).

References p. 253–255.

Chemical Constitution



β-Citraurin

The composition of β-citraurin ($C_{30}H_{40}O_2$), and the ease of oximation which suggests the presence of an aldehyde group, led ZECHMEISTER and TUZSON[1] to suggest that this pigment represents a degradation product of a C_{40} carotenoid. KARRER and SOLMSSEN[2] established the very close relationship between β-citraurin and β-apo-2-carotenal[3]. They proposed the formula shown above, according to which β-citraurin is a 3-hydroxy-β-apo-2-carotenal. The correctness of this formula was later established by KARRER and co-workers[4] and by ZECHMEISTER and VON CHOLNOKY[5]. The first-named authors obtained β-citraurin by the permanganate degradation of zeaxanthin (cf. p. 184), while ZECHMEISTER and VON CHOLNOKY obtained the aldehyde by the hydrolysis of capsanthin (cf. p. 248). β-Citraurin can also be prepared, though only in small amounts accompanied by a large proportion of α-citraurin, by the permanganate degradation of xanthophyll[6]. Finally, KARRER and KOENIG[7] also succeeded in obtaining the aldehyde by permanganate oxidation of capsanthin.

Properties

Crystalline form: β-Citraurin crystallises from a mixture of benzene and petrol in very thin, yellow to orange plates which appear almost colourless under the microscope.

Melting point: 147° (corr., Berl-block, short thermometer).

Solubility: β-Citraurin dissolves easily in acetone, ethanol, ether, benzene and carbon disulphide. The solubility in petrol is very small, even at the boiling point.

Spectral properties:

Solvent	*Absorption maxima*			
Carbon disulphide	525	490	457	mμ (diffuse)
Benzene	497	467		mμ
Petrol	488	459		mμ (sharp)
Hexane	487	458		mμ
Ethanol	diffuse			

Solutions of the pigment in carbon disulphide have a beautiful red colour. Ethanol solutions are red, hexane and petrol solutions straw yellow, and benzene solutions yellowish-brown.

References p. 253–255.

Colour reactions: On treating an ethereal solution of the aldehyde with concentrated aqueous hydrochloric acid, the latter assumes a blue colouration.

Partition test: β-Citraurin is hypophasic on partition between methanol and petroleum ether.

Chromatographic Properties: On chromatography from carbon disulphide, β-citraurin is adsorbed somewhat more strongly than cryptoxanthin and is found above the latter, but below zeaxanthin, on the column.

Detection and estimation: After saponification, the pigment is found in the hypophasic fraction and is separated from other phytoxanthins by means of chromatographic adsorption analysis. It forms a zone with a characteristic red (not violet) colour, which is easily distinguishable from that of any other natural carotenoid. β-Citraurin can be identified by the determination of the absorption maxima and, if necessary, by the prepartion of the oxime.

Derivatives

β-Citraurin oxime $C_{30}H_{41}O_2N$:

This compound is obtained on treating β-citraurin with free hydroxylamine[8]. The oxime crystallises from methanol in thin rods grouped into star-like formations. M.p. 188° (corr.).

Solvent	*Absorption maxima (all sharp)*	
Carbon disulphide	505	473 mμ
Benzene	487	456 mμ
Petrol	474	444 mμ
Hexane	473	443 mμ
Ethanol	476	444 mμ

The oxime is insoluble in cold petrol. The solubility in ethanol and acetone is somewhat greater.

β-Citraurin semicarbazone $C_{31}H_{43}O_2N_3$[8]:

The semicarbazone crystallises from benzene in microscopic reddish-brown leaflets, which melt over a range near 190°. The semicarbazone is easily soluble in ethanol and acetone, but sparingly soluble in petrol.

Solvent	*Absorption maxima*	
Carbon disulphide	517	483 mμ (diffuse)
Benzene	498	463 mμ (diffuse)
Hexane	485	454 mμ (sharp)
Ethanol	486	454 mμ (sharp)

References p. 253–255.

2. RHODOXANTHIN $C_{40}H_{50}O_2$

History

1893 MONTEVERDE[9] observes a new pigment in the reddish-brown leaves of *Potamogeton natans*. The same pigment is later found by TSWETT[10] in a number of conifers, and described under the name "Thujorhodin".

1913 MONTEVERDE and LUBIMENKO[11] isolate rhodoxanthin in the crystalline state. It is investigated in 1925 by PRÁT[12] and in 1926 and 1927 by LIPPMAA[13].

1933 KUHN and BROCKMANN[14] isolate rhodoxanthin from yew trees and propose a constitutional formula for the pigment.

1935 KARRER and SOLMSSEN[15] convert dihydrorhodoxanthin into zeaxanthin and thus confirm the constitution of rhodoxanthin.

Occurrence[16]

Rhodoxanthin is fairly widely distributed in nature in small quantities. It occurs in larger quantities in *Taxus baccata* L. (japonica).

TABLE 47

OCCURRENCE OF RHODOXANTHIN

Source	References
Aloe-species	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643, H. KYLIN, *Z. physiol. Chem. 163* (1927) 229. — H. MOLISCH, *Ber. bot. Ges. 20* (1902) 442.
Bulbina annua	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643.
Buxus	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643.
Chamaecyparis	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643.
Cryptomeria japonica Don.	M. TSWETT, *Compt. rend. 152* (1911) 788.
Cypressus Naitnocki	do.
Encephalartos Hildebrandtii	M. W. LUBIMENKO, *Rev. gén. Bot. 25* (1914) 475; *Compt. rend. 158* (1914) 510.
Equisetum-species	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643. — S. PRÁT, *Biochem. Z. 152* (1924) 495.
Gasteria	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643.
Gnetum-species	N. A. MONTEVERDE and V. N. LUBIMENKO, *Bull. Acad. Sci. Petrograd* [6] *7* (1913) 1105.
Haworthia	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643.
Juniperus virginiana L.	M. TSWETT, *Compt. rend. 152* (1911) 788.
Potamogeton natans	N. A. MONTEVERDE, *Acta Horti Petropol. 13* (1893) 201.
Reseda odorata	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643.
Retinospora plumosa	M. TSWETT, *Compt. rend. 152* (1911) 788.
Scirpus	T. LIPPMAA, *Ber. bot. Ges. 44* (1926) 643.

References p. 253–255.

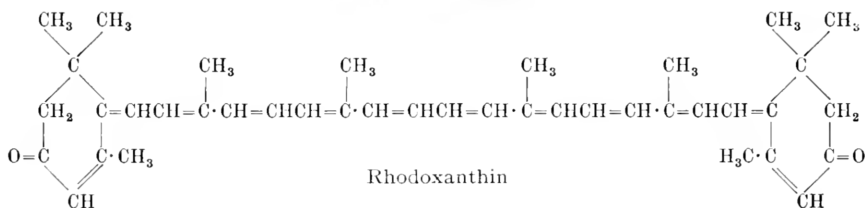
Source	References
Selaginella	N. A. MONTEVERDE and V. N. LUBIMENKO, *Bull. Acad. Sci. Petrograd* [6] *7* (1913) 1105. — T. LIPPMAA, *Ber. bot. Ges.* *44* (1916) 643.
Taxus baccata	R. KUHN and H. BROCKMANN, *Ber. 66* (1933) 828. M. TSWETT, *Compt. rend. 152* (1911) 788.
Thuja orientalis L.	M. TSWETT, *Compt. rend. 152* (1911) 788.

Preparation[17]

The ripe japonica fruit are mashed to a fine pulp and extracted with methanol in portions of 10 kg. The first two extracts are usually colourless to light red, and contain hardly any pigment, while the following methanol extracts are deep red in colour. The residue is then extracted with petroleum ether (b.p. 70–80°). The combined methanol extracts are diluted with water, and the pigments extracted with petrol. This petrol solution is combined with the petrol extract. Considerable purification of the pigment is achieved by repeated partitioning between methanol and petroleum ether. The last petrol solution is concentrated in vacuum almost to dryness. On cooling, rhodoxanthin crystallises. It is boiled first with a little methanol and then with petroleum ether. By this procedure about 70 mg of crude pigment are obtained from 10 kg of japonica fruit.

Rhodoxanthin is purified by recrystallisation from a mixture of one part of benzene and four parts of methanol, or by slowly evaporating an ethanol solution.

Chemical Constitution



Rhodoxanthin

The constitution of rhodoxanthin was elucidated by KUHN and BROCKMANN[17]. Elementary analysis gave the molecular formula $C_{40}H_{50}O_2$. On catalytic hydrogenation the pigment first rapidly absorbs 12 mols of hydrogen; a further 2 mols are taken up on prolonged hydrogenation. This behaviour indicates the presence of 12 double bonds and two carbonyl groups. The presence of the latter was confirmed by the preparation of a dioxime, but whereas polyene aldehydes (e.g. lycopenal) react readily with hydroxylamine, rhodoxanthin only reacts with difficulty. For this reason, KUHN and BROCKMANN concluded that two ketone groups are present in the pigment molecule, conjugated with the system of conjugated double bonds. This conclusion is in accord with the long-wavelength location of the absorption bands.

By means of chromic acid oxidation, KUHN and BROCKMANN[17] established the presence of six side-chain methyl groups.

References p. 253–255.

ZEREWITINOFF determinations indicated the presence of one hydroxyl group, but this result must be due to partial enolisation of the ketone, since no acetyl derivative could be obtained by the action of acetic anhydride in pyridine. (This latter result could be due to the presence of a tertiary hydroxyl group, but this would not be in accord with the general properties of the pigment).

The constitution of rhodoxanthin has been confirmed by investigations of KARRER and SOLMSSEN[18] in the course of which the pigment was converted into zeaxanthin via its dihydro-derivative, and the relationship between the two pigments was thus established. These investigations have already been described on p. 182.

Properties

Crystalline form: Rhodoxanthin crystallises from a mixture of benzene and methanol (1:4) in dark violet needles, combined in rosettes. From aqueous pyridine or ethanol it is obtained in thin, finely branched rods. If an ethanolic solution of rhodoxanthin is allowed to evaporate slowly, the pigment crystallises in well-formed leaflets.

Melting point: 219° (corr., evacuated capillary).

Solubility: The pigment is very easily soluble in pyridine, easily soluble in benzene and chloroform, very sparingly soluble in ethanol and methanol, and insoluble in petrol, hexane, and petroleum ether.

Spectral properties:

Solvent	*Absorption maxima*		
Carbon disulphide	564	525	491 mμ
Chloroform	546	510	482 mμ
Benzene	542	503,5	474 mμ
Ethanol	538	496	(very diffuse)
Petrol	524	489	458 mμ
Petroleum ether	521	487	456 mμ
Hexane	524	489	458 mμ

(cf. Fig. 13 and 15, p. 353 and 354)

Solutions of the pigment in petrol are yellow-red, solutions in methanol wine-red. The same difference was observed in the case of capsanthin. ZECHMEISTER and POLGÁR[19] ascribe this phenomenon to the polar nature of the alcohol.

Colour reactions: Rhodoxanthin dissolves in concentrated sulphuric acid with a deep blue colour. On treating a solution of the pigment in chloroform with antimony trichloride, an intense blue-violet colouration is observed. On shaking an ethereal solution of rhodoxanthin with 25% hydrochloric acid,

the latter is coloured a faint red-violet. With more concentrated acid the colouration is somewhat more intense.

Partition test: On partitioning between petroleum ether and 90 % methanol, rhodoxanthin colours both layers.

Chromatographic behaviour: In contrast to phytoxanthins, rhodoxanthin is not adsorbed on calcium carbonate. It is well adsorbed on alumina from a mixture of benzene and petrol. It forms a deep violet zone in the chromatogram and can be eluted with a mixture of petrol and methanol.

Detection and estimation. After separation from other carotenoids by means of chromatographic analysis on alumina, rhodoxanthin can be identified by its absorption spectrum.

Chemical behaviour: Rhodoxanthin is relatively stable towards atmospheric oxygen.

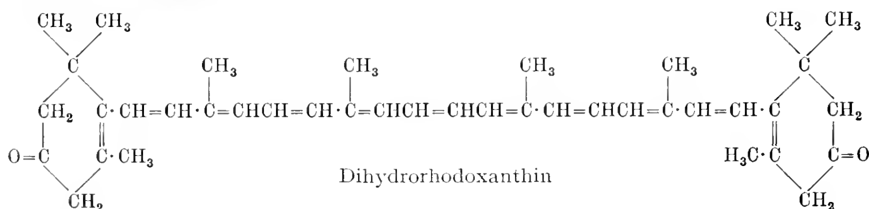
Derivatives

Rhodoxanthin dioxime $C_{40}H_{52}O_2N_2$:

The dioxime is prepared by boiling a solution of rhodoxanthin in a little pyridine with a solution of 6 mols of hydroxylamine containing some sodium hydroxide[17]. The dioxime crystallises from a mixture of pyridine and petrol in red squares, m.p. 227–228° (corr., evacuated capillary). Rhodoxanthin dioxime is less easily soluble in petrol and benzene, but more soluble in ethanol than rhodoxanthin. In contrast to the latter, it can be adsorbed on calcium carbonate from petrol. On alumina it is adsorbed so strongly that it cannot be eluted.

Solvent	*Absorption maxima*		
Carbon disulphide	516	483	453 mμ
Chloroform	527	490	457 mμ
Benzene	527	490	457 mμ
Ethanol	516	483	454 mμ
Petrol	516	483	453 mμ
Hexane	513	479	451 mμ
Petroleum ether	510	477	450 mμ

Dihydrorhodoxanthin $C_{40}H_{52}O_2$:



Dihydrorhodoxanthin

References p. 253–255.

This compound was prepared by KUHN and BROCKMANN[17] by the reduction of a solution of rhodoxanthin in pyridine and glacial acetic acid with zinc. Dihydrorhodoxanthin crystallises from a mixture of benzene and methanol in golden yellow leaflets, m.p. 219° (corr., evacuated capillary). Its solubility is similar to that of rhodoxanthin. The chromophoric system of dihydrorhodoxanthin is the same as that of β-carotene and zeaxanthin, as shown by the similarity of the absorption maxima.

Solvent	*Absorption maxima*					
	Dihydrorhodoxanthin			*Zeaxanthin*		
Carbon disulphide . . .	514	479	448 mμ	517	482	459 mμ
Chloroform	492	460	431 mμ	495	462	429 mμ
Petrol	483	452	425 mμ	483.5	451.5	423 mμ
Ethanol	480	450	422 mμ	483	451	423 mμ
Hexane (cf. Fig. 13, p. 353)	480	449	422 mμ			

Dihydrorhodoxanthin is optically inactive. In solution (e.g. in piperidine or pyridine containing a small proportion of alcoholic potassium hydroxide) it is rapidly dehydrogenated by atmospheric oxygen to rhodoxanthin. On catalytic hydrogenation in decalin, the pigment absorbs 13 mols of hydrogen. By the reduction of dihydrorhodoxanthin KARRER and SOLMSSEN (cf. p. 60, 183) obtained zeaxanthin.

Dihydrorhodoxanthin dioxime $C_{40}H_{54}O_2N_2$:

This derivative is obtained by a procedure analogous to that described for the preparation of rhodoxanthin dioxime (p. 224). The dioxime crystallises from ethanol in reddish-yellow needles, m.p. 226–227° (corr., in vacuum).

Solvent	*Absorption maxima*		
Carbon disulphide	514	479	448 mμ
Petrol	483	451.5	424 mμ
Hexane	480	449	422 mμ

3. MYXOXANTHIN $C_{40}H_{54}O$

History and Occurrence

HEILBRON, LYTHGOE and PHIPERS[20] found a previously unknown epiphasic carotenoid, which they termed myxoxanthin in the algae *Rivularia nitida*. This pigment was later observed in *Oscillatoria rubescens*[21] and in *Calothrix scopulorum*(?)[22].

Preparation

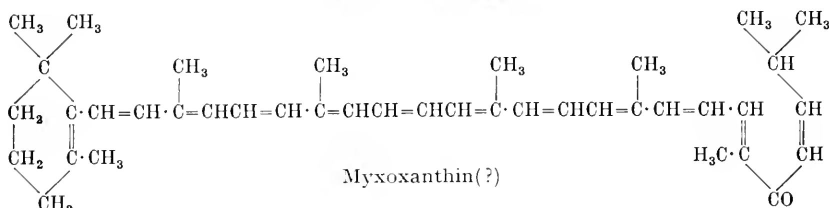
From *Oscillatoria rubescens*[21]: The algae are dehydrated with methanol and then extracted first with methanol and then with ether. The combined extracts are

References p. 253–255.

concentrated under reduced pressure in nitrogen, and the pigments are saponified with aqueous potassium hydroxide and then separated into epiphasic and hypophasic fractions. Myxoxanthin is obtained from the epiphase and myxoxanthophyll can be extracted from the hypophase (p. 228).

The epiphasic petroleum ether extract is washed free from alkali, dried, and allowed to stand for a short time during which a considerable quantity of β-carotene separates. The mother liquors are evaporated to dryness in vacuum, the residue is chromatographed on alumina, and the pigment of the central zone of the chromatogram is chromatographed again from alumina. The myxoxanthin is then dissolved in a mixture of ether and methanol, the solution is concentrated, and colourless impurities are separated out by cooling. On further concentration and cooling, the mother liquors yield myxoxanthin. For further purification, the pigment is repeatedly crystallised from a mixture of pyridine and methanol.

Chemical Constitution



Myxoxanthin(?)

The constitution of myxoxanthin has not yet been fully elucidated. However, the investigations of HEILBRON and LYTHGOE[21] and KARRER and RUTSCHMANN[23] make the above formula very probable.

Elementary analysis gave the empirical formula $C_{40}H_{54}O$. On microhydrogenation, myxoxanthin absorbed 12 mols of hydrogen, while myxoxanthin oxime requires 13 mols of hydrogen for saturation. This shows that the free pigment contains 12 double bonds and one carbonyl group. The latter can be reduced by means of aluminium isopropoxide and isopropylalcohol to give a secondary alcohol, myxoxanthol. This agrees in its spectral properties with γ-carotene and rubixanthin and must therefore contain the same, or a very similar, chromophoric system. Since myxoxanthin exhibits vitamin A activity, it must further contain an unsubstituted β-ionone ring. For reasons of analogy (e.g. astacene), it is assumed that the carbonyl group is cross-conjugated with the system of conjugated double bonds. (Carotenoid ketones which contain a ketone group attached terminally to the unsaturated system generally exhibit an absorption spectrum with 3 bands, whereas astacene and myxoxanthin exhibit only one band).

Properties

Myxoxanthin crystallises from a mixture of pyridine and methanol in deep violet prisms, m.p. 168–169°. The pigment is easily soluble in a mixture of chloroform and ether, but only sparingly soluble in chloroform alone. On

References p. 253–255.

partition between methanol and petroleum ether it is entirely epiphasic. Myxoxanthin is adsorbed on calcium hydroxide and magnesium hydroxide, but not on calcium carbonate, from petroleum ether solution.

Spectral properties:

Solvent	*Absorption maxima*
Carbon disulphide	488 mμ
Chloroform	473 mμ
Ethanol	470 mμ
Petroleum ether	465 mμ

Colour reactions: On adding concentrated sulphuric acid to a chloroform solution of myxoxanthin, the latter is coloured deep blue. Concentrated hydrochloric acid produces no colour change in chloroform. Addition of hydrochloric acid to an ethereal solution produces a greenish-blue colouration.

Myxoxanthin oxime $C_{40}H_{55}ON$:

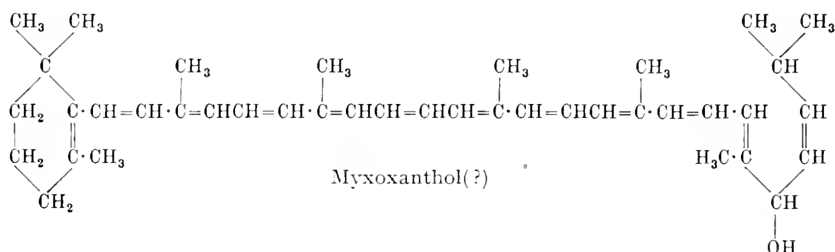
Brilliant, cinnabar-red plates, m.p. 195–196°.

Solvent	*Absorption maxima*
Chloroform	463 mμ

Myxoxanthol $C_{40}H_{56}O$:

Deep red crystals, m.p. 169–172°.

Solvent	*Absorption maxima*		
Carbon disulphide	529	494	464 mμ
Chloroform	508	474	441 mμ
Petroleum ether	495	465	431 mμ



Myxoxanthol(?)

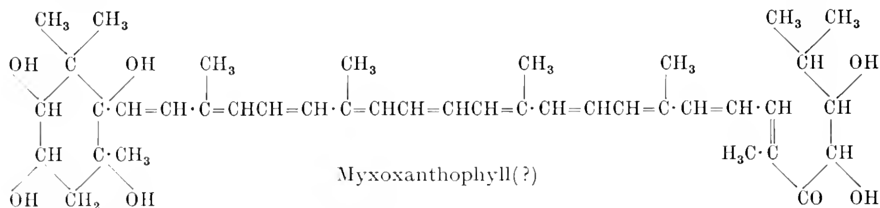
4. MYXOXANTHOPHYLL $C_{40}H_{56}O_7$

In the course of investigations on *Oscillatoria rubescens*, HEILBRON and LYTHGOE[21] discovered a new hypophasic pigment for which they proposed the name myxoxanthophyll. The pigment occurs together with myxoxanthin, xanthophyll and β-carotene. In recent times myxoxanthophyll has been investigated by KARRER and RUTSCHMANN[24].

References p. 253–255.

For the isolation of the pigment the hypophasic portion of oscillatoria-extracts (cf. p. 225) are used. The pigment is dissolved in ether, the solution is washed free from alkali, and dried over sodium sulphate, and the solvent is distilled off in vacuum. The deep red, resinuous residue is dissolved in chloroform, and chromatographed on calcium carbonate. After elution and removal of the solvent the myxoxanthophyll is dissolved in pyridine. Colourless impurities are removed by freezing and the mother liquors are then diluted with petroleum ether, when the pigment crystallises on cooling.

The structure of myxoxanthophyll has been investigated mainly by KARRER and RUTSCHMANN[24]. The molecular formula of the pigment is $C_{40}H_{56}O_7$[21, 24]. On microhydrogenation, 10 mols of hydrogen are absorbed rapidly and an additional mol of hydrogen more slowly[24], indicating the presence of 10 double bonds and one carbonyl group. Of the remaining six oxygen atoms, four are present as secondary hydroxyl groups, since myxoxanthophyll forms a tetraacetate. The latter still contains two free hydroxyl groups, as shown by ZEREWITINOFF determinations, but these cannot be esterified and are therefore probably tertiary in character[24]. It should be remarked that the presence of a carbonyl group has not been definitely established, although the behaviour of the pigment and the long-wavelength absorption renders it very probable. KARRER and RUTSCHMANN[24] tentatively proposed the following formula for myxoxanthophyll, which is in agreement with the properties of the pigment:



Myxoxanthophyll(?)

It should be emphasised, however, that this constitution is by no means certain.

Myxoxanthophyll crystallises from acetone in violet needles, m.p. 182° (uncorr., in vacuum)*. According to HEILBRON and LYTHGOE, the pigment is laevo-rotatory, $[\alpha]_{Cd} = -255°$ (in ethanol). The pigment is readily soluble in pyridine and ethanol, more sparingly soluble in chloroform and acetone, and insoluble in petroleum ether, ether and benzene. Concentrated sulphuric acid produces a deep blue colouration in a chloroform solution of the pigment. No colouration is produced by concentrated hydrochloric acid.

Solvent	*Absorption maxima*		
Pyridine	526	489	458 mμ
Chloroform	518	484	454 mμ
Ethanol	503	471	445 mμ

* I. M. HEILBRON and B. LYTHGOE, *J. Chem. Soc. 1936*, 1376 record m.p. 169–170° C; but the determination was not carried out in vacuum and the sample was probably less pure.

References p. 253–255.

Myxoxanthophyll tetraacetate $C_{48}H_{64}O_{11}$:

The ester is prepared by treating myxoxanthophyll with acetic anhydride in pyridine. It crystallises from methanol in lustrous violet leaflets, m.p. 131–132°. Myxoxanthophyll tetraacetate is entirely hypophasic on partition between methanol and petroleum ether.

Solvent	*Absorption maxima*		
Carbon disulphide	544	508	479 mμ

KARRER and RUTSCHMANN[24] also prepared myxoxanthophyll benzoate, but the compound was not obtained pure because of shortage of material.

5. ASTACENE $C_{40}H_{48}O_4$ AND ASTAXANTHIN $C_{40}H_{52}O_4$

Introduction

The pigments of *crustacea* have long aroused the interest of chemists and zoologists[25]. It is only in recent years, however, that it has been possible to obtain some indication of the nature of these pigments (cf. p. 234), and that the investigations regarding the chemical constitution of these compounds has reached a certain degree of finality. The first pigment of this type was isolated in 1933 in the form of astacene from lobster shell[26]. Astacene was later recognised as a β-carotene tetraketone by KARRER and co-workers[27]. Some years later, KUHN and SÖRENSEN[28] found that the pigment from lobster eggs known as "ovoester" was not an ester of astacene but an entirely new pigment[26]. It was termed astaxanthin, and it was shown that it is readily converted into astacene in alkaline solution under the influence of atmospheric oxygen. It therefore appeared possible that astacene is an artefact formed by the alkaline saponification of astaxanthin esters and that astaxanthin is the natural pigment[28]. This supposition has been proved in a number of cases while in others the necessary experiments have not yet been carried out. It has been found that astaxanthin occurs fairly frequently in the animal organism[29] and also in plants[30].

The close relationship of the two carotenoids and the facile conversion of astaxanthin into astacene under the influence of atmospheric oxygen in alkaline solution are established without doubt. The two pigments will therefore be dealt with together in this chapter.

History

1933 KUHN and LEDERER[31] isolate crystalline astacene from lobster shell. In the animal, the pigment is partly combined with protein and partly esterified.

References p. 253–255.

1934–36 KARRER and co-workers[27] carry out a detailed investigation of the new pigment and establish its constitution.

1938 KUHN and SÖRENSEN[28] show that the eggs of the lobster do not contain esterified astacene ("ovoester"), but a new pigment, astaxanthin. They establish the constitution of astaxanthin and its close relationship to astacene.

Occurrence

It was originally assumed that astacene and astaxanthin are typical animal pigments. More recent investigations show, however, that these pigments are also present in plant organisms[32].

Since the isolation of astacene from lobster (shell, hypodermis and eggs[31]), numerous other sources of this carotenoid and of astaxanthin have been found. The pigment occurs either esterified or combined with protein. The protein adduct appears to be ionic in nature, with the protein as the positively charged component (cf. p. 235).

TABLE 48

OCCURRENCE OF ASTAXANTHIN OR ASTACENE* [32]

Source	References
I. Green algae	
Haematococcus pluvialis+	R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber.* *72* (1939) 1688. — J. TISCHER, *Z. physiol. Chem.* *250* (1937) 147.
II. Protozoa:	
Euglena heliorubescens+	J. TISCHER, *Z. physiol. Chem.* *239* (1936) 257; *267* (1941) 281.
III. Spongiaria:	
Axinella crista-galli	P. KARRER and U. SOLMSSEN, *Helv. chim. Acta* *18* (1935) 915.
IV. Crustacea:	
a) *Malacostraca:*	
1) *Schizopoda Euphausia*	J. C. DRUMMOND and R. MCWALTER, *J. exper. Biol.* *12* (1934) 105.
2) *Decapoda:*	
Astacus gammarus+, Shell, hypodermis, eggs	R. KUHN and N. A. SÖRENSEN, *Ber.* *71* (1938) 1879.
Cancer pagurus	R. FABRE and E. LEDERER, *Bull. Soc. Chim. Biol.* *16* (1934) 105.

* Sources from which astaxanthin was isolated are marked+. In other cases it is not known for certain whether astacene is the natural pigment or a transformation product of astaxanthin.

Source	References
Eupagurus Prideauxii	E. LEDERER, *Bull. Soc. Chim. Biol. 20* (1938) 554, 567, 611.
Leander serratus	R. FABRE and E. LEDERER, *Bull. Soc. Chim. Biol. 16* (1934) 105.
Maja squinado, eggs	R. KUHN, E. LEDERER and A. DEUTSCH, *Z. physiol. Chem. 220* (1933) 229.
Nephrops-species	R. FABRE and E. LEDERER, *Bull. Soc. Chim. Biol. 16* (1934) 105.
Palinurus vulgaris	R. FABRE and E. LEDERER, *Bull. Soc. Chim. Biol. 16* (1934) 105.
Portunus puber	R. FABRE and E. LEDERER, *Bull. Soc. Chim. Biol. 16* (1934) 105.
Potamobius astacus	e.g. H. WILLSTAEDT, *Svensk Kem. Tidskr. 46* (1934) 205, 261.
b) *Copepoda:*	
Calanus finmarchianus+	H. v. EULER, H. HELLSTRÖM and E. KLUSSMANN, *Z. physiol. Chem. 228* (1934) 77. — E. LEDERER, *Bull. Soc. Chim. Biol. 20* (1938) 554, 567, 611.
Heterocope saliens	N. A. SÖRENSEN, *Kgl. Norske, Vid. Selsk. Skr. 1936* No. 1.
c) *Phyllopoda:*	
Holopedium gibberum	do.
d) *Arthrostaca:*	
Gammarus pulex+	do.
V. Mollusca *(Lamellibranchiata):*	
Lima excavata	N. A. SÖRENSEN, *Kgl. Norske Vid. Selsk. Skr. 1936* No. 1.
VI. Echinoderma:	
Ophidiaster ophidianus	P. KARRER and F. BENZ, *Helv. chim. Acta 17* (1934) 412.
Echinaster sepositus	P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 18* (1935) 915.
VII. Tunicata *(Ascidiacea):*	
Dendrodoa grossularia+	E. LEDERER, *Bull. Soc. Chim. Biol. 20* (1938) 554, 567, 611.
Halocynthia papillosa+	do.
VIII. Fish:	
Beryx decadactylus, skin	E. LEDERER, *Compt. rend. Soc. Biol. 118* (1935) 542.
Carassius auratus, skin	E. LEDERER, *Compt. rend. Soc. Biol. 118* (1935) 542.

Source	References
Cyclopterus lumpus, liver	N. A. SÖRENSEN, *Z. physiol. Chem. 235* (1935) 8.
Lophius piscatorius, liver	N. A. SÖRENSEN, *Tidskr. Kjemi Bergves* 1935, 12.
Perca fluviatilis, fins	E. LEDERER, *Bull. Soc. Biol. 20* (1938) 554, 567, 611.
Regalecus glesne+ liver	N. A. SÖRENSEN, *Tidskr. Kjemi Bergves 1935*, 12. — R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber. 72* (1939) 1688.
Salmo salar, muscle	N. A. SÖRENSEN, *Z. physiol. Chem. 235* (1935) 8.
Salmo trutta+, muscle	N. A. SÖRENSEN and J. STENE, *Kgl. Norske Vid. Selsk. Skr. 1938*, No. 9.
Sebastes marinus, skin	E. LEDERER, *Bull. Soc. Biol. 20* (1938) 554, 567, 611.
IX. Reptiles:	
Clemmys insculpata, retina	G. WALD and H. ZUSSMAN, *J. biol. Chem. 122* (1938) 449.
X. Birds:	
Chicken, retina+	R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber. 72* (1939) 1688. —. G. WALD and H. ZUSSMAN, *J. biol. Chem. 122* (1938) 449.
Phasianus colchicus, 'Rosen'+	R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber. 72* (1939) 1688. — H. BROCKMANN and O. VÖLKER, *Z. physiol. Chem. 224* (1934) 193.
XI. Mammals:	
Balaenoptera musculus, Fat	S. SCHMIDT-NIELSEN, N. A. SÖRENSEN and B. TRUMPY, *Kgl. Norske Vid. Selsk. Skr. 5* (1932) 118. — G. N. BURKHARDT, I. M. HEILBRON, H. JACKSON, E. G. PARRY and I. A. LOVERN, *Biochem. J. 28* (1934) 1698.

The occurrence of astaxanthin in the green algae *Haematococcus pluvialis*[33] is of especial interest as it disproves the view that astaxanthin is a purely animal carotenoid.

As early as 1936, TISCHER discovered a pigment in the flagellate *Euglena heliorubescens*[33], which he termed euglenarhodon. Later he also found this pigment in the green algae *Haematococcus pluvialis*[34]. Subsequent investigations by KUHN, STENE and SÖRENSEN[35] showed that euglenarhodon from *Haematococcus pluvialis* is identical with astacene. A reinvestigation of the pigment from *Euglena heliorubescens* by TISCHER[36] led to the same result.

KUHN and his collaborators have recently isolated astaxanthin, as well as β-carotene, from the eggs of rainbow trout, *Salmo irideus*, and have shown that astaxanthin is the chemotactic principle responsible for attracting the trout sperms.

References p. 253–255.

TABLE 49

MODE OF OCCURRENCE OF ASTAXANTHIN IN NATURE*

Mode of Occurrence	Source
Epiphasic ester	*Astacus gammarus*, hypodermis
	**Beryx decadactylus*, skin
	**Carassius auratus*, skin
	Chicken retina
	Euglena heliorubescens
	Haematococcus pluvialis
	**Perca fluviatilis*, fins
	Phasianus colchicus 'Rosen'
	**Sebastes marinus*, skin
Free pigment	**Balaenoptera musculus*, fat
	Calanus finmarchicus (partly esterified)
	Maja squinado, eggs
	Phasianus colchicus
	Regalecus glesne, liver
	Salmo irideus, eggs
	Salmo trutta
Chromoproteid	
blue-black	*Astacus gammarus*, shell
green	*Astacus gammarus*, eggs (ovoverdin)
blue	**Heterocope saliens*
olive-brown or blue	*Gammarus pulex*
violet red	*Dendrodoa grossularia*
olive-brown	**Lophius piscatorius*

* In the cases marked *, only astacene was isolated. It is not yet known for certain whether astacene is the natural pigment or whether it is formed from astaxanthin during the process of isolation.

Preparation of Astacene from Lobster Shells[31]

The shells of freshly killed animals are covered with 2N hydrochloric acid and left to stand until they have turned red. They are then washed with water and the hypodermis are separated. The pigment is then extracted with acetone at room temperature, and transferred into petroleum ether by dilution of the extract with water. The petroleum ether solution is washed with water and with 90% methanol, diluted with 2N sodium hydroxide and sufficient ethanol to produce a homogeneous solution, and left to stand in the dark at room temperature for 5 hours. After this period, sufficient water is added to produce two layers. The ethanolic layer is separated, covered with a little petrol, and the astacene is precipitated by careful acidification with acetic acid. The pigment is washed with hot water, dissolved in a small amount of highly purified pyridine and crystallised by addition of a little water. From 29 animals (12.8 kg live weight), the yield of thrice recrystallised astacene was 0.265 g.

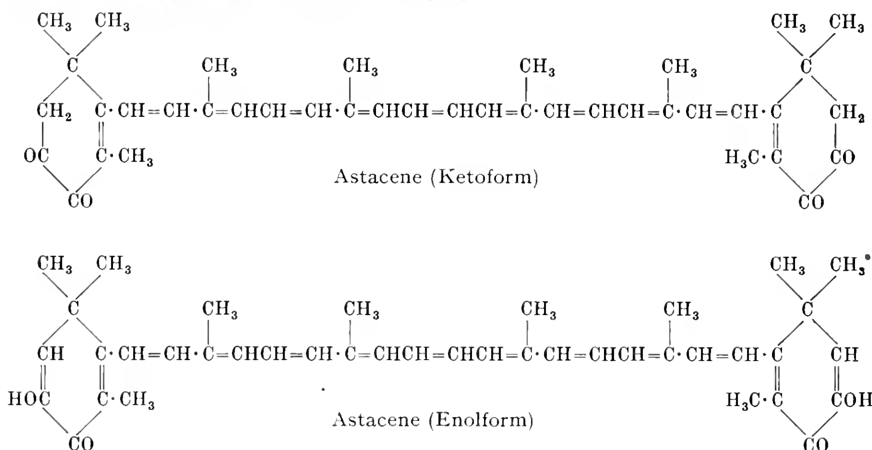
Preparation of Astaxanthin from Lobster Eggs[37]

2.5 Kg of lobster eggs are crushed in a porcelain mortar, with the addition of acetone and solid carbon dioxide. The crushed mass is filtered and repeatedly

References p. 253–255.

extracted on the filter with strongly cooled acetone. The deep red extract is covered with a fifth of its volume of petroleum ether, and diluted carefully with one volume of distilled water, when most of the pigment separates in beautifully glistening plates. After filtering, the petroleum ether solution is extracted with 90 % methanol, the methanol solution is covered with freshly distilled benzene, and a further quantity of astaxanthin is precipitated by careful addition of water. The two fractions are crystallised together from a mixture of pyridine and water[31]. 750 mg of pure pigment are thus obtained. Two other methods of preparation of the two pigments are described by KARRER and co-workers[38].

Chemical Constitution of Astacene $C_{40}H_{48}O_4$:

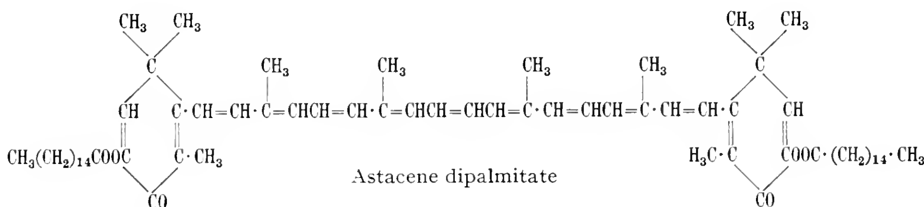


The formula for astacene (3:3′:4:4′ tetraketo-β-carotene) was proposed by KARRER[39]. Elementary analysis of the compound itself and of the dioxime gave the molecular formula $C_{40}H_{48}O_4$ which differs from that of β-carotene only by the presence of 8 fewer hydrogen atoms and 4 additional oxygen atoms. From the molecular formula and the nature of the oxygen atoms, KARRER and co-workers concluded that the constitution of the pigment must be that of a tetraketo-β-carotene. Astacene forms a dioxime which contains 4 active hydrogen atoms. Two of these are derived from the oxime residues, while the other two must be due to the enolisation of the other two carbonyl groups. The four keto groups therefore differ in nature, two being capable of forming an oxime, and the other two undergoing enolisation. Astacene gives a bis-phenazine derivative with *o*-phenylene diamine, which shows that each pair of carbonyl groups must be adjacent. By the oxidation of astacene with permanganate, KARRER and co-workers obtained dimethylmalonic acid. On oxidation of the bis-phenazine derivative, α:α-dimethylsuccinic acid was also isolated, thus establishing the presence of the 4 carbonyl groups in the 3:3′:4:4′-positions. This is in accord with the finding of WILLSTAEDT that astacene can be reduced

by zinc dust and acetic acid in pyridine solution to give a light yellow derivative[40].

Microhydrogenation of astacene showed the presence of 13 double bonds, two of which are formed by enolisation. Free astacene is only slightly enolised as shown by the slow etherification with diazomethane and by ZEREWITINOFF determinations. The weakly acidic nature of the pigment disappears on hydrogenation, as would be expected.

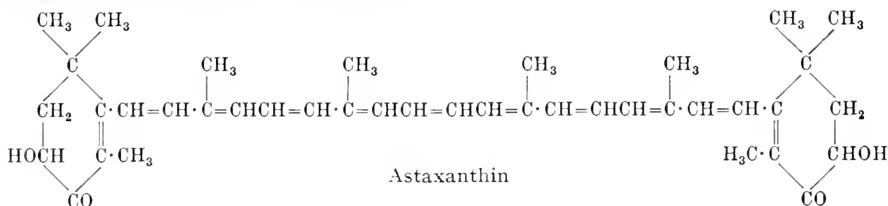
KARRER, LOEWE and HÜBNER[41] investigated the epiphasic astacene ester from lobster and described it as astacene dipalmitate.



Astacene dipalmitate

It is probable that the compound is actually the dipalmitic acid ester of astaxanthin.

Chemical Constitution of Astaxanthin $C_{40}H_{52}O_4$:

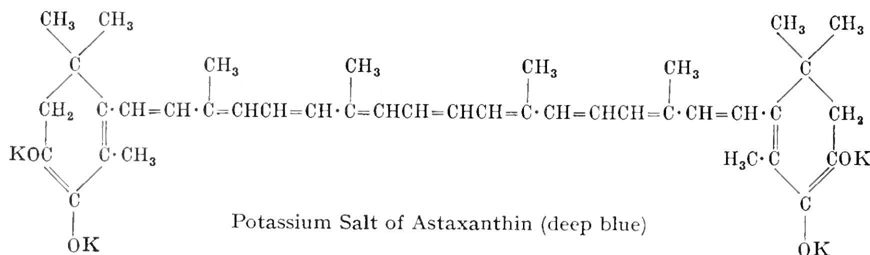


Astaxanthin

The isolation of a crystalline "ovoester" from the eggs of lobster was described by KUHN and LEDERER[31]. It was later shown by KUHN and SÖRENSEN that the "ovoester" is not an ester but a free phytoxanthin. It was named astaxanthin and has the molecular formula $C_{40}H_{52}O_4$[42]. By analogy with the formula of astacene established by KARRER and co-workers, KUHN and SÖRENSEN[42] assigned the structure of a 3:3′-dihydroxy-4:4′-diketo-β-carotene to astaxanthin*. This formulation is based on the fact that, in the absence of air, astaxanthin forms a deep blue salt in potassium hydroxide solution, while under aerobic conditions the pigment absorbs exactly 2 mols of oxygen in alkaline solution and is converted into astacene. The conversion of astaxanthin into astacene represents the autoxidation of a di-α-ketol. In agreement with the

* The alternative structure, 4:4′-dihydroxy-3:3′-diketo-β-carotene, is excluded by the spectral properties of astaxanthin (cf. p. 237).

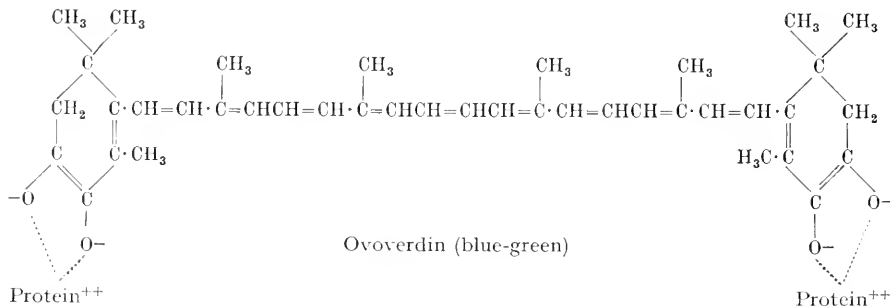
formulation of the pigment, di-esters can be prepared (cf. p. 235). According to KUHN and SÖRENSEN, the deep blue potassium salt of astaxanthin may be compared with the orange coloured potassium derivative of benzoin*, and the corresponding salts of dihydrocrocetin dimethyl ester and dihydrobixin-dimethyl ester[42], and may be formulated as follows:



Potassium Salt of Astaxanthin (deep blue)

Constitution of Ovoverdin[31]

Ovoverdin is the name given by KUHN and LEDERER[31] to the blue-green chromoproteid which is the natural pigment of the lobster shell. According to KUHN and SÖRENSEN[43], ovoverdin is an enol salt somewhat analogous to the potassium derivatives of benzoin and astaxanthin. This formulation would explain the blue-green colour of the compound.



Ovoverdin (blue-green)

It is surprising, however, that ovoverdin is not autoxidisable, in contrast to the potassium derivative of astaxanthin. This fact is explained by KUHN and SÖRENSEN by assuming that the interaction with the protein component is not simply ionic in nature, but that additional binding forces are involved which result in a relatively stable combination of the pigment and the protein in the form of a molecular complex. The nature of the additional binding forces is not specified but a complex derived from *one* protein molecule with two separate links, rather than from two independent protein molecules, appears to be implied.

* See note page 235.

References p. 253–255.

A number of investigations have been made regarding the nature of the protein component. Thus, WYCKOFF[44] determined a molecular weight of about 300.000 for ovoverdin. KUHN and SÖRENSEN[45] worked out a procedure for the purification of ovoverdin which involves the fractional adsorption of the chromoproteid on aluminium hydroxide and the fractional elution with disodium phosphate or ammonium sulphate. The molecular weight of ovoverdin purified in this manner was 144,000. According to STERN and SALOMON[46] the protein component of ovoverdin has albumin character.

Properties and Derivatives of Astacene

Crystalline form: The pigment crystallises from a mixture of pyridine and water in violet needles with a metallic lustre. Sometimes the needles are sickle-shaped.

Melting point: 240–243° (corr., in vacuum, slow heating)[31]; 241°[47], 228°[48].

Solubility: Astacene is insoluble in water, very sparingly soluble in ether, petroleum ether and methanol, sparingly soluble in benzene, ethyl acetate and acetic acid, fairly soluble in carbon disulphide, and easily soluble in chloroform, pyridine and dioxan.

Optical activity: Astacene is optically inactive.

Partition test: On partition between petroleum ether and 90% methanol, astacene is found almost entirely in the lower layer. On addition of a little more water, the pigment is easily transferred to the petroleum ether layer, but remains in the lower layer in the presence of alkali[49].

Chromatographic behaviour: Astacene is hardly adsorbed on calcium carbonate from a mixture of benzene and petrol. It is found in the top zone of the chromatogram on adsorption on alumina from the same solvent mixture. It cannot be eluted from alumina either with a mixture of benzene and methanol or a mixture of pyridine and methanol.

Spectral properties (cf. Fig. 16, p. 354):

Solvent	*Absorption maxima*
Pyridine	about 500 mμ (wide band)
Carbon disulphide	about 510 mμ[47]

Colours of solutions: Concentrated solutions of the pigment in pyridine are blood-red, dilute solutions orange-red.

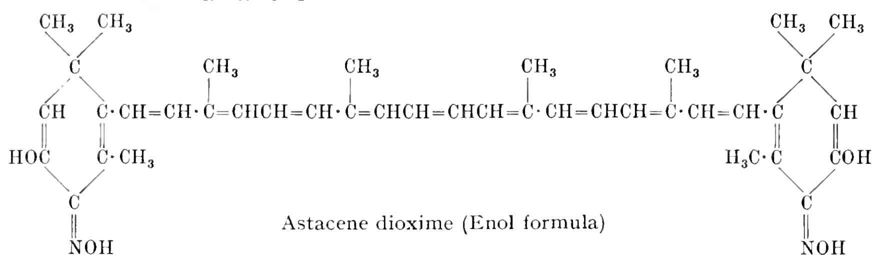
Colour reactions: Astacene dissolves in concentrated sulphuric acid with a deep blue colour. Treatment of the solution of the pigment in chloroform with antimony trichloride results in a blue-green colouration.

References p. 253–255.

Detection and estimation: Astacene differs from other carotenoids by its single-banded spectrum and its behaviour towards alkali.

Chemical behaviour: Astacene is very stable towards atmospheric oxygen. Its general chemical behaviour is characterised by the capacity of two of the carbonyl groups to enolise and of the other two carbonyl groups to undergo ketonic reactions.

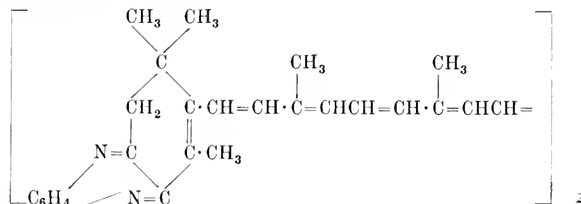
Astacene dioxime $C_{40}H_{50}O_4N_2$[50]:



Astacene dioxime (Enol formula)

The dioxime separates from ethanol in black crystals.

Bis-phenazine derivative $C_{52}H_{56}N_4$[50]:



The bis-phenazine derivative was obtained by KARRER and LOEWE[50] by warming a solution of astacene with o-phenylene diamine in glacial acetic acid for one and a half hours on a water bath. The compound separates from benzene in deep violet crystals. As it cannot enolise, it gives rise to α:α-dimethyl-succinic acid on oxidation with permanganate (cf. p. 47), m.p. 224–225°. It shows an absorption maximum in carbon disulphide at about 515 mμ[51].

Astacene diacetate $C_{44}H_{52}O_6$:

This derivative is obtained by allowing a solution of astacene and acetic anhydride in pyridine to stand for 16 hours[52]. It crystallises from a mixture of pyridine and water in black-violet crystals, m.p. 235° (uncorr., with decomposition).

Astacene dipalmitate $C_{72}H_{108}O_6$[51] (Astacein):

It was shown by KARRER and co-workers (cf. p. 235) that astacene (or astaxanthin) occur in the lobster esterified with palmitic acid. KUHN and co-

workers[51] prepared astacene dipalmitate from astacene and palmitic acid chloride. The ester crystallises from petroleum ether in almost rectangular red leaflets, m.p. 121°. Astacin dipalmitate exhibits epiphasic properties.

Properties and Derivatives of Astaxanthin

Crystalline form: Astaxanthin crystallises from pyridine in lustrous plates.

Melting point: 216° (with decomposition).

Solubility: Only few data are recorded in the literature regarding the solubility of astaxanthin. The pigment is readily soluble in pyridine, from which it can be crystallised on addition of water.

Spectral properties (cf. Fig. 15, p. 354): In contrast to astacene, astaxanthin exhibits an absorption curve in which three definite maxima can be recognised[53]. In pyridine, these maxima are located at 476, 493 and 513 mμ[53].

Optical activity: Astaxanthin is optically inactive[54].

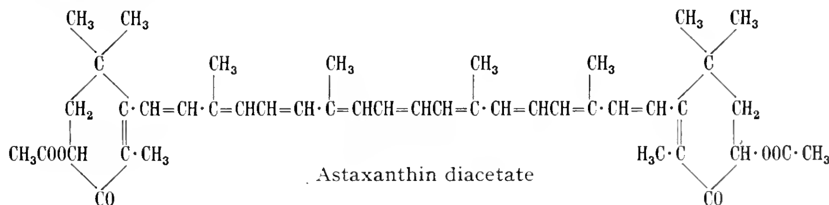
Partition test: Astaxanthin exhibits entirely hypophasic properties.

Chromatographic behaviour: KUHN and co-workers chromatographed astaxanthin on cane sugar from a mixture of benzene and petroleum ether (1:4). Benzene was employed for elution.

Colour reactions: The alkali salts of the pigment have a characteristic deep-blue colour which is only shown, however, if air is excluded. On admission of oxygen, there is an immediate colour change to red and dehydrogenation to astacene occurs.

Detection and estimation: Astaxanthin can be readily identified by the colour reactions described above.

Astaxanthin diacetate $C_{44}H_{56}O_6$[55]:



Astaxanthin diacetate

The diacetate is obtained by treating astaxanthin dissolved in pyridine with acetic anhydride. The ester crystallises from a mixture of pyridine and water in rugged, deep blue-black needles, m.p. 203–205° (Berl-Block, in vacuum, uncorr.). The ester is very little enolised in the cold. In the partition test, it is

References p. 253–255.

found in the lower layer. The absorption maxima are located at slightly shorter wavelengths than those of the parent pigment.

Astaxanthin dicaprylate $C_{56}H_{70}O_6$[55]:

This ester is prepared by the treatment of astaxanthin in pyridine with caprylic acid chloride. It can be purified by chromatography on calcium carbonate from petroleum ether solution. Astaxanthin dicaprylate crystallises from a mixture of petrol and ethanol in dark red crystals, m.p. 121–124° (not sharp, Berl-block, in vacuum). In the partition test with 90% methanol and petroleum ether, the ester is found almost quantitatively in the upper layer. The ester becomes hypophasic only on employing 97% methanol.

Astaxanthin dipalmitate $C_{72}H_{112}O_6$[56]:

This ester is prepared by a procedure analogous to that described for astaxanthin dicaprylate. It crystallises from a mixture of pyridine, methanol and water in flat, violet-red needles, m.p. 71.5–72.5°. Astaxanthin dipalmitate exhibits purely epiphasic behaviour.

Astaxanthin monopalmitate $C_{56}H_{82}O_5$[57]:

This derivative is obtained by the esterification of astaxanthin with the calculated amount of palmitic acid chloride. The ester crystallises from a mixture of benzene and methanol in red spheres, m.p. 113.5–114.5° (corr.). Astaxanthin monopalmitate is entirely epiphasic on partition between 90% methanol and petroleum ether.

Astaxanthin esters from Haematococcus pluvialis.

From *Haematococcus pluvialis*, KUHN and SÖRENSEN[58] isolated various esters of astaxanthin, the composition of which has not yet been definitely determined.

Ovoverdin and Chromoproteids.

The probable structure of ovoverdin has been described above (p. 236). It is very probable that the nature of the protein component is different in different organisms. A common characteristic of all these chromoproteids is their green or blue colour, water solubility, and great sensitivity towards heat, acids and organic solvents (with the exception of petroleum ether). The protein of the chromoproteids is coagulated by these reagents, leaving the free pigment component, astaxanthin.

6. CAPSANTHIN $C_{40}H_{58}O_3$

History

1817 BRACONNOT carries out the first investigations on the pigments of paprika[59].
1869 Thudichum recognises the close relationship of the paprika pigments to the carotenoids[60], a relationship later confirmed by PABST[61] and KOHL[62].

References p. 253–255.

1913 A number of investigators establish the spectroscopic similarity of lycopene to the paprika pigment[63].

1927 ZECHMEISTER and VON CHOLNOKY[64] succeed in obtaining the pigment of *Capsicum annuum* (paprika) in a crystalline form. They propose the name capsanthin for the new carotenoid.

1927–35 ZECHMEISTER and VON CHOLNOKY[65], and KARRER and co-workers[66] elucidate the constitution of capsanthin.

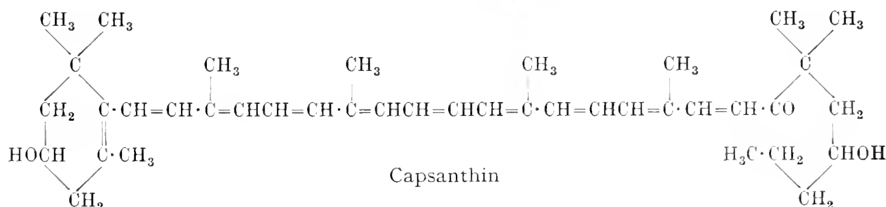
Occurrence

Capsanthin is a rare carotenoid. ZECHMEISTER and VON CHOLNOKY[67] found the esterified pigment in the ripe pods of *Capsicum annuum* and *Capsicum frutescens japonicum*[68] and KARRER and OSWALD[69] observed that the anthers of *Lilium tigrinum* contain capsanthin besides antheraxanthin (cf. p. 191).

Preparation[70]

The paprika pods are freed from their shells and seeds and dried at 35–40°. The finely ground material is then extracted at room temperature with petroleum ether (1 kg of pods requires ¾ l of petroleum ether). The solution is diluted with a threefold volume of ether, 30% methanolic potassium hydroxide is added, and the mixture is allowed to stand for 1–2 days at room temperature. (Concerning the control of the saponification, compare the original communication of ZECHMEISTER and VON CHOLNOKY). At the end of this period the free phytoxanthins are dissolved in ether. The solution is washed until it shows a neutral reaction, dried over sodium sulphate, and most of the solvent is evaporated under reduced pressure. The ethereal residue is diluted with much petroleum ether and allowed to stand in the cold for 24 hours. In this way, 1.2–2 g of crude capsanthin is obtained from 1 kg of high quality paprika. After two recrystallisations from carbon disulphide, the yield amounts to 0.8–1.2 g of crystallised, but still inhomogeneous pigment. The separation from accompanying carotenoids (zeaxanthin, capsorubin) is effected by chromatography on calcium carbonate or zinc carbonate. Carbon disulphide is employed as solvent for developing the chromatogram. A mixture of benzene and ether (1:1) is also suitable for this purpose[71].

Chemical Constitution



Capsanthin

The molecular formula of capsanthin $C_{40}H_{58}O_3$ was determined by ZECHMEISTER and VON CHOLNOKY[72]. The same authors also established that the

References p. 253–255.

pigment contains ten double bonds[73], which must be conjugated in view of the long-wavelengths location of the absorption maxima. ZEREWITINOFF determinations and esterification[74] proved that only two of the three oxygen atoms are present as hydroxyl groups. (This result was confirmed by ZECHMEISTER and VON CHOLNOKY[75]). The third oxygen atom belongs to a carbonyl group which is directly attached to the system of conjugated double bonds and cannot be oximated. The presence of a carbonyl group was indirectly proved by ZECHMEISTER and VON CHOLNOKY[73] by showing that perhydrocapsanthin contains three hydroxyl groups which can be acetylated. This is in agreement with the results of microhydrogenation[76] during which capsanthin takes up 11 mols of hydrogen. The presence of a carbonyl group and its position in the conjugated system was confirmed by the investigations of KARRER and HÜBNER in the course of which capsanthin was converted into the corresponding alcohol, capsanthol, by reduction with aluminium isopropoxide and isopropyl alcohol. Capsanthol is a triol of the formula $C_{40}H_{57}(OH)_3$, the longest wavelength absorption band of which is displaced by only 35 mμ towards shorter wavelengths as compared with capsanthin. This shows that the carbonyl group in capsanthin must be terminally conjugated rather than cross-conjugated, otherwise the displacement of the absorption maxima on reduction would be larger owing to the break in conjugation.

KARRER and co-workers [78] subjected capsanthin to permanganate degradation and obtained α:α-dimethylmalonic acid and α:α-dimethylsuccinic acid. No α:α-dimethylglutaric acid was formed, so that the presence of an unsubstituted β-ionone ring is excluded. These findings are in agreement with the results of biological assay[79] which show that capsanthin has no vitamin A activity. A further confirmation of the open-chain formula of capsanthin was provided by an investigation of KARRER and JUCKER[80], in the course of which a carotenoid containing the chromophoric system present in capsanthin was obtained by a rational partial synthesis (cf. p. 250).

Properties

Crystalline form: Capsanthin crystallises from carbon disulphide in deep carmine-red spheres. From petrol, the pigment is obtained in needles, and from methanol in prisms.

Melting point: 176° (uncorr.)[81], 175–176° (corr.)[82].

Solubility: Capsanthin is readily soluble in acetone and chloroform, less soluble in methanol, ethanol, ether and benzene, only sparingly soluble in carbon disulphide, and almost insoluble in petroleum ether[83].

Spectral properties:

Solvent	*Absorption maxima*	
Carbon disulphide	542	503 mμ
Petrol	505	475 mμ
Benzene	520	486 mμ

(cf. Fig. 14, p. 353)

Solutions of the pigment in ethanol are deep red. Solutions in petrol are lemon to orange-yellow.

Colour reactions: On treating a solution of the pigment in chloroform with concentrated sulphuric acid, the latter assumes a deep blue colouration. On treating an ethereal solution of the pigment with concentrated aqueous hydrochloric acid, *no* colour change is observed. Capsanthin gives a deep blue colouration with antimony trichloride in chloroform. Numerous other colour reactions have been described by ZECHMEISTER[84].

Optical activity: $[\alpha]_{Cd} = +36°$ (in chloroform).

Partition test: On partition between petroleum ether and 90% methanol, capsanthin is found quantitatively in the lower layer.

Chromatographic behaviour: Capsanthin is well adsorbed on calcium carbonate or zinc carbonate from carbon disulphide or from a mixture of benzene and ether (1:1). It is found above violaxanthin on the chromatogram column. Elution is effected by means of methyl or ethyl alcohol or by means of an ether-methanol mixture (5:1)*.

Detection and estimation: For a micro-method for the identification of capsanthin, compare ZECHMEISTER[84]. The simplest method of identifying the pigment is the determination of the absorption spectrum. According to ZECHMEISTER[84] the following reaction is characteristic of the pigment. A layer of 30% methanolic potassium hydroxide is formed under a solution of capsanthin in petroleum ether, and the mixture is allowed to stand undisturbed for one day. At the end of this period, deep red needles are formed.

Physiological properties: According to B. v. EULER, H. v. EULER and KARRER[85] capsanthin has no vitamin A activity.

Chemical behaviour: Capsanthin is not acidic in character but there are certain indications that the pigment is at least partly enolised. Thus, when the pigment is chromatographed on calcium carbonate from benzene, two zones are regularly observed[86]. The same behaviour was observed by KARRER and

* Concerning the observation of L. ZECHMEISTER and L. v. CHOLNOKY that the pigment forms two zones in the chromatogram on calcium carbonate, cf. the section on *cis-trans* isomerism, p. 248.

References p. 253–255.

JUCKER during the chromatographic adsorption of capsochrome[87] (cf. p. 248).

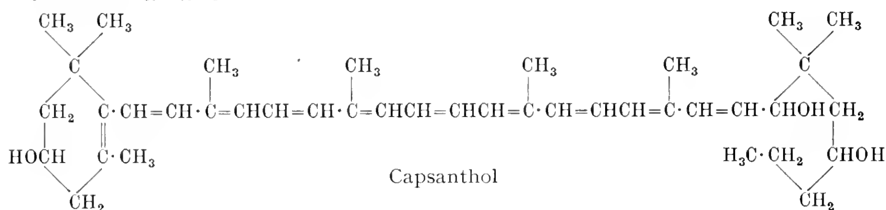
On standing in air, capsanthin is slowly oxidised. In an atmosphere of oxygen, oxidation occurs more rapidly. The uptake of oxygen is complete after about 1 month and amounts to about 29% by weight, corresponding to 10 mols of oxygen. The pigment wax of capsanthin is much more stable towards oxygen. According to PUMMERER and REBMANN[88], 6–8 mols of oxygen are absorbed on treating capsanthin with perbenzoic acid. On treatment with bromine in chloroform solution, 8 mols of bromine are absorbed. The thermal decomposition of the pigment gives rise to *m*-xylene.

Derivatives

Capsanthin diiodide: This compound is obtained on treating capsanthin with iodine in carbon disulphide solution. Capsanthin diiodide crystallises in flat needles which appear yellow-brown to black under the microscope[89]. It is easily soluble in chloroform and acetone, a little less soluble in ethanol and ether and almost insoluble in petroleum ether.

Perhydrocapsanthin: Capsanthin absorbs 10 mols of hydrogen on hydrogenation in acetic acid in the presence of platinum as catalyst; the carbonyl group remains unchanged. The perhydrocapsanthin thus obtained is a viscous, colourless oil which is much more soluble in organic solvents than capsanthin itself. On treating perhydrocapsanthin with sodium and alcohol, the carbonyl group is reduced and the completely hydrogenated triol $C_{40}H_{80}O_3$ is obtained[90].

Capsanthol $C_{40}H_{60}O_3$:



Capsanthol

KARRER and HÜBNER[91] prepared capsanthol by the reduction of capsanthin with aluminium isopropoxide and isopropyl alcohol. The compound was purified by repeated adsorption on calcium hydroxide from benzene solution. Capsanthol crystallises from ethanol in reddish-brown leaflets, which appear yellow under the microscope. Melting point 175–176° (uncorr.). Capsanthol is sparingly soluble in boiling ethanol. Catalytic reduction shows the presence of 10 double bonds.

References p. 253–255.

Solvent	*Absorption maxima*	
Carbon disulphide	508	477 mμ
Pyridine	493	463 mμ
Benzene	492	462 mμ
Chloroform	486	456 mμ
Ethanol	478	448 mμ

Capsanthin diacetate $C_{44}H_{62}O_5$:

This compound was prepared by ZECHMEISTER and VON CHOLNOKY[92] by treating capsanthin with acetyl chloride in pyridine solution. The diacetate is purified by chromatography on calcium carbonate from petrol solution and by crystallisation from methanol. It separates in plates, m.p. 146.5° (corr.).

The pigment is very soluble in chloroform, ether, carbon disulphide and benzene, and somewhat less soluble in methanol. On partition between methanol and petroleum ether, the ester is found quantitatively in the upper layer.

Capsanthin dipropionate $C_{46}H_{66}O_5$:

The dipropionate is prepared in the same way as the diacetate. It separates from a mixture of ethanol and carbon disulphide, or from ethanol alone, in crystals, m.p. 140°.

Capsanthin dicaprate $C_{60}H_{94}O_5$: This compound is prepared in the same way as capsanthin diacetate[93]. M.p. 109° (corr.)*. The ester crystallises from a mixture of benzene and methanol in red plates with a violet tinge. It is readily soluble in petroleum ether, chloroform, ether, carbon disulphide and benzene. It is much less soluble in ethanol than the diacetate. $[\alpha]^{20°}_{656.3} = -61°$ in hexane.

Capsanthin dimyristate $C_{68}H_{110}O_5$: The ester crystallises from a mixture of benzene and methanol in red needles, m.p. 88°. The solubility is similar to that of the dicaprate, except that the dimyristate is insoluble in alcohols.

Capsanthin dipalmitate $C_{72}H_{118}O_5$: The dipalmitate is prepared and purified in the same way as the diacetate[94]. It crystallised from a mixture of benzene and methanol in bordeaux-red plates, m.p. 95° (corr.)** [95]. For the absorption spectra in different solvents compare the original communication by ZECHMEISTER and VON CHOLNOKY**.

Capsanthin distearate $C_{76}H_{126}O_5$: This compound is prepared by the same method as the other esters[94]. M.p. 84°. The distearate shows great similarity to the dipalmitate both in appearance and solubility.

Capsanthin dibenzoate $C_{54}H_{66}O_5$: This ester crystallises from a mixture of benzene and methanol in red needles, and from a mixture of carbon disulphide

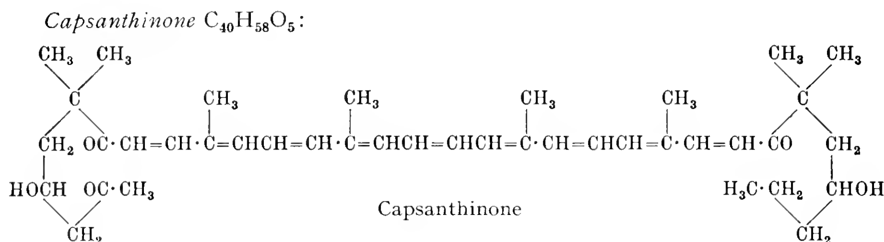
* L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 487* (1931) 210, previously reported m.p. 102° (corr.).

** L. ZECHMEISTER and L. v. CHOLNOKY, *Ann. 509* (1934) 286, reported m.p. 92° (corr.).

References p. 253–255.

and ethanol in leaflets, m.p. 121–122°(?). The dibenzoate is more soluble in alcohols than the fatty acid esters.

Capsanthinone $C_{40}H_{58}O_5$:

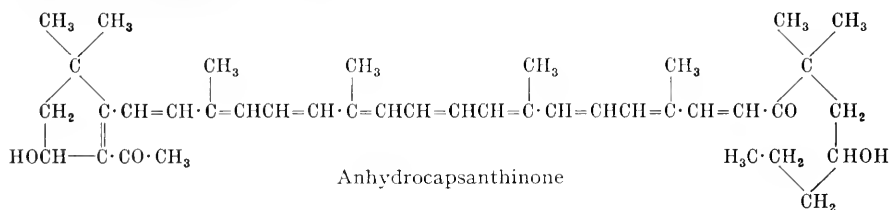


Capsanthinone

Capsanthinone is prepared by the oxidation of capsanthin diacetate with chromic acid[96]. Capsanthinone diacetate crystallises from a mixture of benzene and petrol in glistening needles, m.p. 123–124° (corr.). The acetate is hypophasic. It is readily soluble in ethanol, ether, benzene and carbon disulphide, somewhat less soluble in acetone and practically insoluble in petrol. On treating an ethereal solution of the pigment with concentrated aqueous hydrochloric acid, the latter is coloured deep blue.

Solvent	*Absorption maxima*		
Carbon disulphide	541	503	468 mμ
Benzene	524	487	454 mμ
Hexane	503	472	440 mμ

Anhydrocapsanthinone $C_{40}H_{56}O_4$:



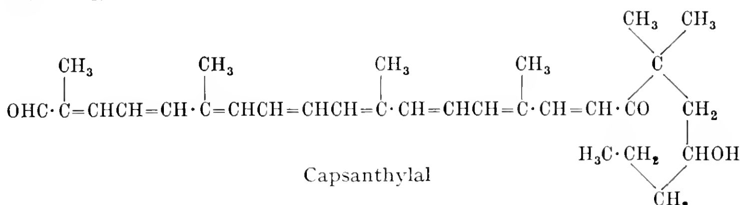
Anhydrocapsanthinone

KUHN and BROCKMANN[97] obtained anhydro-β-semicarotenone by splitting off water from β-semicarotenone (cf. p. 140). Anhydrocapsanthinone is obtained by an analogous reaction from capsanthinone diacetate[96]. The compound crystallises from methanol in small red needles which have no sharp melting point. On partition between methanol and petroleum ether, the pigment is found in the lower layer. In contrast to capsanthinone, the reaction with hydrochloric acid is negative. The spectral properties are in agreement with the proposed structure, the chromophoric system of which differs only by an additional conjugated double bond from that of capsanthinone. This results in the displacement of the maxima towards longer wavelengths by 16 mμ compared with capsanthinone.

References p. 253–255.

Solvent	*Absorption maxima*		
Carbon disulphide	557	517	483 mμ
Benzene	537	499	467 mμ
*Cyclo*hexane	524	489	458 mμ
Hexane	518	483	453 mμ

Capsanthylal $C_{30}H_{42}O_3$[96,98]:



Capsanthylal

Capsanthylal is formed by the oxidation of capsanthin diacetate with chromic acid[98]. If an excess of oxidising agent is employed, smaller degradation products are also obtained. The aldehyde crystallises from 80 % methanol in needles which are grouped in star-like formations. M.p. 127° (corr.).

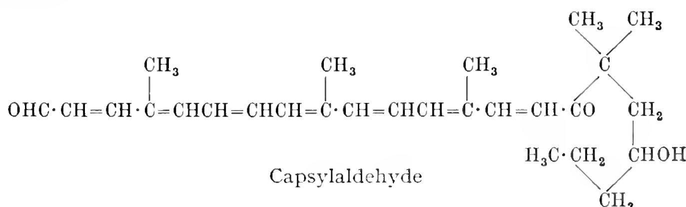
Solvent	*Absorption maxima*		
Carbon disulphide	518	483	452 mμ
Hexane	483	452	mμ

Capsanthylal is readily soluble in benzene, ethanol and carbon disulphide, but only slightly soluble in petroleum ether.

Capsanthylal monoxime $C_{30}H_{43}O_3N$: Crystallises from methanol in needles, which are similar in colour to zeaxanthin. M.p. 184°. The oxime is readily soluble in benzene and carbon disulphide and somewhat less soluble in hexane and petrol.

Solvent	*Absorption maxima*	
Hexane	483	452 mμ

Capsylaldehyde $C_{27}H_{38}O_3$:



Capsylaldehyde

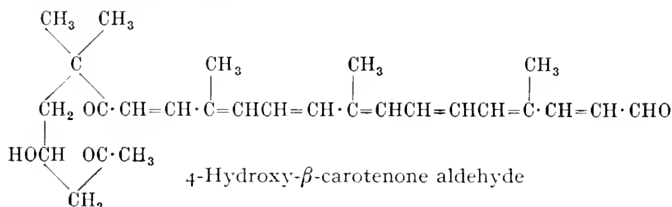
Capsylaldehyde has only been obtained crystalline in the form of its oxime. The formula shown above is assigned to this compound by ZECHMEISTER and VON CHOLNOKY[98]. The oxime crystallises in lemon-yellow needles, m.p. 172°.

References p. 253–255.

It is hardly soluble in petrol, but somewhat more soluble in methanol, benzene and carbon disulphide.

Solvent	*Absorption maxima*					
	Capsylaldehyde			β-Carotenone aldehyde		
Carbon disulphide . .	491	459	430 mμ	490	459	430 mμ
Benzene	476	446	420 mμ	478	448	421 mμ
Hexane	458	431	mμ	458	431	mμ

4-Hydroxy-β-carotenone aldehyde $C_{27}H_{36}O_4$[96]:



4-Hydroxy-β-carotenone aldehyde

This aldehyde is formed besides capsylaldehyde and capsanthylal by the chromic acid oxidation of capsanthin diacetate. Only the oxime has so far been obtained in the crystalline state. It forms yellow cigar-shaped crystals, m.p. 189° (corr.). The oxime is readily soluble in benzene, methanol, hot petrol and hot hexane, but only sparingly in carbon disulphide. The absorption spectrum of the aldehyde coincides with that of the oxime, and is hardly different from that of capsylaldehyde.

Solvent	*Absorption maxima*		
Carbon disulphide	490.5	459.5	429 mμ
Benzene	477	449	420 mμ
Hexane	459	433	408 mμ

ZECHMEISTER and VON CHOLNOKY[99] subjected capsanthin to a different kind of degradation, namely hydrolysis with aqueous alcoholic sodium hydroxide in a sealed tube. The product obtained in this way was identified with citraurin (cf. p. 219), and the structures of both pigments were thus confirmed.

Cis-Trans Isomers

As early as 1937, ZECHMEISTER and VON CHOLNOKY[96] observed that pure capsanthin forms 2 zones in the chromatogram. This observation was first explained as due to the formation of the enol form of the pigments. These investigations were continued by the same two authors in 1940, and it was found that not only 2, but several zones appeared on the adsorption column[100]. Following the investigations by GILLAM and EL RIDI[101] and ZECHMEISTER and co-workers[102], the phenomenon was then ascribed to *cis-trans* isomerisation.

References p. 253–255.

By applying the usual methods (cf. p. 39), it is possible to isomerise capsanthin and capsanthin dipalmitate[103] into various compounds which according to ZECHMEISTER and VON CHOLNOKY[103] and POLGÁR and ZECHMEISTER[104] are *cis-trans* isomers of the two pigments. Neo-capsanthin A could be obtained in a micro-crystalline form, but no data are available regarding its melting point and elementary analysis.

Solvent	*Absorption maxima*					
	Neo-A		*Neo-B*		*Neo-C*	
Carbon disulphide	(532)	(495) mμ				
Benzene	(513)	(481) mμ	(513)	(481) mμ	(508)	(479) mμ
Hexane	496	465 mμ				

The different isomers have the following optical rotations in benzene:

Capsanthin	$[\alpha]_C = \pm\ 0°\ (\pm\ 5\text{–}10°)$
Neocapsanthin A	$[\alpha]_C = +\ 89°$
Neocapsanthin B	$[\alpha]_C = +\ 21°\ (\pm\ 5°)$
Neocapsanthin C	$[\alpha]_C = +\ 27°\ (\pm\ 10°)$

Analogous experiments with capsanthin dipalmitate gave rise to two transformation products which exhibited the following absorption maxima:

	Carbon disulphide		*Benzene*		*Hexane*	
Capsanthin dipalmitate	541.5	502 mμ	(519)	(488) mμ	506	473 mμ
Neocapsanthin dipalmitate I	535	499 mμ	(512)	(483) mμ	502	470 mμ
Neocapsanthin dipalmitate II	533	497 mμ	(510)	(482) mμ	496	465 mμ

Optical rotations in petrol:

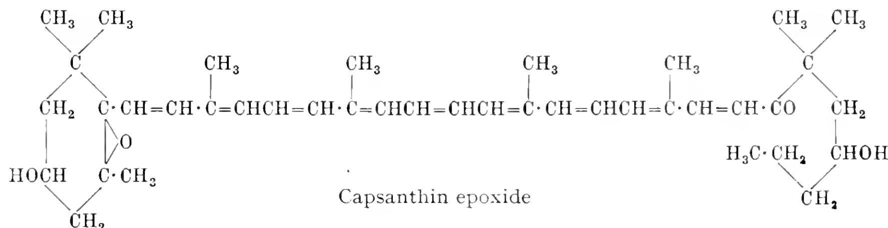
Capsanthin dipalmitate	$[\alpha]_C = -30°$
Neocapsanthin dipalmitate I	$[\alpha]_C = -22°$
Neocapsanthin dipalmitate II	$[\alpha]_C = -20°$

After iodine catalysis, the neo-capsanthins exhibit a well defined 'cis-peak' near 363 mμ in petrol.

The possible configurations of the different isomers are discussed by POLGÁR and ZECHMEISTER[104].

Capsanthin mono-epoxide and Capsochrome

KARRER and JUCKER[105] subjected capsanthin diacetate to oxidation with monoperphthalic acid and obtained a crystalline mono-epoxide $C_{40}H_{58}O_4$:

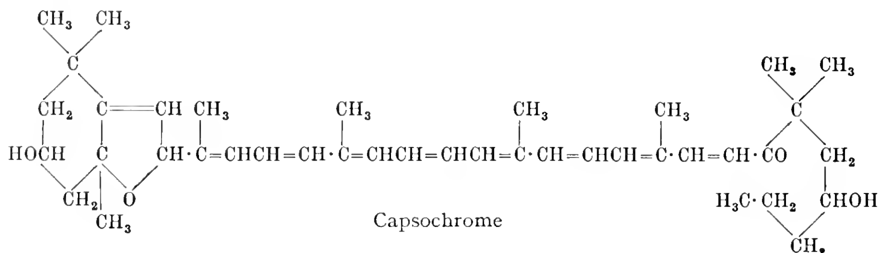


Capsanthin epoxide

References p. 253–255.

The compound crystallises from a mixture of benzene and petroleum ether in leaflets and needles, m.p. 189° (uncorr., in vacuum). On shaking the ethereal solution of the pigment with concentrated aqueous hydrochloric acid, the latter assumes an unstable deep blue colouration. In the partition test the pigment is found quantitatively in the lower layer.

On treating capsanthin epoxide with very dilute hydrochloric acid, it is isomerised into the stabler furanoid oxide, capsochrome.



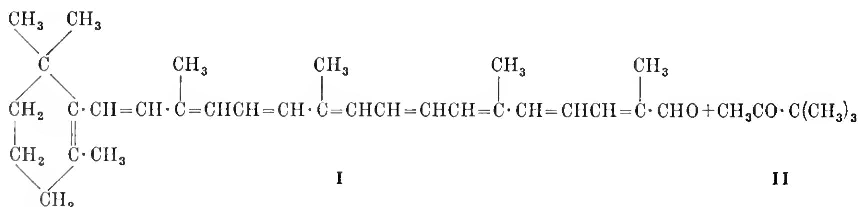
Capsochrome

Capsochrome crystallises well from a mixture of benzene and petroleum ether. Melting point 195° (uncorr., in vacuum). The pigment exhibits the same behaviour towards hydrochloric acid as capsanthin epoxide. It is hypophasic on partition between methanol and petroleum ether.

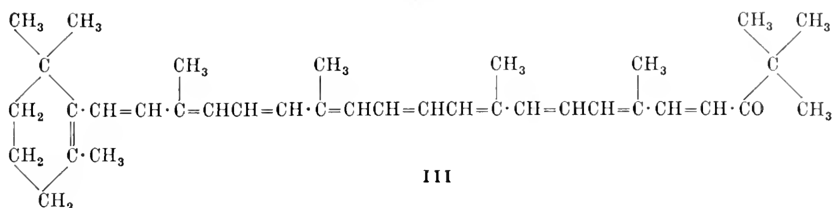
Solvent	*Absorption maxima*			
	Capsanthin epoxide		Capsochrome	
Carbon disulphide	534	499	515	482 mμ
Chloroform	511	481	492	462 mμ (not sharp)
Benzene	514	483	496	464 mμ

Partially Synthetic Polyene Ketone Containing the Chromophoric System of Capsanthin

By condensing β-apo-2-carotenal[106] (I) with pinacolone (II), KARRER and JUCKER[107] obtained the polyene ketone (III) which possesses the same chromophoric system as is present, according to ZECHMEISTER and VON CHOLNOKY[108], in capsanthin. The absorption bands of the two pigments in the visible region are completely identical, which supports the formula proposed for capsanthin.



References p. 253–255.



Solvent	Absorption maxima			
	Polyene Ketone (III)		Capsanthin	
Carbon disulphide	543	503	543	503 mμ
Petroleum ether	503	473	503	475 mμ

Most of the colour reactions exhibited by capsanthin on treatment with different acids and metallic chlorides[84] are also given by the polyene ketone. The two compounds, however, differ somewhat in their behaviour towards concentrated aqueous hydrochloric acid: the acid layer is coloured red on shaking with an ethereal solution of capsanthin but remains colourless in the case of the polyene ketone.

7. CAPSORUBIN $C_{40}H_{60}O_4$

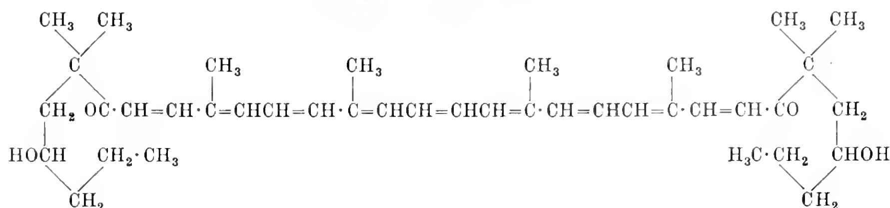
History and Occurrence

In 1934, ZECHMEISTER and VON CHOLNOKY[109] isolated a new pigment, capsorubin, during the chromatographic purification of capsanthin. Capsorubin has so far only been found in *Capsicum annuum*.

Preparation

The paprika pods are pre-treated with ethanol, and then extracted with petroleum ether. The combined extracts are concentrated in vacuum and the pigment esters are chromatographed on calcium carbonate. After repeated chromatographic adsorption, the capsorubin ester is saponified with methanolic potassium hydroxide, and the pigment is finally chromatographed repeatedly on calcium carbonate from carbon disulphide solution. For further purification, the pigment is crystallised from a mixture of benzene and petrol. The yield of analytically pure pigment amounts to about 130 mg from 5 kg of pods.

Chemical Constitution[108, 109]



References p. 253–255.

The formula for capsorubin proposed by ZECHMEISTER and VON CHOLNOKY[108, 109] has not been completely established, but is in complete accord with all the properties of the pigment. The molecular formula of capsorubin is $C_{40}H_{60}O_4$. It contains 9 conjugated double bonds and 2 carbonyl groups. Acetylation shows the presence of two hydroxyl groups which, for reasons of analogy, are assigned to the same positions as in xanthophyll (p. 201) zeaxanthin (p. 183) and capsanthin (p. 242). The products of oxidation with chromic acid indicate the presence of 4 side-chain methyl groups.

Properties[108, 109]

Capsorubin crystallises from a mixture of benzene and petrol in violet-red needles. From carbon disulphide the pigment is obtained in rhombic plates. It is readily soluble in alcohol and acetone, more sparingly soluble in ether, benzene and carbon disulphide, and almost insoluble in petroleum ether. The chromatographic behaviour of capsorubin is similar to that of capsanthin. It is well adsorbed on calcium carbonate from carbon disulphide and is found above capsanthin on the column. Melting point 201° (corr.).

On treating an ethereal solution of capsorubin with concentrated hydrochloric acid, the latter immediately assumes a violet colouration, which later turns deep blue. With 25 % hydrochloric acid, no colour change is observed. The pigment is entirely hypophasic in the partition test.

Solvent	*Absorption maxima*		
Carbon disulphide	541.5	503	468 mμ
Benzene	520	486	455 mμ
Petrol	506	474	444 mμ

Capsorubin diacetate $C_{44}H_{64}O_6$:

This compound is obtained by the acetylation of capsorubin in pyridine with acetyl chloride. The ester crystallises from methanol in square leaflets, m.p. 179° (corr.). It is readily soluble in petrol, benzene and methanol.

Cis-Trans Isomers

ZECHMEISTER and VON CHOLNOKY[110] examined the behaviour of capsorubin on treatment with iodine, on heating or on standing over long periods. They found that the pigment undergoes similar transformations as capsanthin (cf. p. 248). They were not able, however, to isolate any of the transformation products, which they regard as *cis-trans* isomers, in the crystalline state. The only data available are the absorption spectra and optical rotations.

References p. 253–255.

Solvent	*Absorption maxima*									
	Carbon disulphide			Benzene			Hexane			
Capsorubin	541	502	467	524	489	455	502	470	471	mμ
Neocapsorubin A . .	533	495	460	517	483	451	498	466	(435)	mμ
Neocapsorubin B . .	535	497	462	518	484	453	500	467	(436)	mμ
Capsorubin dipalmitate	541.5	502.5	467	524	489	455	507	474	442	mμ
Neocapsorubin dipalmitate I . . .	536	496	463	521	486	452	502	470	439	mμ
Neocapsorubin dipalmitate II . . .	533	495	460	518	484	450	499	467	437	mμ

The optical rotations of the various transformation products in benzene are as follows:

Capsorubin	$[\alpha]_c = 0°$
Neocapsorubin A	$[\alpha]_c = -134°$
Neocapsorubin B	$[\alpha]_c = -69°$
Capsorubin dipalmitate	$[\alpha]_c = 0°$
Neocapsorubin dipalmitate I	$[\alpha]_c = -75°$
Neocapsorubin dipalmitate II	$[\alpha]_c = -15°$

REFERENCES

1. L. Zechmeister and P. Tuzson, *Ber.* *69* (1936) 1878; *70* (1937) 1966.
2. P. Karrer and U. Solmssen, *Helv. chim. Acta* *20* (1937) 682.
3. P. Karrer and U. Solmssen, *Helv. chim. Acta* *20* (1937) 682, 1020 (cf. p. 205).
4. P. Karrer and co-workers, *Helv. chim. Acta* *21* (1938) 448.
5. L. Zechmeister and L. v. Cholnoky, *Ann.* *530* (1937) 291.
6. P. Karrer, H. Koenig and U. Solmssen, *Helv. chim. Acta* *21* (1938) 445.
7. H. Koenig, *Dissertation*, Zürich 1940.
8. L. Zechmeister and L. v. Cholnoky, *Ann.* *530* (1937) 291.
9. N. A. Monteverde, *Acta Horti Petropol.* *13* (1893) 121.
10. M. Tswett, *Compt. rend.* *152* (1911) 788.
11. N. A. Monteverde and V. N. Lubimenko, *Bull. Acad. Sci. Petrograd.* *7* (1913) 1105.
12. S. Prát, *Biochem. Z.* *152* (1924) 495.
13. T. Lippmaa, *Compt. rend.* *182* (1926), 867, 1040; *Ber. bot. Ges.* *44* (1926) 643.
14. R. Kuhn and H. Brockmann, *Ber.* *66* (1933) 828.
15. P. Karrer and U. Solmssen, *Helv. chim. Acta* *18* (1935) 477.
16. Cf. T. Lippmaa, *Das Rhodoxanthin*, University of Tartu (1925).
17. R. Kuhn and H. Brockmann, *Ber.* *66* (1933) 828.
18. P. Karrer and U. Solmssen, *Helv. chim. Acta* *18* (1935) 477.
19. A. Polgár and L. Zechmeister, *J. Am. Chem. Soc.* *66* (1944) 186.
20. I. M. Heilbron, B. Lythgoe and R. F. Phipers, *Nature* *136* (1935) 989.
21. I. M. Heilbron and B. Lythgoe, *J. Chem. Soc.* *1936*, 1376.
22. J. Tischer, *Z. physiol. Chem.* *251* (1938) 109.
23. J. Rutschmann, *Dissertation*, Zürich 1946; *Helv. chim. Acta* *27* (1944) 1691.
24. P. Karrer and J. Rutschmann, *Helv. chim. Acta* *27* (1944) 1691.
25. G. Pouchet, *J. Anat. Physiol.* *12* (1876) 1–90, 113–165. — R. Maly, *Sitzgsber. Akad. Wiss. Wien* *83* (1881) 1126; *Mh. Chem.* *2* (1881) 351. — C. F. W. Kruckenberg, *Vergleichend-physiologische Studien* *92* (1882). — W. Zopf, *Beitr. Phys. Morph. nied.*

Org. *3* (1893) 26. — M. NEWBIGIN, *J. Anat. u. Physiol.* *21* (1897) 237. — G. VEGEZZI, *Dissertation*, Fribourg, 1916. — J. VERNE, *Compt. rend. Soc. Biol. Paris* *83* (1920) 963; *94* (1926) 1349; *97* (1927) 1290; *Arch. Morph.* *16* (1923) 1. — E. CHATTON, A. LWOFF and M. PARAT, *Compt. rend. Soc. Biol. Paris* *94* (1926) 567. — E. LÖNNBERG and H. HELLSTRÖM, *Ark. Zool.* A. *26* (1932) No. 7, etc.
26. R. KUHN and E. LEDERER, *Ber.* *66* (1933) 488.
27. P. KARRER and co-workers, *Helv. chim. Acta* *17* (1934) 412, 745; *18* (1935) 96; *19* (1936) 479.
28. R. KUHN and N. A. SÖRENSEN, *Z. angew. Chem.* *51* (1938) 465; *Ber.* *71* (1938) 1879.
29. R. KUHN J. STENE and N. A. SÖRENSEN, *Ber.* *72* (1939) 1688.
30. J. TISCHER, *Z. physiol. Chem.* *267* (1941) 281.
31. R. KUHN and E. LEDERER, *Ber.* *66* (1933) 488.
32. R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber.* *72* (1939) 1688. — J. TISCHER, *Z. physiol. Chem.* *267* (1941) 281. — Cf. p. 232.
33. R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber.* *72* (1939) 1688. — J. TISCHER, *Z. physiol. Chem.* *239* (1936) 257.
34. J. TISCHER, *Z. physiol. Chem.* *250* (1937) 147; *252* (1938) 225.
35. R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber.* *72* (1939) 1688.
36. J. TISCHER, *Z. physiol. Chem.* *267* (1941) 281.
36*a*. M. HARTMANN, F. GRAF MEDEN, R. KUHN and H. J. BIELIG, *Z. Naturforsch.* *2b* (1947) 330.
37. R. KUHN and N. A. SÖRENSEN, *Ber.* *71* (1938) 1879.
38. P. KARRER and co-workers, *Helv. chim. Acta* *17* (1934) 412, 745; *19* (1936) 479. — W. JAFFÉ, *Dissertation, Zürich, 1939.*
39. P. KARRER and co-workers, *Helv. chim. Acta* *17* (1934) 412; *17* (1934) 745; *18* (1935) 96, *19* (1936) 479.
40. H. WILLSTAEDT, *Svensk Kem. Tidskr.* *46* (1934) 205.
41. P. KARRER, L. LOEWE and H. HÜBNER, *Helv. chim. Acta* *18* (1935) 96.
42. R. KUHN and N. A. SÖRENSEN, *Z. angew. Chem.* *51* (1938) 465; *Ber.* *71* (1938) 1879.
43. Literature references are given by R. KUHN and N. A. SÖRENSEN, *Ber.* *71* (1938) 1882.
44. R. W. G. WYCKOFF, *Science* *86* (1937) 311.
45. R. KUHN and N. A. SÖRENSEN, *Z. angew. Chem.* *51* (1938) 465.
46. K. G. STERN and K. SALOMON, *J. biol. Chem.* *122* (1938) 461.
47. P. KARRER and F. BENZ, *Helv. Chim. Acta* *17* (1934) 412.
48. R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber.* *72* (1939) 1688. In the recent literature, the value 228° is always given.
49. R. KUHN, E. LEDERER and A. DEUTSCH, *Z. physiol. Chem.* *220* (1933) 231.
50. P. KARRER and L. LOEWE, *Helv. chim. Acta* *17* (1934) 745.
51. Cf. R. KUHN and co-workers, *Ber.* *72* (1939) 1688.
52. R. KUHN ,E. LEDERER and A. DEUTSCH, *Z. physiol. Chem.* *220* (1933) 231. — Cf. P. KARRER and L. LOEWE, *Helv. chim. Acta* *17* (1934) 745.
53. R. KUHN and N. A. SÖRENSEN, *Ber.* *71* (1938) 1879.
54. Cf. *Ber.* *71* (1938) 1886.
55. R. KUHN and N. A. SÖRENSEN, *Ber.* *71* (1938) 1887.
56. R. KUHN and N. A. SÖRENSEN, *Ber.* *71* (1938) 1888.
57. R. KUHN, J. STENE and N. A. SÖRENSEN, *Ber.* *72* (1939) 1701.
58. R. KUHN and N. A. SÖRENSEN, *Ber.* *72* (1939) 1699.
59. H. BRACONNOT, *Ann. Chim.* *6* (1817) 122, 133.
60. J. L. THUDICHUM, *Proc. Roy. Soc.* (London) *17* (1869) 253.
61. T. PABST, *Arch. Pharm.* *230* (1892) 108.
62. F. G. KOHL, *Untersuchungen über das Carotin und seine physiologische Bedeutung in Pflanzen*, Leipzig 1902.
63. A. TSCHIRCH, *Ber. bot. Ges.* *22* (1904) 414. — B. M. DUGGAR, *Washington, Univ. Stud.* *1* (1913) 22. — N. A. MONTEVERDE and C. N. LUBIMENKO, *Bull. Acad. Sci Petrograd.* Ser. 6, 7. II (1913) 1105.

64. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *454* (1927) 54.
65. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *455* (1927) 70; *465* (1928) 288; *Ber.* *61* (1928) 1534; *Ann.* *487* (1931) 197; *489* (1931) 1; *509* (1934) 269; *516* (1935) 30.
66. P. KARRER and co-workers, *Helv. chim. Acta* *14* (1931) 614; *19* (1936) 474.
67. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *454* (1927) 57; *487* (1931) 197.
68. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *489* (1931) 1.
69. P. KARRER and A. OSWALD, *Helv. chim. Acta* *18* (1935) 1303.
70. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *454* (1927) 54; *509* (1934) 269.
71. P. KARRER ans E. JUCKER, *Helv. chim. Acta* *28* (1945) 1145.
72. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *509* (1934) 269.
73. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *516* (1935) 30.
74. P. KARRER and co-workers, *Helv. chim. Acta* *14* (1931) 614.
75. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *454* (1927) 54; *509* (1934) 269.
76. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *516* (1934) 30.
77. P. KARRER and H. HÜBNER, *Helv. chim. Acta* *19* (1936) 474.
78. P. KARRER and co-workers, *Helv. chim. Acta* *14* (1931) 614.
79. B. v. EULER, H. v. EULER and P. KARRER, *Helv. chim. Acta* *12* (1929) 278.
80. P. KARRER and E. JUCKER, *Helv. chim. Acta* *27* (1944) 1588.
81. P. KARRER and A. OSWALD, *Helv. chim. Acta* *18* (1935) 1305.
82. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *509* (1934) 287.
83. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *509* (1934) 269; *454* (1927) 67.
84. L. ZECHMEISTER, *Carotinoide*, Berlin 1934.
85. B. v. EULER, H. v. EULER and P. KARRER, *Helv. chim. Acta* *12* (1929) 278.
86. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *530* (1937) 291.
87. P. KARRER and E. JUCKER, *Helv. chim. Acta* *28* (1945) 1143.
88. R. PUMMERER and L. REBMANN, *Ber.* *61* (1928) 1099.
89. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *478* (1930) 103.
90. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *516* (1935) 38.
91. P. KARRER and H. HÜBNER, *Helv. chim. Acta* *19* (1936) 476.
92. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *487* (1931) 210.
93. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *509* (1934) 285.
94. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *487* (1931) 209.
95. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *543* (1940) 248.
96. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *523* (1936) 101.
97. R. KUHN and H. BROCKMANN, *Ann.* *516* (1935) 95.
98. R. KUHN and H. BROCKMANN, *Ann.* *516* (1935) 95.
99. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *530* (1937) 291.
100. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *543* (1940) 248.
101. A. E. GILLAM and M. S. EL RIDI, *Nature* *136* (1935) 914; *Biochem. J.* *30* (1936) 1735; *31* (1937) 1605.
102. L. ZECHMEISTER and co-workers, *Nature* *141* (1938) 249; *Biochem. J.* *32* (1938) 1305; *Ber.* *72* (1939) 1340; *Ber.* *72* (1939) 1678; 2039.
103. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *543* (1940) 248.
104. A. POLGÁR and L. ZECHMEISTER, *J. Am. Chem. Soc.* *66* (1944) 186.
105. P. KARRER and E. JUCKER, *Helv. chim. Acta* *28* (1945) 1143.
106. P. KARRER and U. SOLMSSEN, *Helv. chim. Acta* *20* (1937) 682.
107. P. KARRER and E. JUCKER, *Helv. chim. Acta* *27* (1944) 1588.
108. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *516* (1935) 30.
109. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *509* (1934) 269.
110. L. ZECHMEISTER and L. v. CHOLNOKY, *Ann.* *543* (1940) 248.

CHAPTER XIII

Carotenoid carboxylic acids

I. BIXIN $C_{25}H_{30}O_4$

History

1825 BOUSSINGAULT[1] is the first to describe bixin, the pigment of orleans, which has since been investigated by numerous workers[2].
1878 ETTI[3] succeeds in crystallising bixin.
1917 HEIDUSCHKA and PANZER[4] carry out careful elementary analyses of bixin and are the first to assign the correct empirical formula to the pigment.
1928–33 KUHN and co-workers[5] propose a structural formula for bixin which is confirmed by KARRER and co-workers by the total synthesis of perhydronorbixin[6].

Occurrence

Bixin has only been found in *Bixa orellana*. The fresh seeds of this plant are surrounded by an orange-red mass which contains most of the pigment. After drying, the seeds are surrounded by a brown-red crust. Bixin also occurs in other organs of the plant, e.g. the secretionary cells of the leaves, and appears in the form of numerous brown spots on the lower side of the leaf.

Preparation[7]

a) From commercial orleans. The commercial preparation is finely ground and allowed to stand for several days covered with acetone. The material purified in this way is dried in air and the pigment is extracted with chloroform in a Soxhlet apparatus. It is crystallised from the same solvent, or from ethyl acetate or acetic acid. By this method good preparations of the labile bixin are obtained but the yield is decreased by the pre-extraction with acetone.

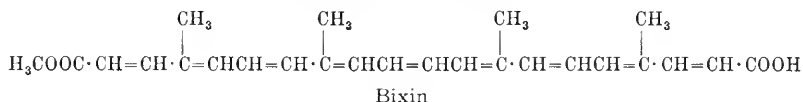
b) From "pâte de rocou". The red dough is stirred up with methanol and the bixin is converted into its ammonium salt by addition of ammonia. The salt is extracted with water and the solution is filtered. The filtrate is acidified with acetic acid, when bixin is precipitated as a red powder. It is filtered, washed with methanol and extracted with chloroform. The crude preparation is then recrystallised. In this way about 500 g of bixin are obtained from 25 kg of "pâte de rocou".

References p. 290–294.

c) From bixa-seeds*. The seeds are covered with water and allowed to stand for several hours. They are then stirred mechanically and filtered through a sieve. The turbid solution is allowed to stand overnight in large percolators and the lower layer is separated and centrifuged. The residue is broken up and dried, first in air and then in vacuum over calcium chloride, until it is not brittle but can be pressed. After grinding in a mill, 100 kg of seeds yield about 5–6 kg of a material which contains 15–30% of bixin.

This material, in portions of 200 g, is immediately covered with portions of two litres of ethanol, warmed to 60–65° on a waterbath, and ammonia is passed in until the colour change is complete and the solution contains free ammonia. The reaction mixture is allowed to stand for 20 minutes, filtered warm, and the residue is stirred up with 1 litre of ethanol. Into this mixture, ammonia is again passed at 60°. It is then allowed to stand for 1 hour and filtered. The combined filtrates precipitate ammonium bixate on cooling. In order to complete the precipitation, 1 ml of acetic acid is added to each litre of solution and the solution is vigorously stirred. After a short time, a dark red resin separates on the stirrer and on the walls of the vessel. The resin is separated from the mother liquor and treated with acetic acid, with vigorous mechanical stirring. After some hours, free bixin separates and can be filtered and dried in vacuum over sodium hydroxide and calcium chloride. It is then crystallised from acetic acid. (About 18 g of boiling glacial acetic acid are required to dissolve 1 g of crude bixin). About 120–160 g of pure bixin are obtained from 100 kg of seeds.

Chemical Constitution



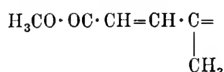
Bixin

The elucidation of the constitution of bixin extended over several years. HEIDUSCHKA and PANZER[8] determined the correct molecular formula $C_{25}H_{30}O_4$ An unsymmetrical structural formula was first proposed, but was abandoned when KUHN and WINTERSTEIN[9] put forward the now accepted formula of bixin. This has been confirmed by the investigations of KARRER and co-workers[10], in the course of which perhydronorbixin was synthesised.

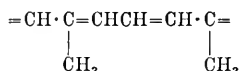
HERZIG and FALTIS[11] recognised that bixin was the monomethyl ester of an unsaturated dicarboxylic acid. Bixin absorbs 9 mols of hydrogen on catalytic hydrogenation and is thus converted into the half-ester of a saturated dicarboxylic acid. From the deep red colour of the pigment it can be concluded that the 9 double bonds are conjugated. By the ozonisation of methyl bixin RINKES and VAN HASSELT[12] obtained the methyl ester of β-acetylacrylic acid and methylglyoxal. Methylglyoxal must be derived from the grouping $=CH.C(CH_3)=$, while the first product indicates the presence of the grouping:

* A detailed description is given by R. KUHN and L. EHMANN, *Helv. chim. Acta 12* (1929) 904, and E. FORCÁT, *Dissertation*, Zürich, 1930.

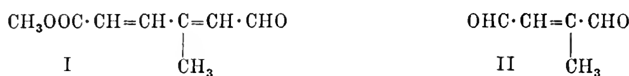
References p. 290–294.



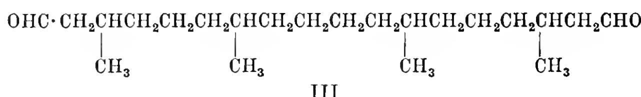
As early as 1909, VAN HASSELT[13] observed the formation of *m*-xylene during the dry distillation of bixin. This finding was later confirmed by HERZIG and FALTIS[14] and indicates the presence of the grouping:



KUHN and co-workers subjected bixin to oxidative degradation with permanganate and, later, with chromic acid, and deduced the presence of 4 side chain methyl groups in both cases[15]. When the structure of the ozonisation products I and II obtained by RINKES[16]



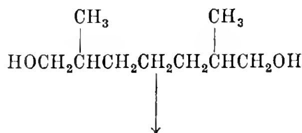
had been determined, KUHN and WINTERSTEIN[17] suggested the above structural formula for bixin, which is also based on the recognition of the symmetrical structure of carotene, lycopene and squalene (KARRER). The correctness of this formula was proved by the investigations of KARRER, in the course of which the position of the two terminal methyl groups was established by the oxidative degradation of partially hydrogenated bixin[18]. By the degradation of perhydronorbixin, KARRER and co-workers[18] obtained 3:7:12:16-tetramethyloctadecan-1:18-dial III,

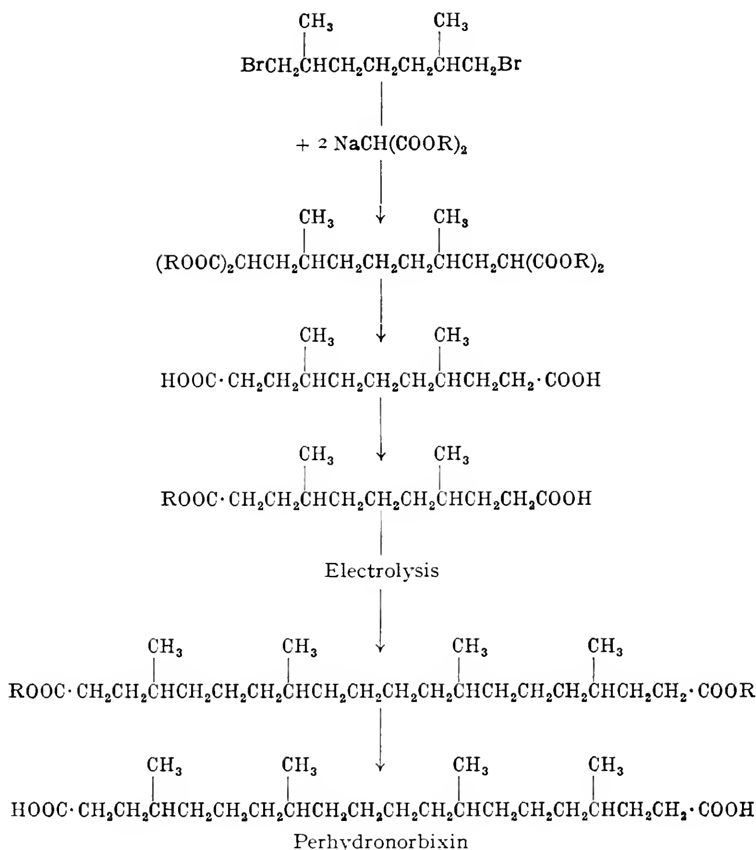


which was oxidised to the dicarboxylic acid and converted into perhydrocrocetin[19] (cf. p. 280).

The elucidation of the constitution of bixin was completed by the synthesis of perhydronorbixin by KARRER and co-workers[18] and by the conversion of perhydrocrocetin into perhydronorbixin. The structure of both these pigments was thus confirmed[20]. The formation of *m*-toluic acid by the thermal decomposition of bixin[21], is also in accord with the above formula.

The synthesis of perhydronorbixin was carried out as follows:





Perhydronorbixin

With regard to the conversion of perhydrocrocetin into perhydronorbixin, see p. 278.

Stereochemistry of Bixin

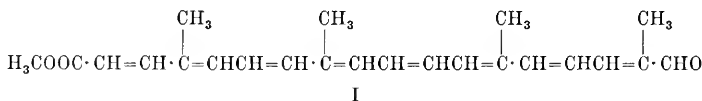
The first observation concerning a stereoisomer of bixin was made in 1913. In the course of the isolation of the pigment, HERZIG and FALTIS[22] accidentally obtained a new, higher melting form which they termed β-bixin. It was later suggested by KARRER and collaborators[23], that the two forms may be *cis-trans* isomers. By the treatment of natural labile bixin with iodine[24], these workers obtained the stable form identical with the β-bixin of HERZIG and FALTIS. The same transformation was also achieved with methyl bixin (p. 268). It was thus shown that *two* series of compounds exist, one of which is derived from the labile natural bixin, and the other from the stable β-bixin. A uniform nomenclature for bixin derivatives was proposed by KARRER and KUHN and is employed in the sequel.

TABLE 50

NOMENCLATURE OF BIXIN DERIVATIVES[25]

Formula	M.p.	Configuration	New name	Old name	
				KARRER	HERZIG and FALTIS
$C_{22}H_{26}(COOH)_2$	254-255°	cis	Labile norbixin	Norbixin	Norbixin
$C_{22}H_{26}\begin{cases}COOCH_3\\COOH\end{cases}$	196°	cis	Labile bixin	Bixin	Bixin
$C_{22}H_{26}(COOCH_3)_2$	163-164°	cis	Labile methyl bixin	Bixin methyl ester	Bixin methyl ester
$C_{22}H_{26}(COOH)_2$	>300°	trans	Stable norbixin	Isonorbixin	β-Norbixin
$C_{22}H_{26}\begin{cases}COOCH_3\\COOH\end{cases}$	220°	trans	Stable bixin	Isobixin	β-Bixin
$C_{22}H_{26}(COOCH_3)_2$	200-201°	trans	Stable methyl bixin	Isobixin-methyl ester	β-Bixin methyl ester

KARRER and SOLMSSEN have attempted to solve the question as to which double bond of the bixin molecule possesses the *cis*-configuration[26]. They subjected the bixins to permanganate degradation* and compared the products obtained from labile bixin and stable bixin. Each isomer yielded a different apo-1-norbixinal methyl ester I and, very probably, a different apo-2-norbixinal methyl ester II**, distinguished by their melting points and absorption spectra. Both bixins also give rise to an identical apo-3-norbixinal methyl ester III. These results show that the isomerism of the two bixins probably depends on the different configuration of the third double bond in the chain, counting from the unesterified carboxyl group. In view of the incompletely established difference of the apo-2-norbixinal methyl esters, it is possible that *cis-trans* isomerism of the second double bond may also be involved.



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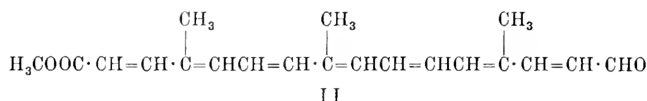
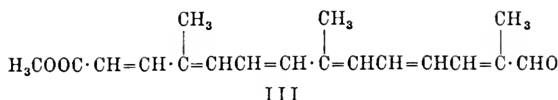


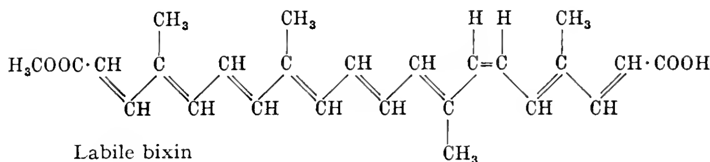
II

* Cf. p. 47.

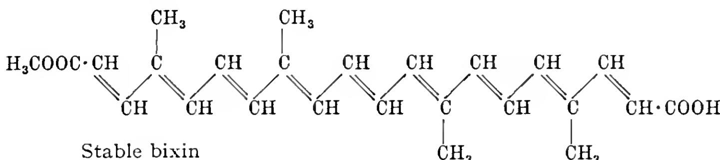
** The identity of the two compounds could not be established with certainty because the apo-2-norbixinal methyl esters were only obtained crystalline in the form of their oximes.



III



Labile bixin

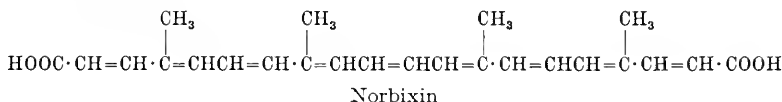


Stable bixin

The stereochemical configuration of the bixins has recently been investigated by ZECHMEISTER and ESCUE[27]. Instead of natural, labile bixin they chose the more easily chromatographed labile methylbixin for investigation, and subjected it to heat treatment, iodine catalysis and illumination with sunlight. These experiments yielded a number of transformation products, two of which, neomethylbixin A and neomethylbixin C, were obtained in a crystalline state. The other isomers could be distinguished by their optical properties.

Neomethylbixin A crystallises from a mixture of benzene and methanol in long, narrow plates, m.p. 190–192° (corr.). It is more soluble and less stable than labile methyl bixin and exhibits maxima at 485 and 453 mμ in petroleum ether solution. Neomethylbixin C is obtained from a mixture of benzene and methanol in small, clustered needles, m.p. 150–151° (corr.). It exhibits absorption maxima at 479 and 448.5 mμ in petroleum ether solution.

1. *Stable norbixin* $C_{24}H_{28}O_4$:



Norbixin

The potassium salt of stable norbixin is obtained by boiling labile bixin with excess 10 % potassium hydroxide. The free acid separated on addition of hydrochloric acid to the aqueous solution[28]. The dinitrile of norbixin can be obtained from bixin dialdehyde dioxime (cf. under lycopene, pp. 118 and 123) and yields norbixin on hydrolysis with methanolic potassium hydroxide.

Stable norbixin crystallises from pyridine in glistening blue-red leaflets. It does not melt below 300°. It is fairly easily soluble in pyridine, very sparingly soluble in acetic acid and amyl alcohol, and almost insoluble in other organic solvents.

References p. 290–294.

Solvent	*Absorption maxima*		
Carbon disulphide	527.5	492	457.5 mμ
Chloroform	509	474,5	442 mμ

On adding alkali to a suspension of stable norbixin in water, it is converted into a very sparingly soluble, crystalline yellow salt. The action of diazomethane on norbixin yields a stable methylbixin. *Trans*-norbixin is stable in air. It dissolves in concentrated sulphuric acid with a greenish-blue colour.

Stable bixin, the monomethyl ester of stable norbixin $C_{25}H_{30}O_4$:

KARRER and co-workers[29] obtained stable bixin by allowing labile natural bixin to stand in chloroform solution in the presence of iodine. It crystallises from acetic acid or pyridine, or from acetone in flakes. M.p. 216–217° (uncorr., with decomposition). The solubility in organic solvents is considerably smaller than that of labile bixin.

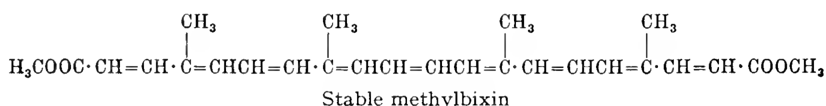
Solvent	*Absorption maxima*		
Carbon disulphide	526.5	491	457 mμ
Chloroform	509.5	475	443 mμ

(cf. Fig. 17, p. 355)

On shaking a solution of stable bixin in acetic acid and pyridine with zinc dust for a short time, dihydrobixin is obtained. The same dihydrobixin is also formed from labile bixin[30] under the same conditions.

Stable methylbixin, the dimethyl ester of stable norbixin $C_{26}H_{32}O_4$:

Stable methylbixin can be prepared either by the isomerisation of labile methylbixin in the presence of iodine[30, 31] or by the esterification of stable norbixin. The ester is also formed by shaking in air a solution of dihydromethylbixin in piperidine, or in pyridine containing a little sodium hydroxide[32].



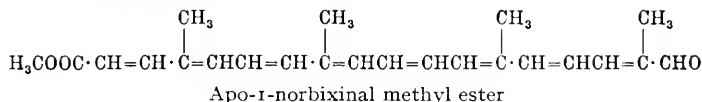
Stable methylbixin

Stable methylbixin crystallises from a mixture of chloroform and ethanol in stout, blue-violet needles, m.p. 205–206° (corr.). On shaking a solution in pyridine containing a little acetic acid with zinc dust at 50°, the same dihydromethylbixin is obtained as from labile methylbixin.

Solvent	*Absorption maxima*			
Carbon disulphide . . .	525.5	490	456.5 mμ	
Chloroform	509.5	475.5	444 mμ	
Hexane	484	450	425	405 mμ (cf. Fig. 19, p. 356)

Quantitative extinction measurements are reported by HAUSSER and SMAKULA[33]. The fluorescence spectrum is described by HAUSSER, R. KUHN and E. KUHN[34].

Stable apo-1-norbixinal methyl ester $C_{23}H_{28}O_3$:



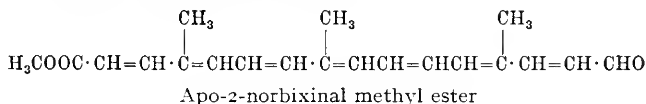
Apo-1-norbixinal methyl ester

This ester is obtained by the controlled permanganate oxidation of stable bixin[35]. The stable aldehyde is also formed by the isomerisation of labile apo-1-norbixinal methyl ester with iodine[35]. It crystallises in small prisms, m.p. 167°.

Solvent	*Absorption maxima*	
Carbon disulphide	509	478 mμ
Ethanol	487	456 mμ
Petroleum ether	472.5	445 mμ

Solvent	*Absorption maxima of oxime*	
Carbon disulphide	509	478 mμ
Ethanol	483	452 mμ
Petroleum ether	475	446 mμ

Stable apo-2-norbixinal methyl ester $C_{20}H_{24}O_3$:



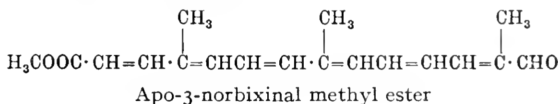
Apo-2-norbixinal methyl ester

This ester is obtained by the controlled oxidation of stable methylbixin with potassium permanganate[35]. Only the oxime and semicarbazone, but not the compound itself, has so far been obtained in a crystalline state.

Solvent	*Absorption maxima*	
Carbon disulphide	483.5	453 mμ
Petroleum ether	450	424 mμ

Solvent	*Absorption maxima of oxime*	
Carbon disulphide	481	451 mμ
Ethanol	459	mμ
	Absorption maxima of semicarbazone	
Carbon disulphide	493	462 mμ
Ethanol	471	mμ

Apo-3-norbixinal methyl ester $C_{18}H_{22}O_3$[35]:



Apo-3-norbixinal methyl ester

References p. 290–294.

The same apo-3-norbixinal methyl ester, m.p. 147° is formed by the controlled permanganate oxidation of stable or labile methylbixin (cf. p. 260).

It forms an oxime, m.p. 188° and a semicarbazone, m.p. 215°. On shaking an ethereal solution of the pigment with concentrated aqueous hydrochloric acid, no blue colouration is observed.

Solvent	*Absorption maxima*		
Carbon disulphide	455	427 mμ	
Petroleum ether	425	mμ	
Ethanol . . . . about	440	mμ	(cf. Fig. 17, p. 355)
	Absorption maxima of semicarbazone		
Carbon disulphide	472	443 mμ	
Ethanol	449	mμ	
	Absorption maxima of the oxime		
Carbon disulphide	458	428 mμ	
Petroleum ether	428	408 mμ	

2. *Labile norbixin* $C_{24}H_{28}O_4$:

Labile norbixin is obtained by the saponification of labile bixin[36] or of labile methylbixin[37]. It crystallises from acetic acid in stout red needles, m.p. 254–255°. Labile norbixin is readily soluble in pyridine, fairly readily soluble in acetic acid, ethanol and methanol, sparingly soluble in chloroform and methyl acetate, and almost insoluble in ether[36]. It readily dissolves in aqueous alkalis. Labile norbixin is autoxidisable in air[36]. By boiling a solution of the sodium salt with excess ammonia and 2.3 mols of titanium trichloride, KARRER and co-workers[36] obtained dihydronorbixin. If a larger proportion of titanium trichloride is used, or on prolonged boiling, tetrahydro- or hexahydro-norbixin is formed.

On prolonged heating with aqueous potassium hydroxide (less smoothly by employing ethanolic potassium hydroxide), labile norbixin is converted into stable norbixin[36]. By boiling with 3% methanolic hydrochloric acid, KARRER and TAKAHASHI[37] obtained stable methylbixin, whereas labile methylbixin is formed by the methylation of labile bixin with diazomethane[38]. On methylation of labile norbixin with dimethyl sulfate, labile bixin and labile methylbixin are obtained[39]. For the action of chlorine and hydrogen chloride on the pigment, compare the communication of HEIDUSCHKA and RIFFART[40].

Labile norbixin dissolves in concentrated sulphuric acid with a blue-green colour[41].

The mono-potassium salt of labile norbixin is micro-crystalline. It is insoluble in water and sparingly soluble in ethanol. The di-potassium salt forms brown-red needles, readily soluble in water. When moist, it is readily oxidised in air.

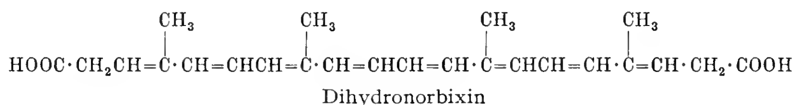
References p. 290–294.

Solvent	Absorption maxima		
Carbon disulphide	527	491	458 mμ
Chloroform	503	469.5	440 mμ

Dihydronorbixin $C_{24}H_{30}O_4$:

KARRER and co-workers prepared dihydronorbixin by boiling labile norbixin dissolved in 2.1 mols of dilute sodium hydroxide with excess ammonia and 2.3 mols of titanium trichloride[42]. Dihydronorbixin crystallises from ether in yellow clusters, which sinter at 197°. It readily dissolves in acetic acid, acetone and chloroform, but is only sparingly soluble in ether and almost insoluble in ligroin. It is readily oxidised in air.

The large displacement (about 70 mμ) of the absorption maxima on passing from norbixin to dihydronorbixin indicates that the two carboxyl groups are no longer conjugated with the system of conjugated double bonds and that the addition of hydrogen takes place at the 1:18 positions.



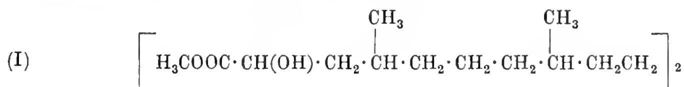
Dihydronorbixin

Solvent	Absorption maxima	
Carbon disulphide	454	428 mμ
Chloroform	435	410 mμ

Perhydronorbixin $C_{24}H_{46}O_4$ (3:7:12:16-tetramethyloctadecane-1:18-dicarboxylic acid):

This compound was obtained by HERZIG and FALTIS[43] by boiling perhydromethylbixin with potassium hydroxide. The total synthesis was achieved by KARRER and BENZ[44] by the method described above (p. 258). Perhydronorbixin is a viscous, colourless oil. B.p. 250°/0.3 mm., 245.5°/0.24 mm., 227°/0.03 mm.[45]. D_4^{20} 0.953; n_D^{20} 1.468[45]. It is insoluble in water. By esterification with diazomethane, or with a mixture of methanol and hydrogen chloride, HERZIG and FALTIS[46] obtained perhydromethylbixin.

α:α'-Dihydroxyperhydronorbixin dimethyl ester $C_{26}H_{50}O_6$ (I):

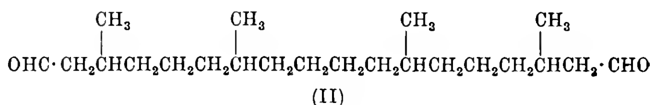
(I) 

KARRER and co-workers[47] converted perhydronorbixin into α:α'-dihydroxyperhydronorbixin dimethyl ester (I) by the action of bromine and red phosphorus followed by potassium hydroxide and diazomethane. The ester is obtained as an almost colourless oil, b.p. 213–216°/0.14 mm.

References p. 290–294.

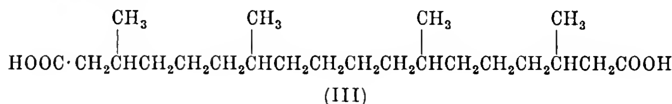
3:7:12:16-Tetramethyloctane-1:18-dial $C_{22}H_{42}O_2$ (II):

The dialdehyde is obtained from α:α′-dihydroxyperhydronorbixin dimethyl ester by the action of methyl magnesium iodide, followed by oxidation with lead tetraacetate[48].



The compound is a yellow oil with an intensive odour, reminiscent of ozone. B.p. 185°/0.3 mm.

2:6:11:15-Tetramethylhexadecane-1:16-dicarboxylic acid $C_{22}H_{42}O_4$ (III):

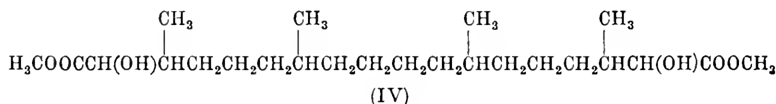


This dicarboxylic acid is obtained by the oxidation of 3:7:12:16-tetramethyloctadecane-1:18-dial with chromic oxide in acetic acid[49]. B.p. 220°/0.1.mm.

The diamide, $C_{22}H_{44}O_2N_2$ is prepared by converting the acid (III) by means of thionyl chloride into the acid chloride and treating the latter with concentrated aqueous ammonia[49]. It separates from ethyl acetate in crystals, m.p. 127°.

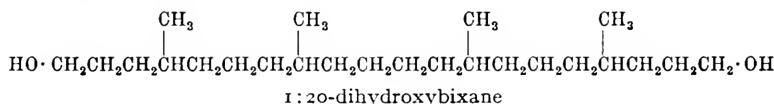
1:16-Dihydroxy-2:6:11:15-tetramethylhexadecane-1:16-dicarboxylic acid dimethyl ester $C_{24}H_{46}O_6$ (IV):

This ester was obtained by RAUDNITZ and PESCHEL[50] from 2:6:11:15-tetramethylhexadecane-1:16-dicarboxylic acid by the action of bromine and red phosphorus, followed by potassium hydroxide and diazomethane.



The ester (IV) is a colourless oil which can be distilled in high vacuum. Concerning its conversion into perhydrocrocetin, see p. 280.

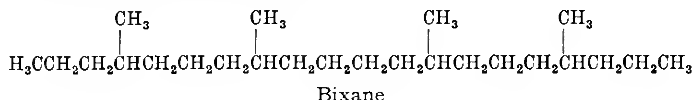
4:8:13:17-Tetramethyleicosane-1:20-diol (1:20-dihydroxybixane) $C_{24}H_{50}O_2$:



This diol was prepared by KUHN and EHMANN[51] by heating perhydromethylbixin with sodium and amyl alcohol. It is a pale yellow oil which partly

solidifies in the cold. B.p. 198°/0.12 mm. It is readily soluble in chloroform and benzene, but only dissolves in acetic acid, ethanol and petroleum ether on warming.

4:8:13:17-Tetramethyleicosane (Bixane) $C_{24}H_{50}$:



Bixane

Bixane can be prepared by heating 1:20-dihydroxybixane with 66% aqueous hydrobromic acid for 15 hours in a sealed tube at 230° and then warming the dibromide formed with activated zinc and 60% acetic acid at 100° for 14 hours. It is a mobile colourless liquid, which boils at 162°/0.51 mm (corr.) D_4^{20} 0.8054; n_D^{20} 1.4502. Bixane is readily soluble in chloroform, carbon disulphide and petroleum ether, and more sparingly soluble in ethanol and acetic acid.

Labile bixin, monomethyl ester of labile norbixin $C_{25}H_{30}O_4$:

Labile (natural) bixin crystallises from acetic acid in deep violet, dichroic prisms. From ethyl acetate the pigment is obtained in rhombs. On rapid heating it melts at 198°, on slow heating at 191.5°. 100 Ml of chloroform dissolve only 0.5 g of labile bixin at 18°. The pigment is even less soluble in ethanol, ether and cold acetic acid but readily soluble in boiling acetic acid, pyridine and nitrobenzene. 1 Litre of boiling ethyl acetate dissolves 4 g of labile bixin.

Solvent	*Absorption maxima*		
Carbon disulphide	523.5	489	457 mμ
Chloroform	503	469.5	439 mμ

Bixin remains unchanged for long periods on keeping in air, but undergoes some decomposition on heating to 110°. By heating above the melting point, VAN HASSELT[52] obtained *m*-xylene (cf. p. 49). The isomerisation of labile bixin into the stable form has been described on p. 259[53]. PUMMERER, REBMANN and REINDEL[54] found that only about six of the double bonds in bixin are saturated by reaction with perbenzoic acid.

With regard to a new method of oxidation of labile bixin with manganese acetate, see a communication by VIEBÖCK[55].

By the reduction of labile bixin with sodium amalgam, KARRER and co-workers[56] obtained a light yellow oil which on oxidation with alkaline potassium permanganate gave succinic acid.

With regard to the action of chlorine and bromine[57], iodine in benzene[58] iodine chloride[54], hydrogen chloride[59] and thiocyanogen[54] see the original communications.

References p. 290–294.

On treating labile bixin with methanolic potassium hydroxide, the potassium salt[60] is first obtained, but this is converted by prolonged shaking, or by boiling for a short time, into labile norbixin. By boiling with aqueous ethanolic potassium hydroxide, stable norbixin can be obtained besides the labile form[61].

Investigations by B. VON EULER, H. VON EULER and KARRER[62] have shown that labile bixin has no vitamin A activity.

The pigment dissolves in concentrated sulphuric acid with a cornflower-blue colour. For further colour reactions see the communication by KUHN and co-workers[63].

The sodium salt of labile bixin crystallises from 70 % ethanol in dark, copper-red crystals[64]. The potassium salt forms deep violet needles which are readily soluble in ethanol and methanol, but insoluble in water.

Dihydrobixin $C_{25}H_{32}O_4$:

KARRER and co-workers[65] prepared this compound by a method analogous to that employed for the preparation of dihydronorbixin (p. 265). KUHN and WINTERSTEIN[66] prepared dihydrobixin by brief shaking of labile or stable bixin in pyridine with zinc dust and acetic acid. M.p. 207–208° (uncorr.)[65] (For the constitution of dihydrobixin, see p. 265.)

Solvent	*Absorption maxima*	
Carbon disulphide	454	428 mμ
Chloroform	435	410 mμ

(cf. Fig. 20, p. 356)

Perhydrobixin, perhydronorbixin mono-methyl ester $C_{25}H_{48}O_4$:

This compound is formed by the catalytic hydrogenation of labile bixin in acetic acid in the presence of a palladium-barium sulfate catalyst[67]. It is a colourless oil, b.p. 213–217°/0.3 mm.[68] D_4^{20} 0.9368; n_D^{20} 1.4615.

Labile methylbixin, dimethylester of labile norbixin $C_{26}H_{32}O_4$:

Labile methylbixin can be obtained by the esterification of labile bixin or labile norbixin with dimethyl sulphate[69]. Labile methylbixin is also formed by the action of diazomethane on labile bixin dissolved in chloroform[70], or on labile norbixin[71]. It crystallises from ethyl acetate in red, pleochroic rhombs, m.p. 163° (uncorr.)[70]. It is fairly readily soluble in chloroform, acetic acid and ethyl acetate, sparingly in ethanol and very sparingly in methanol. For quantitative extinction measurements, see the communication by HAUSSER and SMAKULA[72].

Labile methylbixin can be converted into the stable form by the action of iodine in the same way as labile bixin[73]. Numerous investigations have been made regarding its oxidative degradation. For details, the original literature should be consulted[74].

By brief shaking of a solution of labile methylbixin in pyridine with acetic acid and zinc dust, KUHN and WINTERSTEIN[75] obtained dihydromethylbixin. KARRER and TAKAHASHI[76] found that by the saponification of labile methyl bixin with ethanolic sodium hydroxide (1 mol) at 65°, stable bixin is formed besides labile bixin. Labile methylbixin dissolves in concentrated sulphuric acid with an intense blue colour.

Dihydromethylbixin $C_{26}H_{34}O_4$:

This compound is obtained by briefly shaking a solution of labile or stable methylbixin in pyridine and acetic acid with zinc dust[77]. It crystallises in orange yellow leaflets, m.p. 180–182° (corr.). A piperidine solution of dihydromethyl bixin oxidises in air to give stable methylbixin[78]. (For constitution, see p. 265).

Solvent	*Absorption maxima*	
Carbon disulphide	454	428 mμ
Chloroform	435	410 mμ

(cf. Fig. 20, p. 356)

Perhydronorbixin dimethyl ester, perhydromethylbixin $C_{26}H_{50}O_4$:

Perhydromethylbixin was prepared by HERZIG and FALTIS by the catalytic hydrogenation of labile methylbixin[79]. It is also obtained by the methylation of perhydronorbixin with diazomethane, or with methanol and hydrogen chloride[79]. KARRER and co-workers[80] methylated perhydrobixin to the dimethyl ester by treatment with dimethylsulphate and aqueous potassium hydroxide in acetone. B.p. 211°/0.3 mm. D_4^{20} 0.9234; n_D^{20} 1.4568[81]. By heating with sodium and amyl alcohol, perhydromethylbixin is converted into 4:8:13:17-tetramethyleicosane-1:20-diol[82].

Perhydronorbixin diethyl ester $C_{28}H_{54}O_4$:

The total synthesis of this ester by KARRER and co-workers[83] has already been mentioned (p. 259). The compound is a colourless oil, b.p. 207°/0.3 mm.

Perhydronorbixin diamide $C_{24}H_{48}O_2N_2$:

Perhydronorbixin is converted into the acid chloride by means of phosphorous pentachloride or thionyl chloride and the acid chloride is treated with concentrated aqueous ammonia[84]. The diamide separates from ethyl acetate or ether in colourless crystals, m.p. 111°. It is almost insoluble in ether but soluble in ethanol and chloroform.

Perhydronorbixin bis-(2:4:6-tribromoanilide) $C_{36}H_{50}O_2N_2Br_6$:

The acid chloride of perhydronorbixin (see above) is reacted with 2:4:6-tribromoaniline[85] and the product is recrystallised from methyl acetate. The tribromanilide melts at 83°.

References p. 290–294.

Norbixin mono-ethyl ester, ethylnorbixin $C_{26}H_{32}O_4$:

Ethylnorbixin was prepared by VAN HASSELT by the saponification of labile bixin with ethanolic potassium hydroxide and treatment of the reaction product with diethyl sulphate. The diethyl norbixin which separated was then filtered and washed with dilute acid[86]. The ester crystallises from ethyl acetate in red needles with a green lustre, m.p. 176°. It forms a potassium salt which crystallises in needles and is insoluble in water.

Methylethylnorbixin $C_{27}H_{34}O_4$:

a) M.p. 149°. This form is obtained by the methylation of ethylnorbixin[87]. It crystallises in red rhombic crystals.

b) M.p. 138° (ethylbixin). This form is obtained by treating labile bixin in ethanol solution with one mol of potassium hydroxide and diethyl sulphate in the presence of ethyl acetate[88]. It separates from ethanol in red rhombic crystals. It is readily soluble in chloroform, ethyl acetate and acetone.

Diethylnorbixin $C_{28}H_{36}O_4$:

The preparation of this ester is similar to that of ethylnorbixin. Diethylnorbixin separates from acetone in blue crystals m.p. 121° [88].

Methyl-n-octylnorbixin (n-octyl bixin ester) $C_{33}H_{46}O_4$:

This ester was obtained by KARRER and OSWALD[89] by the action of *n*-octyl iodide on potassium bixinate. Dark violet crystals from ethanol, m.p. 132°.

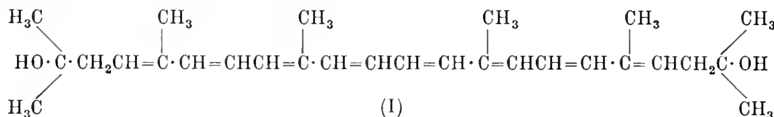
Methyl-n-butylnorbixin (n-butyl bixin ester) $C_{29}H_{38}O_4$:

This ester is prepared in the same way as the previously described derivative. Dark crystals, m.p. 160°.

Methyl-n-octadecyl norbixin (n-octadecyl bixin ester) $C_{43}H_{66}O_4$:

Dark crystals, m.p. 118° [89].

1:1:20:20-Tetramethyldihydrobixinol $C_{28}H_{42}O_2$ (I):



This compound was obtained by KARRER and RÜBEL[90] by reacting dihydrobixin methyl ester with methyl magnesium iodide. It crystallises from ethyl acetate in golden-yellow needles, m.p. 166–167° (uncorr.). It is entirely hypophasic in the partition test.

References p. 290–294.

Solvent	*Absorption maxima*	
Carbon disulphide	455	429 mμ
Chloroform	435	410 mμ

Labile apo-1-norbixinal methyl ester $C_{23}H_{28}O_3$:

KARRER and SOLMSSEN[91] subjected labile bixin to controlled permanganate degradation and obtained 3 different aldehydes which were identified as apo-1-, apo-2-, and apo-3-norbixinal methyl ester*. Apo-1-norbixinal methyl ester is obtained in the largest yield. M.p. 156°. Oxime, m.p. 186°. Semicarbazone, m.p about 225°.

Solvent	*Absorption maxima*	
Carbon disulphide	505	475 mμ
Petroleum ether	470	441 mμ
Ethanol	about 484	mμ

	Absorption maxima of the oxime	
Carbon disulphide	501	470 mμ
Ethanol	479	448 mμ

	Absorption maxima of the semicarbazone	
Carbon disulphide	515	487 mμ
Ethanol	487	460 mμ

Labile apo-2-norbixinal methyl ester $C_{20}H_{24}O_3$:

This compound was only formed in very small amounts[92] and could not be obtained in a crystalline state.

Solvent	*Absorption maxima*	
Carbon disulphide	479.5	449 mμ
Petroleum ether	446.5	421 mμ
Ethanol	wide bands	

	Absorption maxima of the oxime	
Carbon disulphide	478	449 mμ
Ethanol	456	mμ
Petroleum ether	447	mμ

	Absorption maxima of the semicarbazone	
Carbon disulphide	488.5	458 mμ
Ethanol	465.5	mμ

* The apo-3-norbixinal methyl ester was only obtained in one form.

2. CROCETIN $C_{20}H_{24}O_4$

History

1818 ASCHOFF[93] investigates the pigment of saffron and terms it crocin.
1852–1914 Different workers[94] carry out investigations on crocin. The glycosidic nature of crocin is recognised.
1915 DECKER[95] isolates the still inhomogeneous aglycon (crocetin) from crocin.
1927–33 KARRER and SALOMON[96] and KARRER and co-workers[97] elucidate the constitution of crocin and crocetin[98].

Occurrence

Crocin is the colouring principle of saffron which has been employed in different countries since early times. Crocin was shown by KARRER and MIKI[9] to be the digentiobiose ester of crocetin. Besides crocin, *Crocus sativus* contains small quantities of crocetin* and also of β-carotene, γ-carotene, lycopene and zeaxanthin. A colourless glycoside, picrocrocin (saffron bitter) which is closely related to crocin, has also been isolated[100].

TABLE 51

OCCURRENCE OF CROCETIN

Source	References
a) In blossoms:	
Crocus sativus L.	B. WEISS, *J. prakt. Chem.* (1) *101* (1867) 65; *Jb. Fortschr. d. Chem. 1867*, 733. — cf. B. QUADRAT, *J. prakt. Chem. 56* (1852) 68; *Jb. Fortschr. d. Chem. 1851*, 532.
Crocus albiflorus Kit., var. Neapolitanus hort.	R. KUHN, A. WINTERSTEIN and W. WIEGAND, *Helv. chim. Acta 11* (1928) 718.
b) In fruit:	
Gardenia grandiflora Lour.	F. ROCHLEDER and L. MAYER, *J. prakt. Chem.* (1) *72* (1857) 394; *74* (1858) 1; *Jb. Fortschr. d. Chem. 1857*, 490; *1858*, 475. — R. KUHN and co-workers, *Helv. chim. Acta 11* (1928) 718.
c) In blossom leaves:	
Cedrela Toona Roxb.	E. G. HILL and A. P. SIKKAR, *J. Chem. Soc. 91* (1907) 1501. — A. G. PERKIN, *J. Chem. Soc. 101* (1912) 1540. — R. KUHN and A. WINTERSTEIN, *Helv. chim. Acta 12* (1929) 496.
Crocus luteus	R. KUHN, A. WINTERSTEIN and W. WIEGAND, *Helv. chim. Acta 11* (1928) 718.
Nyctanthes Arbor-tristis	E. G. HILL and A. P. SIKKAR, *J. Chem. Soc. 91* (1907) 1501.
Verbascum phlomoides L.	L. SCHMID and E. KOTTER, *Monatsh. 59* (1932) 346, 353.

* Concerning the stereochemistry of natural crocetin from *Crocus sativus*, see R. KUHN and A. WINTERSTEIN, *Ber. 66* (1933) 209; *67* (1934) 348.

References p. 290–294.

Stereochemistry of Crocetin

The first observations on stereoisomers of crocetin are due to KUHN and WINTERSTEIN[101] who obtained, besides the known crocetin dimethyl ester of m.p. 222°, an isomer of m.p. 141°. The lower melting isomer is converted to the higher-melting, stable ester on illumination. The isomerisation can also be brought about by other means, e.g. heat, iodine catalysis, and *via* the dihydro-derivative. KUHN and co-workers regarded the isomerisation as a *cis-trans* change.

From the ease of conversion of the lower into the high-melting form, KUHN and WINTERSTEIN concluded that the former is a *cis* and the latter a *trans* isomer of crocetin. The following nomenclature has been proposed[102].

TABLE 52

NOMENCLATURE OF CROCETINS

New Nomenclature	M.p.	Configuration	Old name
Stable crocetin	285°	trans	α-Crocetin
Stable crocetin monomethyl ester	218°	trans	β-Crocetin
Stable crocetin dimethyl ester	222°	trans	γ-Crocetin
Labile crocetin dimethyl ester	141°	cis	Pigment of KUHN

Preparation

a) *Crocin:* According to KARRER and SALOMON[111], saffron is dried at 90° and pre-extracted with ether. The material is then extracted with ethanol and oily components are precipitated with ether. After further addition of ether, crocin gradually separates out in a microcrystalline form. Appreciable amounts of oily material are also obtained; these consist largely of crocin, but their crystallisation is rather difficult because of the presence of resinous components and takes a long time. By repeated dissolution of this oil in hot ethanol, seeding with crocin and standing, several crops of crystals can be obtained which are combined and re-crystallised from the same solvent.

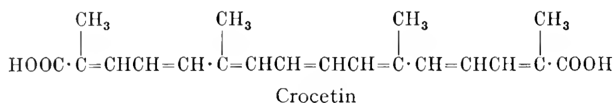
b) *Crocetin*[104]: 500 g of dried saffron is pre-extracted with ether. The residue is dried in air and extracted with 70% ethanol. About half the solvent is evaporated and the residual solution is strongly diluted with water. It is saponified with a solution of 30 g of potassium hydroxide and 500 ml of water. After acidification with hydrochloric acid, a thick yellow precipitate separates which is dried on porous tile and subsequently saponified with 10% ethanolic potassium hydroxide. The potassium salt of crocetin thus obtained is filtered and treated with acetic acid. For further purification, crude crocetin is recrystallised from pyridine.

With regard to the isolation of crocetin from blossom leaves of *Crocus luteus*, see the communication of KUHN and co-workers[105]. The isolation of crocetin dimethyl ester from saffron is described by KARRER and HELFENSTEIN[106].

References p. 290–294.

Chemical Constitution of Crocetin

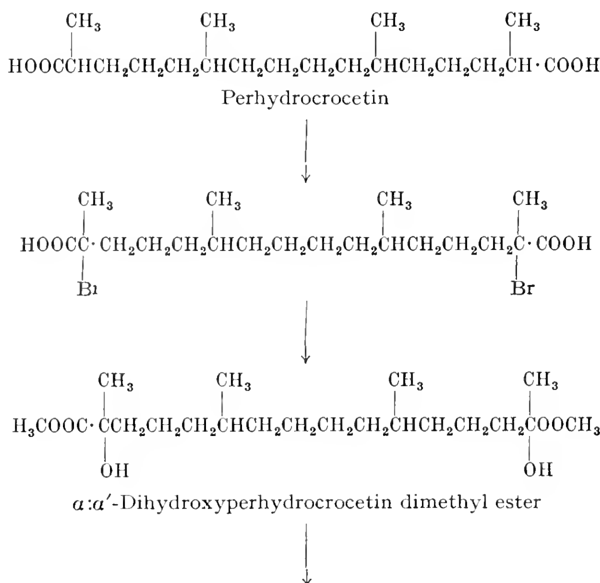
The constitution of crocetin was elucidated by KARRER, SALOMON and co-workers[96, 97, 98]. As a result of this work (and the investigations on bixin) a real insight into the principles governing the structure of carotenoids was obtained for the first time.



Crocetin

The main features of the constitution of crocetin were determined by KARRER and SALOMON[107]. These authors recognised the polyene nature of the pigment and the presence of a system of conjugated double bonds with side-chain methyl groups, and identified perhydrocrocetin as an aliphatic, saturated, dicarboxylic acid. The molecular formula $C_{20}H_{24}O_4$ was proposed by KUHN and L'ORSA[108] and confirmed by KARRER and co-workers. KARRER and SALOMON[107] established the presence of 7 double bonds in the crocetin molecule by means of catalytic hydrogenation and KUHN and L'ORSA[109] established the presence of 4 side-chain methyl groups by chromic acid degradation. The latter authors proposed an 'unsymmetrical' structure for crocetin, whereas KARRER and co-workers[110] put forward the formula shown above, the correctness of which was proved by the degradation of perhydrocrocetin to 6:11-dimethylhexadecane-2:15-dione[111] and by the total synthesis of perhydrocrocetin[112].

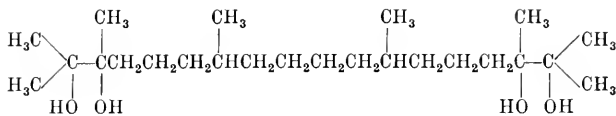
Degradation of perhydrocrocetin to 6:11-dimethylhexadecane-2:5-dione:

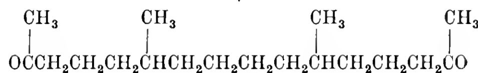


Perhydrocrocetin

α:α'-Dihydroxyperhydrocrocetin dimethyl ester

References p. 290–294.





6:11-Dimethylhexadecane-2:15-dione

Total synthesis of perhydrocrocetin[112]:

$$HOCH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2OH$$

$$\downarrow$$

$$HOCH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2OC_2H_5$$

$$\downarrow$$

$$BrCH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2OC_2H_5$$

$$\downarrow$$

$$(HOOC)_2CHCH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2OC_2H_5$$

$$\downarrow$$

$$HOOC\cdot CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2OC_2H_5$$

Electrolysis

$$\downarrow$$

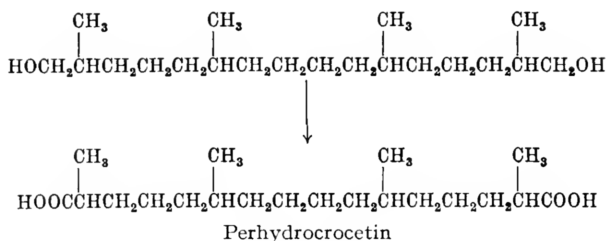
$$H_5C_2OCH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2OC_2H_5$$

$$\downarrow$$

$$BrCH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2Br$$

$$\downarrow$$

References p. 290–294.



Perhydrocrocetin

Properties

Stable Crocetin $C_{20}H_{24}O_4$:

The pigment is obtained from acetic anhydride in brick-red rhombs, m.p. 285°. It is very sparingly soluble in the common organic solvents, but fairly soluble in pyridine and in very dilute alkalis. Crocetin is fairly stable in air in the solid state; the surface of the crystals is decolourised, however, under the influence of light[113]. In alkaline solution on the other hand, the pigment absorbs oxygen from the air even at 20°; this oxidation is considerably accelerated by haemin[114]. With concentrated sulphuric acid, crocetin gives a deep blue colour which soon changes into violet and eventually into brown*. No colouration is observed with hydrochloric acid.

For the separation of crocetin from other carotenoids, see the communication by KUHN and BROCKMANN[115]. A microchemical method of identification is described by TUNMANN[116].

Solvent	*Absorption maxima*			
Carbon disulphide	482	453	426	mμ
Pyridine	464	436	411	mμ
Chloroform	463	434,5		mμ
Petrol	450.5	424.5		mμ
Hexane	445	420	400	mμ

(cf. Fig. 22, p. 357)

Disodium salt of crocetin $C_{20}H_{22}O_4Na_2$:
Orange-yellow needles[117].

Dipotassium salt of crocetin $C_{20}H_{22}O_4K_2$:
This compound is obtained from aqueous ethanol in yellow crystals[117].

Diammonium salt of crocetin $C_{20}H_{22}O_4(NH_4)_2$:
Red needles from ammoniacal aqueous ethanol[117].

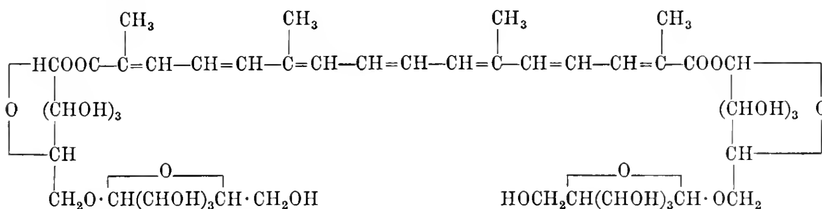
Dipyridine salt of crocetin $C_{20}H_{24}O_4 . 2C_5H_5N$:
Dark red plates from aqueous pyridine[118].

Crocin was first obtained in the crystalline state by KARRER and SALOMON[119]. It was recognised as the digentiobiose ester of crocetin by KARRER and MIKI[120].

* Further colour reactions are described by P. KARRER and co-workers, *Helv. chim. Acta* 11 (1928) 1201.

The sugar residues of crocin are very readily split off by alkalis. On working in aqueous media, crocetin is obtained, whereas in the presence of methanol, methyl esters of the pigment are formed[121].

Crocin $C_{44}H_{64}O_{24}$:



Crocin dissolves readily in water to give an orange-red solution. M.p. 186° (with frothing). Crystals of crocin contain water of crystallisation which is only given up on prolonged drying in vacuum at 100°.

Stable crocetin monomethyl ester (β-crocetin) $C_{21}H_{26}O_4$:

This mono-ester is obtained either by the replacement esterification of crocin with 70% methanol and potassium hydroxide, or by the esterification of crocetin with dimethyl sulphate[122]. The ester crystallises from chloroform in rectangular leaflets, m.p. 218°.

Stable crocetin dimethyl ester (γ-crocetin) $C_{22}H_{28}O_4$:

This ester is best prepared by the replacement esterification of crocin[122], but it can also be obtained by the esterification of crocetin with diazomethane[123]. Hexagonal leaflets, m.p. 222.5° (corr.)[124]. (With regard to the isomerisation of labile crocetin dimethyl ester, see p. 273). Crocetin dimethyl ester can be distilled unchanged in vacuum. The dry-distillation is described by KUHN and WINTERSTEIN[125].

Solvent	*Absorption maxima*[126]	
Petrol	450.5	424.5 mμ
Chloroform	463	434.5 mμ

(cf. Fig. 18, p. 355)

At 20°, one part of crocetin dimethyl ester dissolves in 100,000 parts of methanol. The solubility in ether is also very small.

Labile crocetin dimethyl ester $C_{22}H_{28}O_4$:

According to KUHN and WINTERSTEIN[127], labile crocetin occurs esterified with gentiobiose in the blossoms of saffron (*Crocus sativus*). It has only been isolated in the form of its dimethyl ester.

The labile dimethyl ester is formed together with the stable form by the action of dilute sodium hydroxide on a methanolic extract of saffron. The

separation of the two forms depends on the greater solubility of the labile form in ether. The ester crystallises from methanol in elongated rectangular plates, m.p. 141°. Under the microscope the crystals appear yellow. One part of the labile ester dissolves in 5900 parts of methanol. Its behaviour towards light, iodine, or heat has already been described on p. 273. Reduction with zinc dust and acetic acid in pyridine yields a dihydroderivative which is identical with that obtained from the stable ester. By saponification with warm ethanolic potassium hydroxide, stable crocetin is formed.

Solvent	*Absorption maxima*	
Petrol	445	422 mμ
Chloroform	458	432.5 mμ

Tricyclocrocetin $C_{22}H_{24}O_4$(?):

This compound was obtained by KUHN and WINTERSTEIN[128] by the dry-distillation of stable crocetin dimethyl ester in vacuum, followed by chromatography and saponification. It crystallises from methanol in colourless needles, m.p. 263–264°. On oxidation with chromic acid it yields 2.5 mols of acetic acid. Catalytic hydrogenation shows the presence of 4 double bonds. For the light absorption curve, the original communication should be consulted.

Perhydrocrocetin dimethyl ester $C_{22}H_{42}O_4$:

This perhydro-compound was obtained by KARRER and SALOMON by the catalytic reduction of stable crocetin dimethyl ester[129]. It is a viscous oil which partly solidifies in the form of crystals, m.p. 27° [130].

Perhydrocrocetin dimethyl ester boils at 198–210° at 1 mm and 180–185° at 0.05 mm. For the light absorption curve, see the communication by KARRER and SALOMON[131].

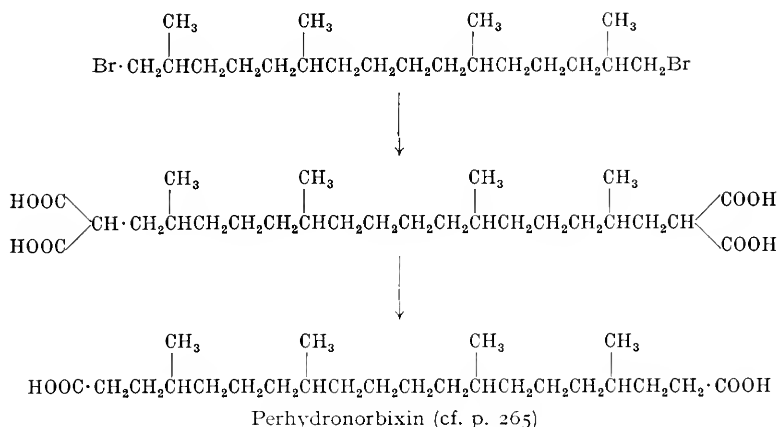
2:6:11:15-Tetramethylhexadecane-1:16-diol $C_{40}H_{42}O_2$:

This diol was obtained by KARRER and co-workers[132] by the reduction of perhydrocrocetin dimethyl ester with sodium in alcohol according to Bouveault-Blanc.

Colourless oil, b.p. 180–181°/0.1 mm (uncorr.).

2:6:11:15-Tetramethylhexadecane-1:16-diol is converted by the action of hydrogen bromide into 1:16-dibromo-2:6:11:15-hexadecane, from which perhydronorbixin can be obtained by condensation with sodium malonic ester, saponification of the product and decarboxylation by heating to 200°[133].

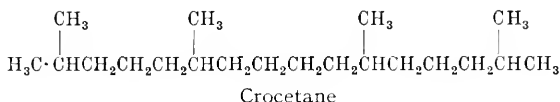
References p. 290–294.



Perhydronorbixin (cf. p. 265)

2:6:11:15-Tetramethylhexadecane (Crocetane) $C_{20}H_{42}$:

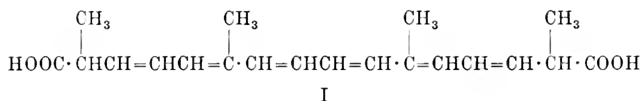
KARRER and GOLDE prepared crocetane from 1:16-dibromo-2:6:11:15-tetramethylhexadecane by reduction with coppered zinc and dilute acetic acid[134].



Crocetane

Crocetane is a colourless oil, b.p. 135°/0.5 mm. It is readily soluble in petroleum ether, chloroform and carbon disulphide and more sparingly soluble in ethanol and acetic acid.

6:11-Dimethylhexadeca-3:5:7:9:11:13-hexaen-2:15-dicarboxylic acid. Dihydrocrocetin $C_{20}H_{26}O_4$ (I):



I

Dihydrocrocetin was prepared by KARRER, HELFENSTEIN and WIDMER[135] by the reduction of crocetin with titanium trichloride in dilute sodium hydroxide and aqueous ammonia. It crystallises from ether in stout yellow needles, m.p. 192–193°. It is readily soluble in ethanol and acetic acid, more sparingly soluble in ether, and very sparingly soluble in water, ligroin and benzene. Dihydrocrocetin is rapidly oxidised in air. With concentrated sulphuric acid, a wine-red colouration with a blue tinge is produced. Quantitative extinction measurements in ethanol solution are reported by HAUSSER and SMAKULA[136] (cf. Fig. 21, p. 356).

References p. 290–294.

Dihydrocrocetin dimethyl ester $C_{22}H_{30}O_4$:

This ester was obtained by KARRER and HELFENSTEIN[137] by the action of diazomethane on dihydrocrocetin and by KUHN and WINTERSTEIN by hydrogenation of stable[138] or labile[139] crocetin dimethyl ester in pyridine with zinc dust and acetic acid.

This compound separates from ether in sulphur-yellow crystals, m.p. 96°. On shaking a solution in piperidine with air, stable crocetin dimethyl ester is rapidly formed[138]. If the pigment is dissolved in pyridine and little sodium hydroxide is added, a deep blue colouration is immediately produced. This rapidly changes to orange-red on contact with air, due to the oxidation of the dihydroderivative to the stable crocetin dimethyl ester[140].

Dihydrocrocetin diethyl ester $C_{24}H_{34}O_4$:

This ester is prepared by treating dihydrocrocetin with diazoethane[141]. M.p. 62°. Dihydrocrocetin diethyl ester is very readily soluble in organic solvents.

Hexahydrocrocetin $C_{20}H_{30}O_4$:

Hexahydrocrocetin was prepared by KARRER and co-workers[142] by the reduction of crocetin with excess titanium trichloride. It is a light yellow oil which is very stable towards atmospheric oxygen. On catalytic hydrogenation it is converted into perhydrocrocetin. On addition of concentrated sulphuric acid a brown-red colouration is produced.

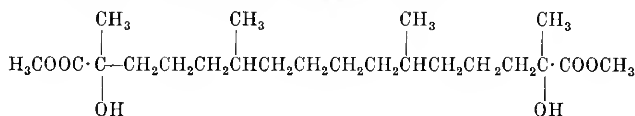
6:11-Dimethylhexadecane-2:15-dicarboxylic acid, perhydrocrocetin $C_{20}H_{38}O_4$:

The total synthesis of this compound has already been described (p. 275). It is also formed by the catalytic hydrogenation of crocetin[143]. Perhydrocrocetin has also been prepared from 1:16-dihydroxy-2:6:11:15-tetramethylhexadecane-1:16-dicarboxylic acid dimethyl ester (cf. p. 266)[144]. The ester was treated with methyl magnesium iodide, the tetrahydroxyderivative formed was reacted with lead tetraacetate, and the resulting dialdehyde was oxidised with chromic acid and acetic acid.

Perhydrocrocetin diamide $C_{20}H_{40}O_2N_2$:

Perhydrocrocetin is converted into the acid chloride by means of thionyl chloride and the acid chloride is reacted with concentrated aqueous ammonia[144, 145]. M.p. 130°.

a:a'-Dihydroxyperhydrocrocetin dimethyl ester $C_{22}H_{42}O_6$ (I):



This ester is prepared by the action of bromine and red phosphorous on perhydrocrocet in, conversion of the dibromide into the diol, and esterification with diazome th ane. It is a viscous, colourless oil, b.p. 165°/0.04 mm.

6:11-Dimethylhexadecane-2: 15-dione $C_{18}H_{34}O_2$:

This compound is prepared from the diester (I)[146] described above. The diketone is a liquid with a weakly aromatic smell. B.p. 132–135°/0.05 mm. It forms a disemicarbazone, $C_{20}H_{40}O_4N_6$, which separates from ethanol in crystals, m.p. 168°.

Crocetin tetrabromide $C_{20}H_{24}O_4Br_4$:

This compound is obtained from crocetin by the action of bromine dissolved in chloroform[147]. It separates from a mixture of ethanol and ether in yellow crystals, m.p. 103–104° (with decomposition). It is readily soluble in chloroform, ethanol, ether and acetic acid.

3. AZAFRIN $C_{27}H_{38}O_4$

History

1885 MAISCH[148] is the first to describe the pigment of the roots of *Escobedia scabrifolia*. He names the pigment escobedine.

1911 LIEBERMANN[149] succeeds in isolating escobedine in the crystalline form. He proposes a new name, azafrin, for the pigment.

1913–16 LIEBERMANN and SCHILLER[150] and LIEBERMANN and MÜHLE[151] attempt to elucidate the constitution of azafrin.

1931–33 KUHN and co-workers[152] carry out investigations on azafrin in the course of which a structural formula is proposed and proved.

Occurrence

Up to the present time, azafrin, has only been found in two South American plants, *Escobedia scabrifolia* and *Escobedia linearis*[153]. Most of the pigment is contained in the roots, but some is also present in the stalks. Under the name of "azafran" or "azafranillo", azafrin is used in Paraguay for the colouring of fats.

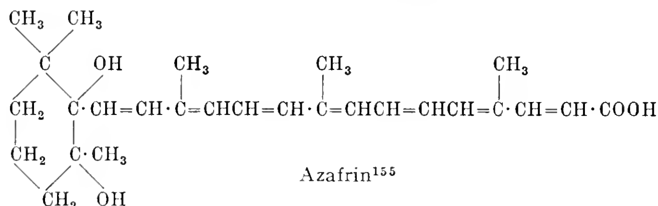
Preparation[154]

The finely ground material is extracted with benzene or chloroform in an extraction apparatus, the solution is strongly concentrated and set aside in the cold. After about 24 hours the red pigment crystallises out. It is dissolved in 0.1 N alcoholic potassium hydroxide, the solution is filtered, and the pigment is precipitated with dilute acetic acid and recrystallised from toluene. From 3 kg of azafran, 7.5 g of pure pigment is obtained.

References p. 290–294.

According to a more recent procedure due to KUHN and WINTERSTEIN[155], the escobedia roots are first roughly ground, then finely ground in a spherical mill and exhaustively extracted with acetone in a Soxhlet apparatus. The dark brown solution is allowed to stand overnight, filtered to separate gelatinous material, and evaporated to a small volume in vacuum. The liquid residue soon solidifies to a crystalline mass which is filtered after addition of toluene. For further purification, the crude pigment is twice recrystallised from a mixture of acetone and toluene. Azafrin can also be purified by chromatographic adsorption on calcium carbonate from a petrol-benzene mixture. A less wasteful procedure consists of extracting the impurities with petrol from an alkaline solution of azafrin.

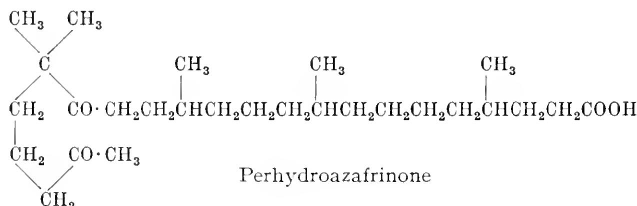
Chemical Constitution



Azafrin[155]

The formula of azafrin was proposed by KUHN and co-workers and is proved by the following facts:

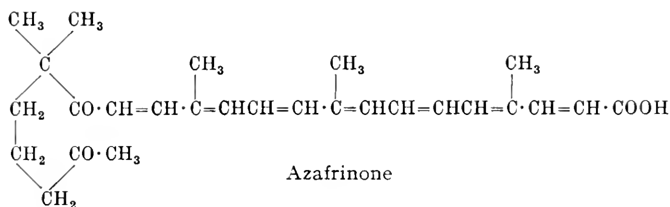
Azafrin is a monocyclic carboxylic acid containing 7 double bonds which must be conjugated amongst themselves and with the carboxyl group[156]. The other two oxygen atoms are present in the form of 2 hydroxyl groups. These are tertiary in nature and must occupy neighbouring positions since on treatment with lead tetraacetate according to CRIEGEE[157], tetradecahydroazafrin yields a diketone (perhydroazafrinone)[155]:



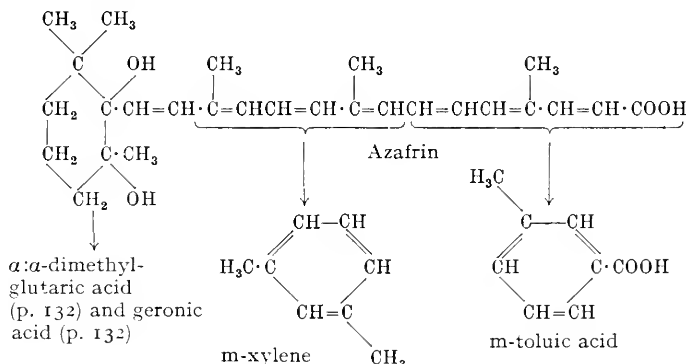
Perhydroazafrinone

On careful oxidation with chromic acid, azafrin yields a diketone which was termed azafrinone by KUHN and DEUTSCH[158]. Since this compound is optically inactive, it can be concluded that the rotatory power of azafrin is due to the two hydroxyl-bearing carbon atoms. Azafrinone absorbs at slightly longer wavelengths than azafrin. As the difference is of about the same magnitude as between β-carotene and β-semicarotenone (p. 139), it may be concluded that one of the carbonyl groups of azafrinone is in conjugation with the system of conjugated double bonds.

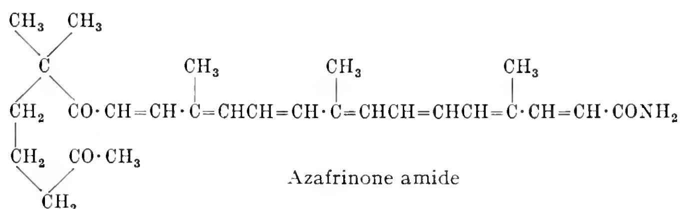
References p. 290–294.



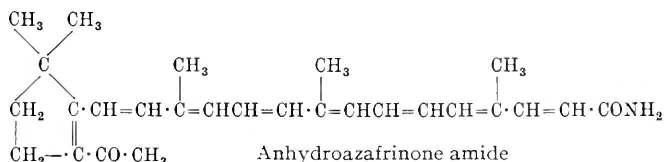
On ozonisation azafrin yields α:α-dimethylglutaric acid and geronic acid. Chromic acid oxidation shows the presence of four side-chain methyl groups, the position of which follows from the nature of the three products obtained on thermal decomposition, namely *m*-xylene, toluene and *m*-toluic acid[159].



The formula of azafrin was further confirmed by KUHN and BROCKMANN by the conversion of azafrin into anhydroazafrinone amide which has also been obtained from β-carotene (cf. p. 134), thus proving the relation between the two pigments[160]. Chromic oxidation of azafrin yields azafrinone, which is converted via the acid chloride into azafrinone amide:



By the action of potassium hydroxide, azafrinone amide is converted into anhydroazafrinone amide.



References p. 290–294.

Properties

Crystalline form: Azafrin crystallises from benzene in microscopic orange-red needles combined into clusters. From toluene it crystallises in prisms.

Melting point: 212–214° (corr.).

Solubility: The pigment is insoluble in water, but dissolves in dilute alkali or alkali carbonate solutions. Azafrin is fairly soluble in chloroform, ethanol, acetic acid and benzene, but only very sparingly in ether.

Solvent	*Absorption maxima*	
Chloroform	458	428 mμ
Pyridine	458	428 mμ
Sodium hydroxide	447	422 mμ

Optical activity: $[\alpha]^{20}_{643.8} = -75°$ (in ethanol, c = 0.28). The optical rotation is not appreciably altered by the addition of boric acid[161].

Colour reactions:* Azafrin dissolves in concentrated sulphuric acid with an intense blue colour which changes to violet on addition of alcohol. On dissolving the pigment in acetic acid and adding concentrated hydrochloric acid, a violet colouration is produced after short boiling or on standing for several hours. On passing hydrogen chloride into a saturated chloroform solution, the latter assumes a cornflower-blue colour. Antimony trichloride in chloroform solution produces an emerald green colouration which changes into blue.

Partition test: Azafrin exhibits entirely hypophasic properties.

Chromatographic behaviour: Azafrin can be chromatographed on calcium carbonate from benzene-petrol solution. The chromatogram is developed with benzene. A mixture of benzene and methanol, or of pyridine and methanol is used for elution[162].

Derivatives

Azafrinone $C_{27}H_{36}O_4$ (Formula p. 283):

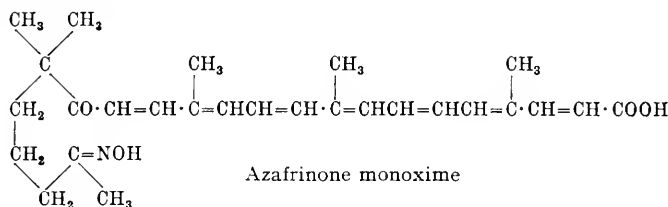
KUHN and DEUTSCH[163] obtained azafrinone by the oxidation of azafrin in acetic acid and benzene with 0.1 N chromic acid solution. The diketone can also be prepared by the saponification of azafrinone methyl ester[163]. It separates from acetone in orange-red plates or needles, m.p. 191° (corr.). On catalytic hydrogenation, azafrinone absorbs 9 mols of hydrogen. Azafrinone is optically inactive.

* R. KUHN, A. WINTERSTEIN and H. ROTH compare the colour reactions of azafrin, crocetin and norbixin, *Ber. 64* (1931) 333.

References p. 290–294.

Solvent	Absorption maxima	
Carbon disulphide	483	452 mμ
Chloroform	472	440 mμ
Petrol	454	429 mμ

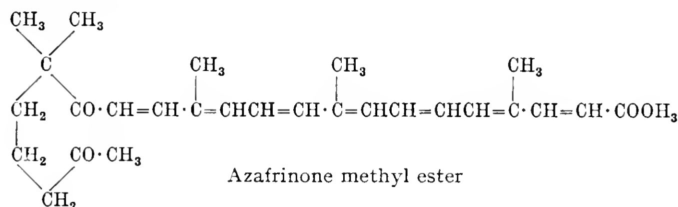
Azafrinone monoxime $C_{27}H_{37}O_4N$:



Azafrinone monoxime

Like semi-β-carotenone, azafrinone only forms a monoxime. It separates from acetone in crystals, m.p. 194° (corr.)[163].

Azafrinone methyl ester $C_{28}H_{38}O_4$:



Azafrinone methyl ester

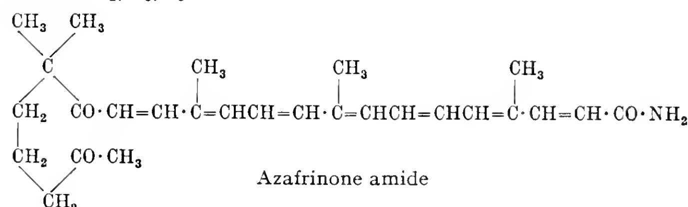
This compound is obtained by the esterification of azafrinone suspended in ether with diazomethane[164], or by the oxidation of azafrin methyl ester with chromic acid[164].

The ester crystallises from petrol in red needles, m.p. 112° (corr.). It is sparingly soluble in hexane, somewhat more soluble in ethanol, ether and acetic acid, and easily soluble in chloroform, acetone, benzene, and pyridine. The chromatographic behaviour of azafrinone methyl ester is similar to that of azafrin methyl ester.

Solvent	Absorption maxima	
Carbon disulphide	483	452 mμ
Chloroform	472	440 mμ
Petrol	454	429 mμ

(cf. Fig. 23, p. 357)

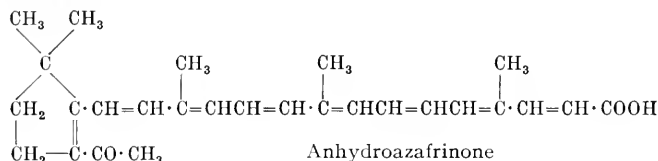
Azafrinone amide $C_{27}H_{37}O_3N$:



Azafrinone amide

KUHN and BROCKMANN[165] converted azafrinone into the acid chloride by means of thionyl chloride and allowed the acid chloride to stand in benzene solution with ammonia. The reaction product was purified by chromatography on a calcium carbonate column from benzene solution. The amide crystallises from methanol in red needles, m.p. 177–178°. It is sparingly soluble in hexane, petroleum ether and petrol, but readily soluble in chloroform, carbon disulphide and hot benzene or methanol.

Anhydroazafrinone $C_{27}H_{34}O_3$:



Anhydroazafrinone

Anhydroazafrinone is obtained by the action of potassium hydroxide on azafrinone[166]. It crystallises from dilute methanol in dark red prisms, m.p. 196°. The solubility in hexane, petroleum ether and petrol is very small; it is somewhat better in chloroform, carbon disulphide, hot benzene or hot methanol.

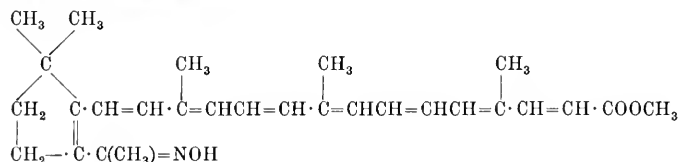
Solvent	*Absorption maxima*		
Carbon disulphide	511	476	447 mμ
Hexane	478	449	420 mμ
Benzene	493	460	430 mμ
Petroleum ether	477	447	419 mμ
Chloroform	493	459	433 mμ
Ethanol	(479)	(449)	mμ (diffuse)

Anhydroazafrinone methyl ester $C_{28}H_{36}O_3$[167]:

This ester is prepared by the esterification of anhydroazafrinone with diazomethane. It separates from dilute methanol in crystals, m.p. 153°.

Solvent	*Absorption maxima*		
Petrol	479	448	420 mμ

Anhydroazafrinone oxime methyl ester $C_{28}H_{37}O_3N$[167]:



Anhydroazafrinone oxime methyl ester is obtained by treating anhydroazafrinone methyl ester with excess hydroxylamine and a little alkali. For

References p. 290–294.

purification, the reaction mixture is chromatographed on alumina from benzene solution. The derivative crystallises from dilute methanol in dark red leaflets with a violet lustre. M.p. 149–150°.

Solvent	*Absorption maxima*		
Petrol	476	447	420 mμ

Anhydroazafrinone amide $C_{27}H_{35}O_2N$:[168]

The preparation of anhydroazafrinone amide from β-carotene or azafrinone has already been described (pp. 134 and 283). The amide crystallises from methanol in red-violet needles, m.p. 215°. It is sparingly soluble in hexane, petroleum ether and petrol, but somewhat more easily soluble in chloroform and hot benzene.

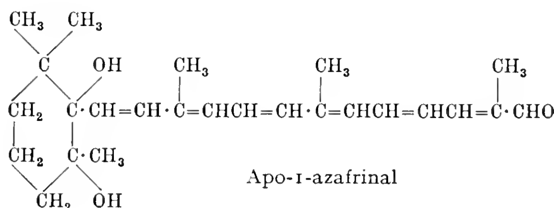
Solvent	*Absorption maxima*		
Carbon disulphide	508	474	444 mμ
Hexane	475	444	419 mμ
Petroleum ether	473	443	418 mμ
Benzene	477	447	420 mμ
Chloroform	492	459	430 mμ
Ethanol	(481)	(450)	mμ

Perhydroazafrin $C_{27}H_{52}O_4$:

Perhydroazafrin can be obtained either by the catalytic hydrogenation of azafrin[169] or by the saponification of perhydroazafrin methyl ester[170]. It is a colourless, viscous oil which can be distilled in high vacuum.

Optical rotation in ethanol: $[\alpha]_D^{20} = -6.7°$ (c = 3.2)[169].

Apo-1-azafrinal $C_{25}H_{36}O_3$:



Apo-1-azafrinal

This aldehyde was obtained by KARRER, OBST and SOLMSSEN[171] by the careful permanganate oxidation of azafrin. It crystallises from benzene in orange-yellow needles, m.p. 171°.

Solvent	*Absorption maxima*
Carbon disulphide	461 mμ
Petroleum ether	431 mμ
Ethanol	diffuse

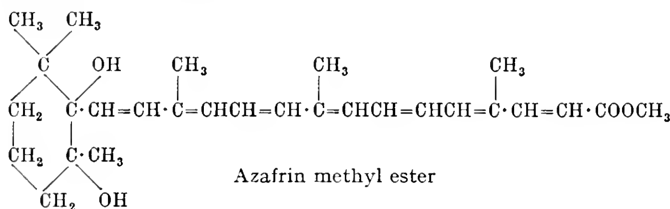
References p. 290–294.

Apo-1-azafrinal oxime $C_{25}H_{37}O_3N$[171]:

Melting point: 185°.

Solvent	*Absorption maxima*	
Carbon disulphide	445	416 mμ
Petroleum ether	415	mμ
Ethanol	423	mμ

Azafrin methyl ester (methylazafrin) $C_{28}H_{40}O_4$[172]:



Azafrin methyl ester

This ester is prepared by the esterification of azafrin with dimethylsulphate and potassium hydroxide.

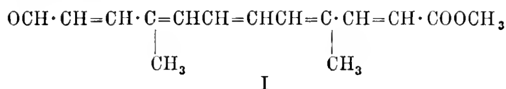
The ester crystallises from methanol, or from a mixture of methanol and ether or acetic acid, in yellow-red leaflets or needles, m.p. 191°. It is very readily soluble in chloroform, and easily soluble in all organic solvents with the exception of petroleum ether and ligroin. Optical rotation in chloroform $[\alpha]^{22}_{643.8} = -32°$.

Solvent	*Absorption maxima*		
Petrol	447	442.5	mμ
Chloroform	458	428	mμ
Carbon disulphide	476	445.5	419 mμ

(cf. Fig. 23, p. 357)

With hydrogen chloride, hydrogen bromide, hydrogen iodide, perchloric acid, sulphuric acid or trichloracetic acid in acetic acid solution, azafrin methyl ester gives coloured addition products from which it cannot be regenerated[172]. With methyl magnesium iodide, 2 mols of methane are evolved[173]. In biological tests methylazafrin exhibits no vitamin A activity[174].

3:8-Dimethylundecapentaene-11-al-1-carboxylic acid methyl ester ("azafrinal I" methyl ester) $C_{15}H_{18}O_3$ (I):



I

This ester is formed besides other products by the chromic acid oxidation of methylazafrin[175].

References p. 290–294.

It crystallises from dilute methanol in light yellow needles, m.p. 159–160°. It is readily soluble in chloroform, ethanol, carbon disulphide and benzene, and somewhat less readily soluble in petrol, petroleum ether and hexane.

Solvent	*Absorption maxima*
Carbon disulphide	421 mμ
Chloroform	411 mμ
Benzene	410 mμ

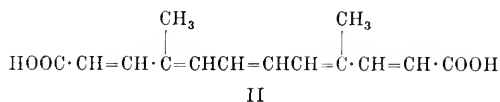
The *oxime* is obtained from the aldehyde on standing with hydroxylamine in ethanol. It crystallises from dilute methanol in yellow needles with a blue surface lustre.

Melting point: 206–207°.

Solvent	*Absorption maxima*
Carbon disulphide	425 mμ
Chloroform	413 mμ
Benzene	412 mμ

3:8-Dimethyldecapentaene-1:10-dicarboxylic acid $C_{14}H_{16}O_4$ (II):

The oxime described above is converted to the corresponding nitrile by boiling with acetic anhydride[176]. Hydrolysis of the nitrile yields the acid II.



It forms yellow needles, m.p. 267–268°. It is insoluble in hexane, petroleum ether and petrol, sparingly soluble in chloroform, ethanol, carbon disulphide and benzene, and readily soluble in pyridine.

Solvent	*Absorption maxima*
Carbon disulphide	419 mμ

The potassium salt forms pale yellow leaflets.

Dimethyl ester $C_{16}H_{20}O_4$:

Formed by the esterification of the acid II with diazomethane[176]. Melting point: 175–176°.

Solvent	*Absorption maxima*
Carbon disulphide	419 mμ

Methyl ester nitrile $C_{15}H_{17}O_2N$:

The preparation of this nitrile is described under 3:8-dimethyldecapentaene-1:10-dicarboxylic acid. Golden yellow prisms from dilute methanol. Melting

References p. 290–294.

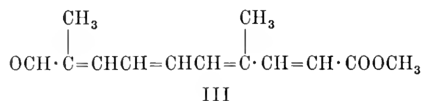
point 165° [177]. The nitrile is readily soluble in chloroform, benzene and hot methanol and sparingly soluble in cold methanol, petrol and petroleum ether.

Solvent	*Absorption maxima*
Carbon disulphide	413 mμ

Mono-amide $C_{14}H_{17}O_3N$:

The amide is formed by boiling the methyl ester nitrile with potassium hydroxide[177]. It crystallises from methanol in yellow prisms, m.p. 256–257°.

2:7-Dimethylnonatetraen-1-al-9-carboxylic acid methyl ester ("azafrinal-II" methyl ester) $C_{13}H_{16}O_3$ (III):



This compound is formed besides other products during the chromic acid oxidation of azafrin methyl ester[178]. It is purified by repeated chromatography on alumina. It crystallises from 70 % methanol in light yellow prisms, m.p. 106°.

It forms an oxime, $C_{13}H_{17}O_3N$, which crystallises from dilute methanol in light yellow prisms, m.p. 194° [178].

Perhydroazafrin methyl ester $C_{28}H_{54}O_4$:

KUHN, WINTERSTEIN and ROTH[179] prepared this perhydroester by the catalytic hydrogenation of azafrin methyl ester. Colourless, viscous oil, b.p. 180–200°/1 mm. $[\alpha]_D^{20}$ = —9.0° (in ethanol).

Azafrin ethyl ester $C_{29}H_{42}O_4$:

Azafrin ethyl ester is obtained by esterification of azafrin with diethyl sulphate[180]. It crystallises from ethanol in red prisms, m.p. 182° (corr., with decomposition).

REFERENCES

1. J. B. BOUSSINGAULT, *Ann. Chim.* (2) *28* (1825) 440.
2. Cf. WEHMER, *Pflanzenstoffe*, 2nd edition, vol. 2, 796. — F. CZAPEK, *Biochemie*, 2nd ed. vol. 3, 576. — KARNOT, *Dissertation*, Leipzig 1849; *Jahresber.* 1849, 457. — K. G. ZWICK, *Ber. 30* (1897) 1972. — L. MARCHLEWSKI and L. MATEJKO, *Anz. Akad. Wiss. Krakau, 1905*, 745. — J. F. B. VAN HASSELT, *Chem. Weekbl. 6* (1909) 480; *Rec. 30* (1911) 1; *33* (1914) 192. — A. HEIDUSCHKA and H. RIFFART, *Arch. Pharm. 249* (1911) 43.
3. C. ETTI, *Ber. 11* (1878) 864.
4. A. HEIDUSCHKA and A. PANZER, *Ber. 50* (1917) 546 and 1525.
5. R. KUHN and co-workers, *Helv. chim. Acta 11* (1928) 427; *12* (1929) 64; *Ber. 64* (1931) 1732; *Helv. chim. Acta 12* (1929) 904; *Ber. 65* (1932) 646; *65* (1932) 1873.
6. P. KARRER and co-workers, *Helv. chim. Acta 15* (1932) 1218, 1399.
7. Cf. L. ZECHMEISTER, *Carotinoide*, Berlin 1934.
8. A. HEIDUSCHKA and A. PANZER, *Ber. 50* (1917) 546, 1525.

9. R. Kuhn and A. Winterstein, *Ber.* *65* (1932) 646.
10. P. Karrer and co-workers, *Helv. chim. Acta* *15* (1932) 1218, 1399.
11. J. Herzig and F. Faltis, *Ann.* *431* (1923) 40.
12. I. J. Rinkes, *Chem. Weekbl.* *12* (1915) 996 and I. J. Rinkes and J. F. B. van Hasselt, *Chem. Weekbl.* *13* (1916) 436, 1224, *14* (1917) 888.
13. J. F. B. van Hasselt, *Chem. Weekbl.* *6* (1909) 480.
14. J. Herzig and F. Faltis, *Monatsh.* *35* (1914) 997; *Ber.* *50* (1917) 927; *Ann.* *431* (1923) 40.
15. R. Kuhn, A. Winterstein and L. Karlovitz, *Helv. chim. Acta* *12* (1929) 64. — R. Kuhn and F. L'Orsa, *Ber.* *64* (1931) 1732.
16. I. J. Rinkes, *Rec.* *47* (1928) 934; *48* (1929) 603; *48* (1929) 1093.
17. R. Kuhn and A. Winterstein, *Ber.* *65* (1932) 646. — Cf. R. Kuhn and L. Ehmann, *Helv. chim. Acta* *12* (1929) 904.
18. P. Karrer, F. Benz, R. Morf, H. Raudnitz, M. Stoll and T. Takahashi, *Helv. chim. Acta* *15* (1932) 1218, 1399.
19. H. Raudnitz and J. Peschel, *Ber.* *66* (1933) 901.
20. P. Karrer and F. Benz, *Helv. chim. Acta* *16* (1933) 337.
21. R. Kuhn and A. Winterstein, *Ber.* *65* (1932) 1873.
22. J. Herzig and F. Faltis, *Ann.* *431* (1923) 40.
23. P. Karrer, A. Helfenstein, R. Widmer and T. B. van Itallie, *Helv. chim. Acta* *12* (1929) 741.
24. Cf. R. Kuhn and A. Winterstein, *Ber.* *65* (1932) 646; *66* (1933) 209.
25. Cf. O. Walker, *Dissertation*, Zürich 1935.
26. P. Karrer and U. Solmssen, *Helv. chim. Acta* *20* (1937) 1396.
27. L. Zechmeister and R. B. Escue, *Science* *96* (1942) 229; *J. Am. Chem. Soc.* *66* (1944) 322.
28. P. Karrer and co-workers, *Helv. chim. Acta* *12* (1929) 753.
29. P. Karrer and co-workers, *Helv. chim. Acta* *12* (1929) 754. Cf. Ref. 30.
30. R. Kuhn and A. Winterstein, *Ber.* *65* (1932) 650.
31. P. Karrer and co-workers, *Helv. chim. Acta* *12* (1929) 754. Cf. also R. Kuhn and A. Winterstein, *Ber.* *65* (1932) 650.
32. R. Kuhn and P. J. Drumm, *Ber.* *65* (1932) 1459. — R. Kuhn and co-workers, *65* (1932) 1785.
33. K. W. Hausser and A. Smakula, *Z. angew. Chem.* *47* (1934) 663; A. Smakula, *48* (1935) 152.
34. K. W. Hausser, R. Kuhn and E. Kuhn, *Z. physik. Chem. B* *29* (1935) 452.
35. P. Karrer and U. Solmssen, *Helv. chim. Acta* *20* (1937) 1396.
36. P. Karrer and co-workers, *Helv. chim. Acta* *12* (1929) 752. — Cf. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas* *30* (1911) 6.
37. P. Karrer and T. Takahashi, *Helv. chim. Acta* *16* (1933) 287.
38. P. Herzig and F. Faltis, *Ann.* *431* (1923) 60.
39. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas* *30* (1911) 11.
40. A. Heiduschka and H. Riffart, *Arch. Pharm.* *249* (1911) 47.
41. P. Karrer and co-workers, *Helv. chim. Acta* *12* (1929) 752. — Cf. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas* *30* (1911) 6.
42. P. Karrer and co-workers, *Helv. chim. Acta* *12* (1929) 746, 754. — Cf. P. Karrer and F. Rübel, *Helv. chim. Acta* *17* (1934) 773.
43. J. Herzig and F. Faltis, *Ann.* *431* (1923) 51.
44. P. Karrer and F. Benz, *Helv. chim. Acta* *16* (1933) 337.
45. R. Kuhn and L. Ehmann, *Helv. chim. Acta* *12* (1929) 905.
46. J. Herzig and F. Faltis, *Ann.* *431* (1923) 51.
47. P. Karrer and co-workers, *Helv. chim. Acta* *15* (1932) 1409.
48. P. Karrer and co-workers, *Helv. chim. Acta* *15* (1932) 1410.
49. P. Karrer and co-workers, *Helv. chim. Acta* *15* (1932) 1411.
50. H. Raudnitz and J. Peschel, *Ber.* *66* (1933) 901.

51. R. Kuhn and L. Ehmann, *Helv. chim. Acta 12* (1929) 904.
52. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas 30* (1911) 31.
53. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 754. — Cf. R. Kuhn and A. Winterstein, *Ber. 65* (1932) 650.
54. R. Pummerer, L. Rebmann and W. Reindel, *Ber. 62* (1929) 1417.
55. F. Vieböck, *Ber. 67* (1934) 377.
56. P. Karrer and co-workers, *Helv. chim. Acta 15* (1932) 1417.
57. A. Heiduschka and H. Riffart, *Arch. Pharm. 249* (1911) 43. — J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas 30* (1911) 26.
58. C. Liebermann and G. Mühle, *Ber. 48* (1915) 1657.
59. A. Heiduschka and H. Riffart, *Arch. Pharm. 249* (1911) 43.
60. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas 30* (1911) 17.
61. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 750.
62. B. v. Euler, H. v. Euler and P. Karrer, *Helv. chim. Acta 12* (1929) 278.
63. R. Kuhn and co-workers, *Helv. chim. Acta 11* (1928) 723.
64. L. Marchlewski and L. Matejko, *Anz. Akad. Wiss. Krakau, 1905*, 749 (C. 1906, II, 1265).
65. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 748, 755.
66. R. Kuhn and A. Winterstein, *Ber. 65* (1932) 650.
67. J. Herzig and F. Faltis, *Ann. 431* (1923) 49. — Cf. R. Kuhn and co-workers, *Helv. chim. Acta* 11 (1928) 723; *12* (1929) 910.
68. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 749.
69. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas 30* (1911) 8.
70. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 751.
71. J. Herzig and F. Faltis, *Ann. 431* (1923) 61.
72. K. W. Hausser and A. Smakula, *Z. angew. Chem. 47* (1934) 662; A. Smakula, *Z. angew. Chem. 48* (1935) 152.
73. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 753. — R. Kuhn and A. Winterstein, *Ber. 65* (1932) 650.
74. I. J. Rinkes, *Chem. Centr. 1916*, I, 336. — I. J. Rinkes and J. F. B. van Hasselt, *Chem. Centr. 1917*, I, 208, II, 680. — R. Kuhn, A. Winterstein and L. Karlovitz, *Helv. chim. Acta 12* (1929) 66. — I. J. Rinkes and J. F. B. van Hasselt, *Chem. Centr. 1916*, II, 390; *Chem. Centr. 1917*, I, 208, II, 680. — I. J. Rinkes, *Rec. Trav. chim. Pays-Bas 48* (1929) 1093.
75. R. Kuhn and A. Winterstein, *Ber. 65* (1932) 650.
76. P. Karrer and T. Takahashi, *Helv. chim. Acta 16* (1933) 288.
77. R. Kuhn and A. Winterstein, *Ber. 65* (1932) 650.
78. R. Kuhn and co-workers, *Ber. 65* (1932) 1459, 1785.
79. J. Herzig and F. Faltis, *Ann. 431* (1923) 48.
80. P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 751.
81. R. Kuhn and L. Ehmann, *Helv. chim. Acta 12* (1929) 905.
82. R. Kuhn and L. Ehmann, *Helv. chim. Acta 12* (1929) 905. — P. Karrer and co-workers, *Helv. chim. Acta 15* (1932) 1406.
83. P. Karrer and co-workers, *Helv. chim. Acta 15* (1932) 1404.
84. F. Faltis and F. Vieböck, *Ber. 62* (1929) 706. — P. Karrer and co-workers, *Helv. chim. Acta 12* (1929) 750; *15* (1932) 1416.
85. P. Karrer and co-workers, *Helv. chim. Acta 15* (1932) 1417.
86. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas 30* (1911) 13.
87. J. F. B. van Hasselt, *Chem. Weekbl. 6* (1909) 482; *Rec. Trav. chim. Pays-Bas 30* (1911) 14.
88. J. F. B. van Hasselt, *Rec. Trav. chim. Pays-Bas 30* (1911) 13.
89. A. Oswald, *Dissertation, Zürich 1939.*
90. P. Karrer and F. Rübel, *Helv. chim. Acta 17* (1934) 773.
91. P. Karrer and U. Solmssen, *Helv. chim. Acta 20* (1937) 1396.
92. P. Karrer and U. Solmssen, *Helv. chim. Acta 20* (1937) 1396.
93. Aschoff, *Ber. Jb. 51* (1818) 142.

94. B. QUADRAT, *J. prakt. Chem. 56* (1852) 68. — F. ROCHLEDER, *J. prakt. Chem. 74* (1858) 1. — B. WEISS, *J. prakt. Chem. 101* (1867) 65. — R. KAYSER, *Ber. 17* (1884) 2228. — E. FISCHER, *Ber. 21* (1888) 988. — E. SCHUNCK and L. MARCHLEWSKI, *Ann. 278* (1894) 349. — PFYHL and SCHERZ, *Z. Unters. d. Genussmittel 16* (1906) 237. — F. DECKER, *Arch. Pharm. 252* (1914) 139.
95. F. DECKER ,*Arch. Pharm. 252* (1914) 139.
96. P. KARRER and H. SALOMON, *Helv. chim. Acta 10* (1927) 397; *11* (1928) 513; *11* (1928) 711; *16* (1933) 643.
97. P. KARRER and co-workers, *Helv. chim. Acta 12* (1929) 985; *13* (1930) 392; *15* (1932) 1218; 1399; *16* (1933) 297.
98. Cf. R. KUHN and co-workers, *Helv. chim. Acta 11* (1928) 716; *12* (1929) 64; *Ber. 64* (1931) 1732.
99. P. KARRER and K. MIKI, *Helv. chim. Acta 12* (1929) 985.
100. E. WINTERSTEIN and J. TELECZKY, *Helv. chim. Acta 5* (1922) 376. — R. KUHN and A. WINTERSTEIN, *Naturwissenschaften, 21* (1933) 527; *Ber. 67* (1934) 344.
101. R. KUHN and A. WINTERSTEIN, *Ber. 66* (1933) 209; *67* (1934) 344. — Cf. also *Ber. 65* (1932) 1785.
102. Cf. O. WALKER, *Dissertation Zürich 1935*. — R. KUHN and A. WINTERSTEIN, *Ber. 66* (1933) 209.
103. P. KARRER and H. SALOMON, *Helv. chim. Acta 11* (1928) 513. — Cf. R. KUHN and A. WINTERSTEIN, *Ber. 67* (1934) 344.
104. P. KARRER and co-workers, *Helv. chim. Acta 10* (1927) 397; *11* (1928) 513; *13* (1930) 392.
105. R. KUHN and co-workers, *Helv. chim. Acta 11* (1928) 716.
106. P. KARRER and A. HELFENSTEIN, *Helv. chim. Acta 13* (1930) 392.
107. P. KARRER and H. SALOMON, *Helv. chim. Acta 11* (1928) 513.
108. R. KUHN and F. L'ORSA, *Ber. 64* (1931) 1732; *Z. angew. Chem. 44* (1931) 847.
109. R. KUHN and F. L'ORSA, *Ber. 64* (1931) 1732; *Z. angew. Chem. 44* (1931) 847.
110. P. KARRER and co-workers, *Helv. chim. Acta 15* (1932) 1399.
111. P. KARRER and co-workers, *Helv. chim. Acta 16* (1933) 297.
112. P. KARRER, F. BENZ and M. STOLL, *Helv. chim. Acta 16* (1933) 297.
113. A. G. PERKIN, *J. Chem. Soc. 101* (1912) 1541.
114. R. KUHN and K. MEYER, *Z. physiol. Chem. 185* (1929) 193.
115. R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 206* (1932) 42.
116. O. TUNMANN, *Chem. Centr. 1916*, II, 279; *Apoth.-Ztg. 31* (1916) 237.
117. F. DECKER, *Arch. Pharm. 252* (1914) 147. — R. KUHN and co-workers, *Helv. chim. Acta 11* (1928) 722.
118. P. KARRER and H. SALOMON, *Helv. chim. Acta 10* (1927) 402.
119. P. KARRER and H. SALOMON, *Helv. chim. Acta 11* (1928) 513.
120. P. KARRER and K. MIKI, *Helv. chim. Acta 12* (1929) 985.
121. P. KARRER and A. HELFENSTEIN, *Helv. chim. Acta 13* (1930) 392.
122. P. KARRER and A. HELFENSTEIN, *Helv. chim. Acta 13* (1930) 396. — P. KARRER and H. SALOMON, *Helv. chim. Acta 10* (1927) 397.
123. P. KARRER and co-workers, *Helv. chim. Acta 15* (1932) 1418.
124. R. KUHN and F. L'ORSA, *Ber. 64* (1931) 1732.
125. R. KUHN and A. WINTERSTEIN, *Ber. 65* (1932) 1876; *66* (1933) 1733.
126. Quantitative extinction measurements: K. W. HAUSSER and A. SMAKULA, *Z. angew. Chem. 47* (1934) 663; *48* (1935) 152. — K. W. HAUSSER, *Z. techn. phys. 15* (1934) 13. — P. KARRER and H. SALOMON, *Helv. chim. Acta 11* (1928) 516.
127. R. KUHN and A. WINTERSTEIN, *Ber. 66* (1933) 209; *67* (1934) 348.
128. R. KUHN and A. WINTERSTEIN, *Ber. 66* (1933) 1737.
129. P. KARRER and H. SALOMON, *Helv. chim. Acta 11* (1928) 515, 524.
130. R. KUHN and F. L'ORSA, *Ber. 64* (1931) 1735.
131. P. KARRER and H. SALOMON, *Helv. chim. Acta 11* (1928) 515, 524.
132. P. KARRER and T. GOLDE, *Helv. chim. Acta 13* (1930) 707; cf. P. KARRER and co-workers, *Helv. chim. Acta 15* (1932) 1406.

133. P. Karrer and F. Benz, *Helv. chim. Acta 16* (1933) 337.
134. P. Karrer and T. Golde, *Helv. chim. Acta 13* (1930) 707; cf. P. Karrer and co-workers, *Helv. chim. Acta 15* (1932) 1406.
135. P. Karrer , A. Helfenstein and R. Widmer, *Helv. chim. Acta 11* (1928) 1207.
136. K. W. Hausser and A. Smakula, *Z. angew. Chem. 47* (1934) 663; *48* (1935) 152.
137. P. Karrer and A. Helfenstein, *Helv. chim. Acta 13* (1930) 392.
138. R. Kuhn and P. J. Drumm, *Ber. 65* (1932) 1459, ref. 6.
139. R. Kuhn and A. Winterstein, *Ber. 66* (1933) 209.
140. R. Kuhn and co-workers, *Ber. 65* (1932) 1785.
141. P. Karrer and A. Helfenstein, *Helv. chim. Acta 13* (1930) 397.
142. P. Karrer and co-workers, *Helv. chim Acta 11* (1928) 1207.
143. R. Kuhn and F. L'Orsa, *Ber. 64* (1931) 1735.
144. H. Raudnitz and J. Peschel, *Ber. 66* (1933) 901.
145. P. Karrer, F. Benz and M. Stoll, *Helv. chim. Acta 16* (1933) 297.
146. P. Karrer and co-workers, *Helv. chim. Acta 15* (1932) 1408.
147. F. Decker, *Arch. Pharm. 252* (1914) 155.
148. Dragendorf, *Heilpflanzen 608*, 1885.
149. C. Liebermann, *Ber. 44* (1911) 850.
150. C. Liebermann and W. Schiller, *Ber. 46* (1913) 1973.
151. C. Liebermann and G. Mühle, *Ber. 48* (1915) 1653.
152. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 883. —. R. Kuhn and H. Brockmann, *Ber. 67* (1934) 885. *Ann. 516* (1935) 104.
153. Cf. Y. Takeda and T. Ohta, *Z. physiol. Chem. 258* (1939) 6.
154. R. Kuhn and co-workers, *Ber. 64* (1931) 333; *65* (1932) 1873.
155. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 883.
156. R. Kuhn, A. Winterstein and H. Roth, *Ber. 64* (1931) 333.
157. R. Criegee, *Ber. 64* (1931) 260.
158. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 883.
159. R. Kuhn and A. Winterstein, *Ber. 65* (1932) 1873.
160. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 95.
161. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 883.
162. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 883.
163. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 891. — Cf. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 131.
164. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 891. — Cf. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 131.
165. R. Kuhn and H. Brockmann, *Ber. 67* (1934) 885; *Ann. 516* (1935) 132.
166. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 131.
167. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 132.
168. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 133.
169. R. Kuhn, A. Winterstein and H. Roth, *Ber. 64* (1931) 340. — C. Liebermann and G. Mühle, *Ber. 48* (1915) 1653.
170. R. Kuhn and A. Deutsch, *Ber. 66* (1933) 890.
171. P. Karrer, H. Obst and U. Solmssen, *Helv. chim. Acta 21* (1938) 451.
172. C. Liebermann and W. Schiller, *Ber. 46* (1913) 1977. — R. Kuhn and co-workers, *Ber. 64* (1931) 338.
173. R. Kuhn, A. Winterstein and H. Roth, *Ber. 64* (1931) 333.
174. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 221* (1933) 133.
175. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 104, 134.
176. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 136–137.
177. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 136–137.
178. R. Kuhn and H. Brockmann, *Ann. 516* (1935) 139.
179. R. Kuhn ,A. Winterstein and H. Roth, *Ber. 64* (1931) 340. — Cf. C. Liebermann and G. Mühle, *Ber. 48* (1916) 1653.
180. R. Kuhn, A. Winterstein and H. Roth, *Ber. 64* (1931) 339.

CHAPTER XIV

Carotenoids of partly or completely unknown structure

I. RHODOVIOLASCIN $C_{42}H_{60}O_2$

History

1873 LANKESTER[1] is the first to investigate the pigments of purple bacteria, subsequently studied by other workers[2].

1905 ARCHICHOVSKJI[3] succeeds in separating a green pigment not identical with chlorophyll from the red pigments of purple bacteria.

1907 MOLISCH[4] carries out a detailed investigation of the red pigments.

1935–40 KARRER and co-workers[5] investigate the pigment mixture from rhodovibrio-bacteria and thiocystis-bacteria. They isolate a number of different polyene pigments and partly elucidate the constitution of rhodoviolascin.

Occurrence

Rhodoviolascin has hitherto been found only in rhodovibrio-bacteria[6] and in thiocystis-bacteria[6]. (According to ZECHMEISTER and co-workers[7], spirillo-xanthin from rhodospirillum rubrum[8] is identical with rhodoviolascin).

Preparation[9, 10]

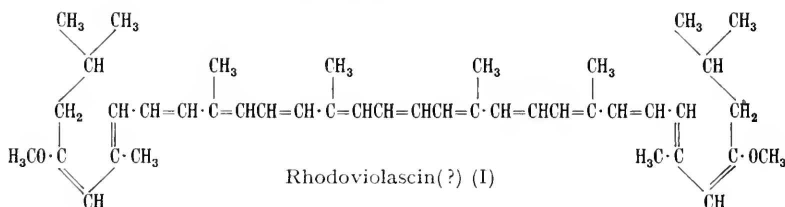
For the method of growing rhodovibrio-bacteria, compare the communication by KARRER and SOLMSSEN[9]. The bacteria are dehydrated with ethanol and exhaustively extracted with carbon disulphide. After removal of the solvent by distillation, an almost black residue remains which still contains much elementary sulphur. (The latter is formed by reduction processes during the growth of the bacteria in the magnesium sulphate-containing nutrient solution). The black residue is dissolved in a ligroin-methanol mixture, the solution is decanted from sulphur and diluted with a little water. Rhodoviolascin is precipitated as a dark-red crystalline powder at the boundary between the ligroin and methanol. It is filtered off and sealed in evacuated ampoules. The ligroin solution, which contains the other carotenoids and also some more rhodoviolascin, is extracted several times with methanol to remove the bacterio-chlorophyll, washed with water, dried over sodium sulphate and concentrated by distillation. The residue is dissolved in a little

References p. 341–343.

benzene and chromatographed on calcium hydroxide. The chromatogram is first developed with a mixture of benzene and petroleum ether and then with petroleum ether alone. The salmon-red zone of rhodoviolascin is eluted with a mixture of methanol and benzene, the solvent is removed by distillation and the remaining pigment is combined with the crystalline rhodoviolascin (see above). The following pigments were obtained from the other chromatogram zones: rhodovibrin, rhodopin, rhodopurpurin, β-carotene(?) and flavorhodin.

For further purification, the crude rhodoviolascin is chromatographed several times on calcium hydroxide and finally recrystallised from benzene. In order to obtain 0.9 g of pigment, Karrer and Koenig worked up 19,320 l of ripe bacteria nutrient solution in the course of two years.

Chemical Constitution



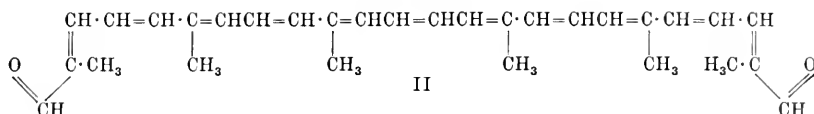
Rhodoviolascin(?) (I)

Karrer and Solmssen[11] proposed a tentative structural formula for rhodoviolascin which was subsequently modified by Karrer and Koenig[12] (Formula I). However, even the modified structure cannot yet be regarded as established with certainty.

The molecular formula of rhodoviolascin is $C_{42}H_{60}O_2$. Methoxyl determinations show the presence of two methoxyl groups. On catalytic hydrogenation, 13 moles of hydrogen are taken up. The long-wavelength location of the absorption maxima (573.5, 534, 496 mμ in carbon disulphide) shows that all the double bonds must be in conjugation. The pigment does not react with hydroxylamine and yields no dihydro-derivative on treatment with pyridine, acetic acid and zinc. In contrast to carotenoid diketones, it exhibits the same absorption spectrum in petrol and methanol.

Karrer and Koenig subjected rhodoviolascin to stepwise degradation with permanganate[12] and obtained at least 6 different oxidation products. From these, bixindialdehyde could be isolated and identified, thus establishing the structure of the central part of the molecule from carbon atom 6 to carbon atom 27. Besides bixindialdehyde, another dialdehyde was obtained in very small yield which was found above bixindialdehyde on the chromatogram and had absorption maxima located at 40 mμ towards longer wavelengths. It must therefore contain 2 conjugated double bonds more than bixindialdehyde. Methoxyl determination gave a negative result and the compound is probably 2:6:10:15:19:23-hexamethyltetracosaundecaene-1:24-dial (II):

References p. 341–343.



The formation of this compound from rhodoviolascin is in agreement with formula I.

Properties

Crystalline form: Rhodoviolascin crystallises from benzene in beautifully glistening, deep red, spindle-shaped crystals.

Melting point: 218°.

Solubility: The pigment is almost insoluble in petroleum ether, ligroin and methanol. It is somewhat more soluble in hot benzene.

Partition test: Rhodoviolascin is entirely epiphasic in character.

Optical activity: At the high dilution of the pigment solution which is necessary in view of the high colour intensity, no optical rotation was observed.

Spectral properties:

Solvent	*Absorption maxima*		
Carbon disulphide	573.5	534	496 mμ
Chloroform	544	507	476 mμ
Benzene	548	511	482 mμ
Ethanol	526	491	(465)mμ

(cf. Fig. 25, p. 358)

Colour reactions: With antimony trichloride in chloroform solution, a blue colouration is produced with an absorption maximum at 642 mμ.

Chromatographic behaviour: Rhodoviolascin can be easily chromatographed on calcium hydroxide from benzene solution. It is adsorbed below rhodopin, but above rhodopurpurin.

2. RHODOPIN $C_{40}H_{58}O$*

Rhodopin was first isolated by KARRER and SOLMSSEN[13] from rhodovibrio-bacteria. Later investigations have shown that this pigment is also present in thiocystis-bacteria[14].

For the isolation of rhodopin one proceeds first as in the preparation of rhodoviolascin (p. 295). After the crystallisation of the rhodoviolascin, the petroleum ether mother liquors are chromatographed on calcium hydroxide. Rhodopin is eluted with a benzene-methanol mixture from the upper part of the chromatogram.

* The formula $C_{40}H_{56}O$ is also in agreement with the analytical data.

References p. 341–343.

The solvent is removed by distillation and the residue is repeatedly adsorbed on calcium hydroxide. Only in this way is it possible to separate rhodopin from rhodoviolascein and rhodovibrin. Finally the pigment is re-crystallised from a mixture of carbon disulphide and petroleum ether.

The structure of rhodopin is not yet definitely known. It has the molecular formula $C_{40}H_{58}O$ or $C_{40}H_{56}O$. ZEREWITINOFF determinations showed the presence of one active hydrogen atom, but no acetyl derivative of rhodopin could be prepared. Microhydrogenation indicated the presence of 12 double bonds, which are probably all conjugated. No carbonyl group was detected and on reduction of the pigment with zinc dust and acetic acid no change in the spectrum was observed. Methoxyl determinations gave a negative result. KARRER and KOENIG[12] attempted to obtain some information regarding the structure of rhodopin by oxidative degradation with permanganate. The amount of material available was, however, too small for the identification of the degradation products.

Rhodopin crystallises from a mixture of carbon disulphide and petroleum ether in deep red crystals which appear as small clusters of needles and prisms under the microscope. Melting point: 171° (after previous sintering). Rhodopin exhibits epiphasic behaviour on partition between petroleum ether and 90 % methanol.

Solvent	*Absorption maxima*		
Carbon disulphide	547	508	478 mμ
Chloroform	521	486	453 mμ
Petroleum ether	501	470	440 mμ
Ethanol (absolute)	505	474	(445)mμ

(cf. Fig. 26, p. 358)

3. RHODOVIBRIN

During the chromatographic purification of rhodopin on calcium hydroxide, KARRER and SOLMSSEN* obtained another hitherto unknown carotenoid for which they proposed the name rhodovibrin. Rhodovibrin is adsorbed somewhat more strongly than rhodopin on the chromatographic column and can thus be separated from the latter. Repeated chromatographic adsorption is necessary, however, as the two pigments are only separated with difficulty. Rhodovibrin crystallises form a mixture of carbon disulphide and petroleum ether in small deep-red crystal clusters which are almost indistinguishable in appearance from those of rhodopin and melt at 168°. The absorption maxima are located at 556 and 517 mμ in carbon disulphide solution. The pigment could not be obtained in a completely pure state as it is only present in very small quantities in

* The literature is summarised under rhodopin, p. 297; cf. H. KOENIG, ***Dissertation***, Zürich, 1940, p. 29.

References p. 341–343.

rhodovibrio-bacteria, but it appears to have the molecular formula $C_{40}H_{58}O_2$ or $C_{40}H_{56}O_2$. It is improbable that both oxygen atoms are present as hydroxyl groups since rhodovibrin, like rhodopin, is epiphasic in the partition test. Methoxyl determinations also gave a negative result.

4. RHODOPURPURIN

Rhodopurpurin occurs in rhodovibrio-bacteria in very small amounts and was isolated from the latter by KARRER and SOLMSSEN* by the procedure described for the preparation of rhodoviolascin and rhodopin (pp. 295 and 297). It is adsorbed below rhodopin in the chromatogram on calcium hydroxide and is isolated by elution, dissolution in petroleum ether and concentration of the solution. The pigment crystallises from petroleum ether in fine microscopic needles which are partly combined in clusters and melt at 161–162°. Ultimate analysis shows that the compound is probably a hydrocarbon of the formula $C_{40}H_{56}$ or $C_{40}H_{58}$.

On partition between methanol and petroleum ether, rhodopurpurin is entirely epiphasic. In its spectral properties it shows great similarity to lycopene; its identity with the latter is still uncertain, however.

Solvent	*Absorption maxima*		
Carbon disulphide	550	511	479 mμ
Chloroform	527	487	(458)mμ
Petroleum ether	502	472	mμ
Benzene	527	490	mμ

5. FLAVORHODIN

This pigment was also obtained by KARRER and SOLMSSEN* from rhodovibrio-bacteria. It is obtained from the lowest zones of the calcium hydroxide-chromatogram in the preparation of rhodopin**.

The constitution of flavorhodin is unknown. The pigment is easily soluble in petroleum ether, benzene, chloroform acetone and ether, but only sparingly soluble in ethanol and methanol. It is entirely epiphasic in the partition test.

Solvent	*Absorption maxima*		
Carbon disulphide	503	472	441 mμ
Chloroform	482	453	mμ
Petroleum ether	470	442	mμ
Ethanol	471	443	mμ

(cf. Fig. 27, p. 359)

In its optical properties, flavorhodin is reminiscent of sarcinin (p. 319), but the question of the identity of these two pigments is still unsettled.

* For a summary of the literature, see under rhodopin, p. 297.
** Cf. p. 297.

6. APHANIN $C_{40}H_{54}O$

History and Occurrence

Although the pigments of blue algae have been repeatedly investigated within recent years, our present knowledge concerning these compounds is still very incomplete. In 1927, KYLIN[15] investigated extracts of the blue algae *Calotrix scopulorum* by capillary-analytical methods, and found, besides carotene, three new pigments. These were not, however, isolated in a pure state and analysed.

In 1936, HEILBRON and LYTHGOE investigated the carotenoids from *Oscillatoria rubescens*[16], and reported the presence of β-carotene and xanthophyll, as well as of two new pigments, myxoxanthin and myxoxanthophyll (cf. p. 225). Very recently, KARRER and RUTSCHMANN[17] reinvestigated the polyene pigments from *Oscillatoria rubescens* and found a previously unknown acidic pigment, oscillaxanthin, besides β-carotene, zeaxanthin, myxoxanthin and myxoxanthophyll, but no xanthophyll (cf. p. 335).

TISCHER[18] investigated the carotenoids of the blue algae *Aphanizomenon flos-aquae* and was able to isolate four new polyene pigments besides β-carotene. He proposed the term aphanin, aphanicin, flavacin and aphanizophyll for these pigments. They have so far only been observed in *Aphanizomenon flos-aquae*.

Preparation[18]

The algae *Aphanizomenon flos-aquae* are collected from their aqueous suspensions by means of nets, freed from impurities and dehydrated with ethanol. The residue, still alcohol-moist, is mixed with sand and ammonium sulphate and extracted at room temperature with ether which has been freshly distilled from sodium. The ether is removed by distillation and the aqueous ethanolic residue is extracted with petroleum ether (Extract A). The petroleum ether-insoluble pigments from the alcoholic layer are obtained in the form of a red flocculent precipitate by salting out with ammonium sulphate. The alcoholic extracts which remain after the dehydration of the algae also yield a small amount of red pigment, which is combined with that obtained by salting-out and dissolved in pyridine (Extract B).

The material remaining after extraction with ether is once more extracted with ethanol at 40° (Extract C).

The petroleum ether extract A is adsorbed on alumina and the chromatogram is developed with petrol. The carotenoids are adsorbed on the column in the following downward sequence: 1. aphanicin, 2. aphanin, 3. flavacin and 4. β-carotene*.

a) *Aphanicin:* The pigment is again chromatographed on alumina, saponified with methanolic potassium hydroxide after elution and once more chromatographed on alumina. Aphanicin is eluted with petrol containing a little ethanol and the solu-

* For a detailed description, see J. TISCHER, *Z. physiol. Chem.* *251* (1938) 109.

tion is washed with water. The solvent is evaporated and the residue is recrystallised from petrol. From 50 kg of fresh moist algae, which yield 2¼ kg of dry material, a total of about 50 mg of pure aphanicin was obtained.

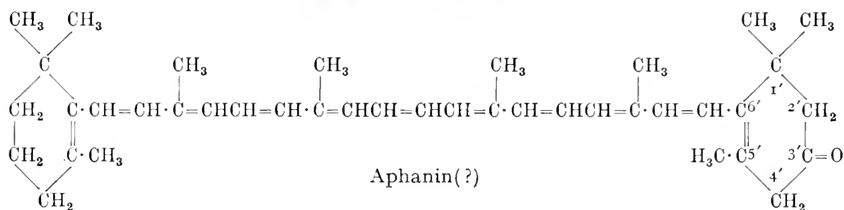
b) *Aphanin:* The aphanin zone in the chromatogram is washed through into the filtrate. After removal of the solvent by distillation, the pigment can readily be obtained in a crystalline state. For further purification, the pigment is adsorbed on alumina and crystallised from a mixture of benzene and methanol (1 : 10). The yield of aphanin from 50 kg of algae amounted to about 110 mg.

c) *Flavacin:* The pigments from the third zone of the first chromatogram are repeatedly chromatographed on alumina. The colourless impurities are frozen out from the petroleum ether solution, and the mother liquors are saponified with methanolic potassium hydroxide, and then extracted with petroleum ether. After evaporation of the solvent a small amount of flavacin crystallises from the residue. It can be purified by repeated re-crystallisation from a mixture of benzene and methanol.

The fourth zone of the chromatogram yields analytically pure β-carotene.

From the pyridine solution B and the ethanol extract C, aphanizophyll is obtained. The pyridine solution B is evaporated to dryness in vacuum and the residue is saponified with ethanolic potassium hydroxide. The reaction mixture is acidified with acetic acid and the pigment extracted with ether. This solution is adsorbed on sodium sulphate and the aphanizophyll is eluted with methanol and crystallised from acetone. For further purification, the pigment is chromatographed on calcium carbonate and repeatedly recrystallised from acetone, and finally from chloroform. From 50 kg of fresh algae, about 10 mg aphanizophyll were obtained in this way. Working up of the extract C yielded a further very small quantity of the pigment[19].

Chemical Constitution[20]



The main features of the constitution of aphanin were established by TISCHER[2] who proposed the above formula. Elementary analysis indicated the molecular formula $C_{40}H_{54}O$. On microhydrogenation the pigment absorbed 11 mols of hydrogen rapidly and an additional mol of hydrogen slowly. This behaviour suggested the presence of a carbonyl group which was confirmed by the preparation of a well-crystallised oxime. It seems almost certain that the carbonyl group is not conjugated with the system of conjugated double bonds as the absorption maxima of the oxime and of the parent compound have the same wavelength location. This is also in agreement with the fact that the absorption maxima in different solvents such as petrol and ethanol are the same, whereas carotenoids containing carbonyl groups conjugated with the

References p. 341–343.

ethylenic double bonds exhibit a different behaviour (cf. p. 56). The test for the presence of an isopropylidene grouping gave a negative result, thus excluding a γ-carotene type structure. On permanganate degradation, almost 5 (4.77) mols of acetic acid were obtained. This result is not in contradiction with the proposed formula for aphanin since cryptoxanthin, which contains six side-chain methyl groups, also gives rise to only 4.85 mols of acetic acid[21]. The reduction of aphanin with aluminium isopropoxide and isopropylalcohol yielded an alcohol, aphanol, with an absorption maximum displaced by about 10 mμ towards shorter wavelengths as compared with aphanin (in petrol). TISCHER suggested that the structure of aphanol is identical with that of cryptoxanthin. If this suggestion is correct, the carbonyl group must be in the 3′-position, but since aphanol was not obtained crystalline and was not analysed, this question must still remain open.

In animal feeding tests, aphanin exhibits half the vitamin A activity of β-carotene, so that it must contain one unsubstituted β-ionone ring according to present views. For that reason the constitution of one half of the molecule must be assumed to correspond to that of β-carotene. The fact that the pigment is optically inactive is also in agreement with the proposed formula.

Properties

Crystalline form: Aphanin crystallises from a mixture of benzene and methanol in large, spear-like, blue-black leaflets which are often combined in rosettes and exhibit a pronounced graphitic lustre. From a mixture of benzene and petrol, aphanin is obtained in stout prismatic crystals.

Melting point: 176° (corr., crystallised from benzene-methanol[22]). 180° (corr., crystallised from benzene-petrol[20]).

Solubility: Aphanin is very readily soluble in carbon disulphide, chloroform and benzene, less readily soluble in pyridine, ether and petrol, and very sparingly soluble in methanol.

Spectral properties:

Solvent	*Absorption range*	*Absorption maxima*	
Carbon disulphide .	475–555	533.5	494 mμ
Chloroform	455–520	504	474 mμ
Benzene	455–520	505	472 mμ
Petrol, b.p. 70–80° .	445–510	494	460 mμ
Pyridine	460–525	507.5	477 mμ
Methanol	445–505	491.5	457 mμ

TISCHER[22] describes the absorption spectrum of aphanin as follows: "The absorption spectrum exhibits two maxima separated by a more weakly ab-

References p. 341–343.

sorbing zone, giving the appearance of a single wide absorption band with 2 maxima. The wide absorption range has no sharp limits and although the two maxima are clearly recognised their centres can only by approximately determined."

Solutions of the pigment in petrol and methanol are yellow, solutions in benzene, chloroform and pyridine are orange, and solutions in carbon disulphide are red-orange.

Optical activity: Aphanin shows no optical rotation.

Partition test: On partition between petroleum ether and 90 % methanol, the pigment is found entirely in the upper layer; if 95 % methanol is used, however, the lower layer is also weakly coloured.

Chromatographic behaviour: Aphanin is adsorbed somewhat more strongly than β-carotene, but more weakly than aphanicin, on alumina from petrol solution. If the chromatogram is developed with ether, the colour changes to brown-red; on washing with benzene or chloroform, it becomes dark violet.

Colour reactions: Concentrated aqueous hydrochloric acid gives no blue colouration with an ethereal solution of aphanin. A chloroform solution of the pigment assumes a deep blue colour which gradually changes to blue-green on addition of concentrated sulphuric acid. With antimony trichloride in chloroform, aphanin exhibits a brownish-violet colour which turns blue-violet on fading*.

Physiological properties: Aphanin exhibits vitamin A activity which is about half as great as that of β-carotene[23].

Detection and estimation: The separation of aphanin from the other carotenoids of *Aphanizomenon flos-aquae* is carried out by means of chromatographic adsorption on alumina. By this means, the pigment can be separated from the more strongly adsorbed aphanicin and can be identified by its absorption spectrum.

Derivatives

Aphanin oxime $C_{40}H_{55}ON$:

This compound is obtained on treating a solution of aphanin in pyridine with free hydroxylamine. For purification, the crystallised oxime is chromatographed on alumina and recrystallised from a mixture of benzene and methanol. M.p. 208° (corr.). The oxime is rather sparingly soluble in benzene.

* For further colour reactions, cf. the original communication, *Z. physiol. Chem.* 251 (1938) 109.

References p. 341–343.

Solvent	*Absorption range*	*Absorption maxima*	
Carbon disulphide .	475–545	530	492 mμ
Chloroform	455–520	504	472 mμ
Benzene	455–520	505	472 mμ
Petrol (70–80°) . .	445–520	494	459 mμ
Pyridine	460–525	509	477 mμ
Methanol	445–505	491	457 mμ

The spectrum of aphanin oxime exhibits two bands. The limits of the wide absorption band are less clear than in the case of aphanin and cannot be determined exactly[18]. For various colour reactions of the oxime, the original communication[18] should be consulted.

Aphanol $C_{40}H_{56}O$:

During the reduction of aphanin with aluminium isopropoxide and isopropylalcohol a compound which shows the absorption spectrum of cryptoxanthin is obtained amongst other products. According to TISCHER[18] this compound is identical in structure with cryptoxanthin.

7. APHANICIN

This pigment was first isolated by TISCHER[22] from *Aphanizomenon flos-aquae**.

Chemical Constitution

Very little is yet known concerning the constitution of aphanicin. Various considerations lead TISCHER to regard aphanicin as a "di-carotenoid" of the empirical formula $C_{80}H_{106}O_3$, consisting of 2 molecules of aphanin combined by an oxygen bridge. As compounds of this type have not otherwise been encountered in nature, nor prepared synthetically, this suggestion can only be accepted with reserve. Experimentally the following results were obtained. The elementary analysis of aphanicin gave the values C, 86.14%; H, 9.35% (Calculated for $C_{40}H_{54}O$: C, 87.20: H, 9.89%). The pigment formed an oxime, found: N, 3.97%. ($C_{40}H_{55}ON$ requires N, 2.48%. Calculated for $C_{40}H_{55}ON \cdot \frac{1}{2}NH_2OH$, 3.61%**). The aphanicin molecule thus contains at least 1 carbonyl group. On microhydrogenation, aphanicin absorbs 12 mols of hydrogen. As the absorption spectrum of aphanicin is very similar to that of aphanin, it may be assumed that the two compounds contain very similar or identical chromophoric systems. Aphanicin has only about half the vitamin A activity of

* For the isolation of the pigment, see under aphanin, p. 300.

** Addition products of free hydroxylamine and carotenoids have been observed, J. TISCHER, *Z. physiol. Chem.* *267* (1941) 281.

aphanin*. Reduction of the pigment with aluminium isopropoxide and isopropyl alcohol yields a product, aphanicol, the absorption maxima of which are displaced by about 10 mμ towards shorter wavelength as compared with aphanicin (in petrol). From this fact and from the identical location of the absorption maxima in petrol and methanol it may be concluded that the carbonyl group is not in conjugation with the system of conjugated double bonds.

Properties

Crystalline form: Aphanicin crystallises from a mixture of benzene and methanol with a little more difficulty than aphanin and forms red-violet prismatic needles with a strong metallic lustre.

Melting point: 190° (corr., crystallised from benzene-methanol). 195° (corr., crystallised from benzene-petrol).

Solubility: The pigment is even less soluble in methanol than aphanin. In other solvents the solubility of the two pigments is about equal.

Spectral properties:

Solvent	*Absorption range*	*Absorption maxima*	
Carbon disulphide .	475–555	533	494 mμ
Chloroform	455–520	504	474 mμ
Benzene	455–520	505	474 mμ
Petrol, b.p. 70–80° .	445–510	494	462 mμ
Pyridine	460–525	507.5	478 mμ
Methanol	445–505	491.5	457 mμ

The colours of solutions of aphanicin in organic solvents are the same as those of aphanin, see p. 303.

Optical activity: No data have been recorded.

Partition test: On partition of aphanicin between petroleum ether and 95 % methanol, a somewhat higher proportion of the pigment is found in the alcoholic layer than in the case of aphanin. Even with 90 % methanol the lower layer is slightly coloured.

Chromatographic behaviour: Aphanicin is more strongly adsorbed than aphanin on alumina from a mixture of benzene and petrol (1:1) and can thus be readily separated from the latter. The two zones are also distinguished by the depth of their colours, the lower zone being a darker bordeaux-red than the upper zone.

* It has recently been shown that steric differences have a great influence on vitamin A activity, cf. L. ZECHMEISTER and co-workers, *Arch. Biochem.* 5 (1944) 107; 7 (1945) 247; 7 (1945) 157.

Colour reactions: Aphanicin shows almost the same colour reactions as aphanin. Table 53 shows the reactions which give different results.

TABLE 53

COLOUR REACTIONS DISTINGUISHING BETWEEN APHANIN AND APHANICIN

Reagent	Colouration	
	Aphanin	Aphanicin
Trichloracetic acid in chloroform	faint brown-violet	red-violet, stable
Arsenic trichloride in chloroform	brown, changing to sepia	brown, changing to violet
Antimony trichloride in chloroform	brown-violet, fading	brown-violet
Antimony trichloride in ether	yellow-brown	reddish-brown

Physiological behaviour[23]: Aphanicin exhibits about half the vitamin A activity of aphanin.

Detection and Estimation: Aphanicin can be separated from the other carotenoids of *Aphanizomenon flos-aquae* by chromatographic adsorption and is identified by means of its spectral properties and its colour reactions.

Derivatives

Aphanicin oxime:

Aphanicin oxime is prepared in the same way as aphanin oxime. M.p. 241°

Solvent	*Absorption range*	*Absorption maxima*	
Carbon disulphide .	460–545	529	492 mμ
Chloroform	450–520	504	474 mμ
Benzene	455–520	505	472 mμ
Petrol b.p. 70–80° .	445–505	493	461 mμ
Pyridine	460–525	508	478 mμ
Methanol	445–505	491	456 mμ

Aphanicol:

This compound is prepared in the same way as aphanol[24]. Aphanicol could not be obtained crystalline.

Solvent	*Absorption maxima*	
Carbon disulphide	516	482 mμ
Petrol	484	454 mμ

References p. 341–343.

8. FLAVACIN

TISCHER[18] discovered flavacin together with aphanin, aphanicin and aphanizophyll in the blue algae *Aphanizomenon flos-aquae.* The isolation of the pigment is described in connection with the preparation of aphanin on p. 301.

TABLE 54

COMPARISON OF SOME PROPERTIES OF MUTATOCHROME AND FLAVACIN

Properties	Flavacin	Mutatochrome
Melting point	155° (corr.) *	163–164° (uncorr.) in vacuum
Absorption maximum in carbon disulphide	490 457 (424) mμ	489.5 459 mμ
Absorption maximum in petrol	458 428 mμ	456 427 mμ
Action of concentrated hydrochloric acid	no blue colouration **	very faint blue colouration which only appears gradually
Partition test	epiphasic	epiphasic
Position in the chromatogram	above β-carotene	above β-carotene

* This melting point was not yet constant.

** J. TISCHER employed 30% hydrochloric acid for this reaction, whereas P. KARRER and E. JUCKER employed 37% hydrochloric acid but still only observed a very weak blue colouration.

Chemical Constitution

Very little is known concerning the structure of flavacin. The pigment is found below aphanin but above β-carotene in the chromatogram. It has purely epiphasic character which suggests that it may be a hydrocarbon. The absorption maxima in carbon disulphide are located at 490 and 457 mμ, so that the chromophoric system of flavacin must contain fewer conjugated double bonds than, for instance, that of β-carotene. Since, however, the latter is more weakly adsorbed on the chromatographic column, flavacin must contain an additional functional group which increases its strength of adsorption. One is thus tempted to compare flavacin with mutatochrome[25]. The two compounds have, in fact, largely the same properties. The question of the identity of the two pigments must still be left open, however, as a direct comparison has not yet been made.

9. APHANIZOPHYLL*

The chemical constitution of aphanizophyll is still unknown. The pigment contains a hydroxyl group which can be esterified and a carbonyl group which reacts with hydroxylamine. Elementary analysis, however, gave very low values for the carbon content (C, 70.15 %; H, 9.42 %), so that no conclusions regarding the structure of the pigment can be drawn on the basis of the analytical results. TISCHER[22] points out the similarity between aphanizophyll and myxoxanthophyll[16], but does not regard the two pigments as identical.

Properties

Crystalline form: The phytoxanthin crystallises from methanol in prismatic crystals combined in rosettes. From pyridine it is obtained in circular, finely tufted crystals.

Melting point: 172–173° corr.

Solubility: Aphanizophyll dissolves readily in pyridine and ethanol and somewhat less readily in acetone, ether and acetic acid. It is completely insoluble in benzene, petrol and carbon disulphide.

Optical rotation: No data have been recorded.

Spectral properties:

Solvent	*Absorption maxima*		
Pyridine	531	494	462 mμ
Chloroform	523	487.5	457 mμ
Methanol	507	475	444 mμ

Solutions of the pigment in methanol are yellow, solutions in chloroform deep red, and solutions in pyridine blood-red.

Partition test: Aphanizophyll is entirely hypophasic on partition between petroleum and methanol.

Chromatographic behaviour: Aphanizophyll is adsorbed so strongly on alumina and calcium hydroxide that it is almost impossible to elute it. It is also readily adsorbed on calcium carbonate or sodium sulphate from chloroform or ether solution but can be completely eluted with methanol.

Colour reactions: A solution of aphanizophyll in chloroform shows the following colour reactions. Concentrated sulphuric acid produces a blue colour. Concentrated nitric acid gives a blue colour which changes to green and eventually fades. Antimony trichloride in chloroform gives a blue colour which

* For the isolation of this pigment, see under aphanin, p. 301.

changes to violet. An ethereal solution of the pigment gives a slowly fading bluish-green colour with 30 % hydrochloric acid.

Detection and estimation: Aphanizophyll can be separated from accompanying carotenoids by means of chromatographic analysis and can be identified by determination of the absorption maxima, by partition between methanol and petroleum ether, and by the blue colouration produced with concentrated hydrochloric acid.

Derivatives

Aphanizophyll can be esterified with palmitic acid chloride. In contrast to the parent pigment, the ester is readily soluble in benzene, petrol and carbon disulphide. It crystallises from methanol in needles, which melt even on warming by hand.

Solvent	*Absorption maxima*		
Carbon disulphide	547	506	474 mμ
Benzene	524	489	457 mμ

The ester exhibits the same absorption maxima in pyridine, chloroform and methanol as the parent compound. It is more weakly adsorbed than aphanizophyll. On alumina, the ester is adsorbed with an orange-red colour and can be readily eluted, but it is not sufficiently adsorbed on calcium carbonate. On partition between petroleum ether and 90 % methanol, it exhibits pronounced epiphasic behaviour. With 95 % methanol, the lower layer is also definitely coloured. It is possible that aphanizophyll occurs as the palmitic ester in algae, as considerable amounts of palmitic acid could be isolated from the hypophasic fraction[26].

Apart from one or more hydroxyl groups, aphanizophyll also contains a carbonyl group since an oxime can be prepared. The oxime is soluble in benzene and is well adsorbed on calcium carbonate. It exhibits the same absorption spectrum as aphanizophyll in different solvents. The absorption maxima in benzene are located at 524, 489 and 457 mμ. In view of the spectroscopic properties of the oxime it may be concluded that the carbonyl group is *not* in conjugation with the system of conjugated double bonds.

Neither the oxime nor the ester could be obtained in the crystalline state and analysed.

10. FUCOXANTHIN $C_{40}H_{56}O_6$

History

1867 ROSANOFF[27] suggests that brown algae contain a yellow pigment besides chlorophyll. The pigment was also observed shortly afterwards by KRAUS and MILLARDET[28] and termed phycoxanthin.

References p. 341–343.

1837 SORBY[29] shows that brown algae contains not only one but, in his view, three yellow pigments which he terms xanthophyll, lichnoxanthin and fucoxanthin.

1906 TSWETT[30] succeeds in separating the pigments of brown algae into carotene, fucoxanthophyll and fucoxanthin by chromatographic adsorption.

1914 WILLSTÄTTER and PAGE[31] isolate fucoxanthin in the crystalline state for the first time and investigate this pigment in detail.

1931–35 KARRER and co-workers[32], KUHN and WINTERSTEIN[33] and HEILBRON and PHIPERS[34] carry out investigations on the constitution of fucoxanthin.

Occurrence

Fucoxanthin has been found mainly in *Phaeophyceae* where it occurs together with chlorophyll (mainly chlorophyll a) and other carotenoids such as carotene and xanthophyll. The following species of brown algae contain the pigment: *Fucus virsoides, Dictyota, Cystosira* and *Laminaria*[31]. According to HEILBRON and PHIPERS[34], dried brown algae (*Fucus vesiculosus*) contain β-carotene and zeaxanthin, whereas fresh algae contain β-carotene and fucoxanthin. More recent investigations[34a] show that β-carotene is the main carotenoid pigment of the male gametes, while fucoxanthin is present mainly in the female gametes. Fucoxanthin also occurs in *Zygnema pectinatum, Polysiphonia nigrescens*[35] and in diatoms[36,37].

Preparation[38]

Air-dried brown algae are minced in a mincing machine and exhaustively extracted at room temperature with 90% methanol. After dilution with water, chlorophyll is extracted from this solution with petroleum ether and the mother liquors are diluted with more water, covered with petroleum ether and allowed to stand for 24 hours. At the end of this period most of the fucoxanthin has separated as a brown precipitate at the boundary between the two solvents, and can be filtered off. The mother liquors which contain very little additional pigment, are discarded. The pigment is purified by crystallisation from methanol and a little water, and a second time from methanol alone. The yield of pure pigment from about 15 kg of air dried algae amounts to about 2 g. If, on the other hand, the algae are several weeks old, the pigment can no longer be isolated and only a very small amount of impure pigment is obtained[39].

Using the procedure of KARRER and co-workers[38] just described, only fucoxanthin is isolated from brown algae. WILLSTÄTTER and PAGE[39] have described a considerably more complicated method of preparation in which xanthophyll is also isolated and subsequently separated from fucoxanthin.

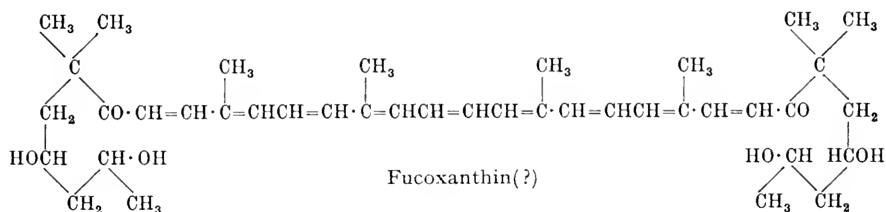
Chemical Constitution

KARRER and coworkers[38] obtained the molecular formula $C_{40}H_{56}O_6$ for fucoxanthin whereas I. M. HEILBRON and R. F. PHIPERS[40] obtained the formula

$C_{40}H_{60}O_6$. The nature of the oxygen atoms in fucoxanthin has not yet been clarified. The results obtained by KARRER and co-workers[41] indicated 4–5 hydroxyl groups, whereas the results of KUHN and WINTERSTEIN[33] indicated 6. On catalytic hydrogenation 10 mols of hydrogen are absorbed[41]; but the analysis of the perhydrocompound gave values in agreement with the formula $C_{40}H_{78}O_2$[40]. On vigorous oxidation with permanganate, four mols of acetic acid are obtained. From these results the number of side-chain methyl groups can be deduced. From the oxidation products of the pigment with alkaline permanganate, α:α-dimethylmalonic acid can be isolated (KARRER and co-workers[41]). The fact that no α:α-dimethylsuccinic acid or α:α-dimethylglutaric acid are formed indicates that the two ends of the fucoxanthin molecule are highly substituted by hydroxyl groups (cf. p. 201).

These results do not, however, lead to any definite constitution for the pigment.

HEILBRON and PHIPERS[40] proposed the following formula for fucoxanthin.



Fucoxanthin(?)

The proposed constitution is not, however, in accord with all the properties of fucoxanthin. The chromophoric system of the proposed structure corresponds to that of β-carotenone (cf. p. 141) and the absorption maxima would be expected to be located at considerably longer wavelengths than those observed. Furthermore, a diketone of this type should be reduced by zinc and acetic acid to a dihydro-derivative, as in the case of β-carotenone. Fucoxanthin does not undergo such a reaction. The proposed formulation of the pigment is thus unacceptable.

Properties

Crystalline form: Fucoxanthin crystallises from methanol in brown-red prisms with a blue lustre. The crystals contain 3 molecules of methanol of crystallisation. From dilute ethanol or acetone, the pigment is obtained in deep red hexagonal plates containing 2 molecules of water. From a mixture of ether and petroleum ether, the pigment crystallises in needles without solvent of crystallisation.

Melting point: 159.5–160.5° (corr., WILSTÄTTER and PAGE[39]), 166–168° (uncorr.)[40].

References p. 341–343.

Solubility: Fucoxanthin is readily soluble in ethanol, somewhat less soluble in carbon disulphide, sparingly soluble in ether, and quite insoluble in petroleum ether. 100 g of hot methanol will dissolve 1.66 g of the pigment.

Spectral properties: Fucoxanthin exhibits an absorption spectrum similar to that of xanthophyll, but the bands are less sharp.

In carbon disulphide	510	477	445 mμ (diffuse)
In chloroform . . .	492	457	mμ (cell thickness 2 mm)[42]

(cf. Fig. 9, p. 351)

A solution of fucoxanthin in ether is orange-yellow, a solution in ethanol is reddish, and a solution in carbon disulphide is red.

Colour reactions: On shaking an ethereal solution of fucoxanthin with 25 % aqueous hydrochloric acid, the latter is coloured deep-blue.

Optical rotation: According to KARRER and co-workers[41] fucoxanthin has a specific rotation of $[\alpha]_D^{18} = +72.5° \pm 9°$ (in chloroform). HEILBRON and PHIPERS[40], on the other hand, find that the pigment does not rotate the plane of polarised light.

Chromatographic behaviour: Hardly any data are available regarding the chromatographic adsorption of fucoxanthin[43]. In order to establish whether the pigments investigated by different workers were identical or mixtures of different substances, a renewed detailed chromatographic investigation of fucoxanthin would be desirable.

Detection and estimation: As far as is known at the present time, the pigment occurs mainly in brown algae. It is accompanied by β-carotene, zeaxanthin and xanthophyll from which it can be easily distinguished by means of its absorption maxima and its colour reaction with hydrochloric acid.

Chemical properties: Although fucoxanthin has no acidic properties, it is not indifferent towards alcoholic alkali since methanolic potassium hydroxide dissolves the pigment much more quickly than methanol alone. On acidification, no fucoxanthin can be regenerated from this solution, but new compounds are obtained which HEILBRON and PHIPERS[40] termed isofucoxanthins. Nothing definite is yet known concerning the nature of these compounds. HEILBRON suggests that isofucoxanthins are formed by an aldol condensation but this suggestion has not been proved. Isofucoxanthins are much more strongly basic than fucoxanthin. Even 0.001 % hydrochloric acid removes the blue colour of an ethereal solution of these new pigments. The absorption maxima of isofucoxanthin are displaced towards shorter wavelengths with respect to fucoxanthin.

The behaviour of fucoxanthin towards hydrochloric acid has already been described on p. 312. WILLSTÄTTER and PAGE[39] showed that a definite amount (4 mols) of hydrochloric acid is consumed in this reaction. The compound formed has the formula $C_{40}H_{54}O_6.4HCl$.

Crystalline fucoxanthin is fairly stable in air, no uptake of oxygen being observed during a period of several weeks. However, the pigment absorbs water from the air and forms various hydrates. If an ethereal solution of the pigment is allowed to stand in the presence of iodine, a tetraiodide of fucoxanthin soon crystallises. This derivative forms short, pointed, violet-black prisms with a coppery lustre. M.p. 134–135° (corr., after slight sintering).

Little is known concerning the pigments[44] designated as "fucoxanthin a, b, c" and their homogeneity is uncertain; the same applies to "neofucoxanthin A" and "neofucoxanthin B"[45].

11. GAZANIAXANTHIN

History and Occurrence

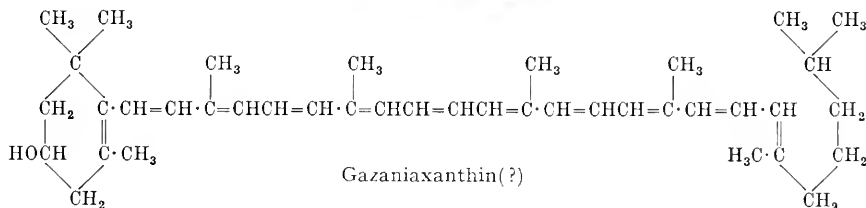
1938 SCHÖN isolates a new phytoxanthin, gazaniaxanthin, from the blossoms of *Gazania rigens*[46].

1943 ZECHMEISTER and SCHROEDER[47] propose a tentative formula for gazaniaxanthin.

Preparation[47]

1 kg of blossom leaves of *Gazania rigens* are dried at 40–50° and extracted with petroleum ether at room temperature. After saponification with methanolic potassium hydroxide, the pigment mixture is chromatographed on calcium hydroxide from petroleum ether solution. Depending on the age of the blossoms, the yield of gazaniaxanthin varies between 380 and 620 mg.

Chemical Constitution



Gazaniaxanthin(?)

According to SCHÖN[46], gazaniaxanthin has the molecular formula $C_{40}H_{54}O$ or $C_{40}H_{56}O$. The oxygen atom is present in the form of a hydroxyl group, as shown by ZEREWITINOFF determinations and the preparation of an acetate. On catalytic hydrogenation gazaniaxanthin absorbs 11 mols of hydrogen[47]. According to ZECHMEISTER and SCHROEDER[47] the molecular formula is $C_{40}H_{58}O$

References p. 341–343.

and ozonisation yields 0.85 mols of acetone. On the basis of these results ZECHMEISTER and SCHROEDER proposed the above formula for gazaniaxanthin. Apart from the uncertainty regarding the position of the hydroxyl group, this formula partly contradicts the experimental results. According to all previous experience in the carotenoid series, acetone is only obtained on ozonisation in the presence of an isopropylidene grouping $(CH_3)_2C{=}C\ldots$ It is not easy to see why the grouping $(CH_3)_2CH_2\ldots$ should also give rise to nearly 1 mol (0.85) of acetone on ozonisation. (ZECHMEISTER and SCHROEDER[47] cite the analogous case of thymol which on ozonisation yields 0.3 mols of acetone). Further experimental study is clearly required for a complete elucidation of the constitution of gazaniaxanthin.

Properties

Gazaniaxanthin crystallises from a mixture of benzene and methanol in glistening red plates, m.p. 133–134°. It is found below rubixanthin but above cryptoxanthin in the chromatogram on calcium hydroxide. It shows the same behaviour in the partition test as other phytoxanthins containing a hydroxyl group.

Solvent	*Absorption maxima*		
Carbon disulphide	531	494.5	461 mμ
Benzene	509	476	447.5 mμ
Petroleum ether	494.5	462.5	434.5 mμ
Ethanol	494.5	462	434.5 mμ

According to the experiments of ZECHMEISTER and SCHROEDER[47], gazaniaxanthin exhibits no vitamin A activity. It may be concluded from this result that the hydroxyl group is substituted in the β-ionone ring.

Gazaniaxanthin monoacetate:

This compound is formed by treating gazaniaxanthin with acetic anhydride in pyridine. It crystallises from benzene or methanol in stout needles and from a mixture of petroleum ether and methanol in curved needles, m.p. 83–85°. The spectral properties correspond to those of gazaniaxanthin.

Cis-trans Isomers

ZECHMEISTER and SCHROEDER[47] converted gazaniaxanthin into a complex mixture of isomers by the usual methods (p. 39). So far it has not been possible to obtain these compounds in a crystalline state and to investigate them in detail.

References p. 341–343.

12. CELAXANTHIN $C_{40}H_{56}O$*

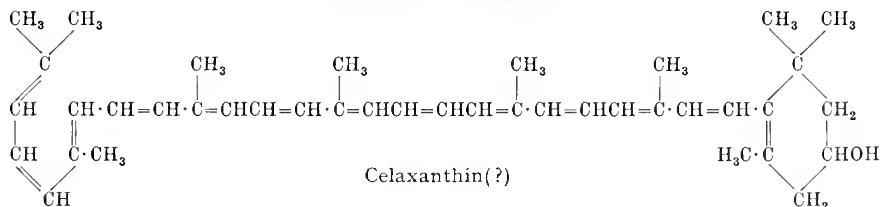
History and Occurrence

In the course of investigations on the red berries of *Celastrus scandens*, A.L. LE ROSEN and ZECHMEISTER[48] found, besides β-carotene, cryptoxanthin, zeaxanthin, rubixanthin(?) and two unknown pigments present in very small amounts, a new phytoxanthin, for which they proposed the name celaxanthin. In its spectral properties the new pigment shows close similarity to rhodoviolascin (p. 297) and torulin (p. 329) so that these pigments are probably closely related.

Preparation[48]

The air-dried, bright red flesh of *Celastrus* berries (230 g) was extracted for a short time with a mixture of petroleum ether and methanol (8:1.5) in a shaker. It was then dried at 40°, finely ground, and again exhaustively extracted with the same solvent mixture. The pigments obtained from the combined extracts were dissolved in petroleum ether, the solution was washed free from methanol, dried over sodium sulphate, and chromatographed on calcium hydroxide. The chromatogram was developed either with benzene or a mixture of benzene and acetone. The celaxanthin esters were found in the second zone from the top of the column, beneath the zone of a phytoxanthin with absorption maxima at very long wavelengths (587, 547.5 mμ in carbon disulphide). The celaxanthin zone was arbitrarily divided into two zones and the pigments were separately subjected to renewed chromatography. They were then eluted with a mixture of benzene and methanol (3:1), the solvent was evaporated and the pigments were precipitated with methanol from a petroleum ether solution. The celaxanthin ester obtained in this manner was saponified at room temperature with methanolic potassium hydroxide. The reaction mixture was worked up in the usual way and the pigment obtained was purified by adsorption on calcium hydroxide from a little benzene, and the column was washed with a mixture of petroleum ether and acetone (10:1). After elution and removal of the solvent by distillation, the celaxanthin was crystallised from a mixture of ethanol and carbon disulphide or benzene.

Chemical Constitution



Celaxanthin(?)

The results of elementary analysis suggests a molecular formula $C_{40}H_{54}O$ or $C_{40}H_{56}O$. The occurrence of a natural celaxanthin ester shows that the oxygen is present in the form of a hydroxyl group. As the pigment does not show the

* Or $C_{40}H_{54}O$.

References p. 341–343.

properties of an enol, the grouping =C.(OH) can be excluded. On ozonisation, LE ROSEN and ZECHMEISTER obtained 0.55 mols of acetone per mol of pigment (after substraction of the blank) thus indicating the presence of an isopropylidene grouping. Finally, the long-wavelength location of the absorption maxima indicates the presence of 13 conjugated bonds. The position of the hydroxyl group is not certain; for reason of analogy it is assumed to be at carbon atom 3′.

The experimental results are in agreement with the proposed formula for celaxanthin, but do not exclude the possibility of other structures.

Properties

Crystalline form: Celaxanthin crystallises from petroleum ether and ethanol in long needles which are combined in rosettes or clusters. In bulk, the pigment has the appearence of a dark red crystalline powder, somewhat reminiscent of lycopene.

Melting point: 209–210° (corr., BERL-BLOCK, capillary filled with carbon dioxide). Another sample of the pigment had m.p. 204–205°.

Solubility: Celaxanthin is sparingly soluble in carbon disulphide and benzene at room temperature. It is slightly soluble in petroleum ether and almost insoluble in methanol and ethanol.

Partition test: On partition between petroleum ether and 85 % methanol, the pigment is entirely epiphasic. With 95 % methanol the lower layer is also coloured.

Optical activity: The optical activity could not be determined because of the high colour intensity.

Spectral properties:

Solvent	*Absorption maxima*			
Carbon disulphide . .	562	521	487	455 mμ
Ethanol	520.5	488	455	mμ
Petroleum ether . . .	520	486.5	456	(429)mμ

Chromatographic behaviour: Celaxanthin is well adsorbed on calcium hydroxide from petroleum ether or from a mixture of petroleum ether and acetone. Benzene, or a mixture of benzene and acetone can also be used as solvents for this adsorbent. The pigment can be eluted with the same solvents, but containing a little methanol.

Cis-trans Isomers

LE ROSEN and ZECHMEISTER found that celaxanthin is reversibly isomerised on warming in the same way as torulin (cf. p. 329). LE ROSEN and ZECHMEISTER

References p. 341–343.

ascribe this change to *cis-trans* isomerism and distinguish three neo-celaxanthins*:

Solvent	*Absorption maxima in petroleum ether*			
Neocelaxanthin A . .	534	497	464	(433.5) mμ
Neocelaxanthin B . .	530	493.5	460	(431.5) mμ
Neocelaxanthin C . .	536	496.5	461	(432) mμ

The neotorulins, also obtained by LE ROSEN and ZECHMEISTER, exhibit spectral properties similar to the neocelaxanthins, but can be easily separated from the latter by chromatography on calcium hydroxide. (The two natural pigments can be separated in the same way).

13. PETALOXANTHIN $C_{40}H_{56}O_3$**

History and Occurrence

The blossoms of *Cucurbita Pepo* were first examined for carotenoids in 1914 by MICHAUD and TRISTAN[49]. In 1936, ZECHMEISTER, BÉRES and UJHELYI[50] isolated from this source a new phytoxanthin, for which they proposed the name petaloxanthin. (The obvious name cucurbitaxanthin could not be used as this had already been employed by SUGINOME and UENO to designate lutein obtained from *Cucurbita maxima* Duch[51]). Petaloxanthin has so far only been found in pumpkin blossoms.

Preparation[50]

1 kg of material (from male pumpkin blossoms) is exhaustively extracted with ether in the cold. The combined extracts are concentrated and the residue is saponified with methanolic potassium hydroxide at room temperature. The reaction mixture is covered with petroleum ether and the pigments are divided into an epiphasic and hypophasic fraction by addition of a little water. The pigments of the hypophasic fraction are extracted with ether and the solution is washed and dried, and the solvent evaporated. The residue is dissolved in a little carbon disulphide, a small portion remaining undissolved. This less soluble fraction is dissolved in more carbon disulphide. The solution is slightly cooled to precipitate colourless impurities and the mother liquors are twice chromatographed on calcium carbonate. The petaloxanthin is eluted with methanol-containing ether and crystallised from a mixture of carbon disulphide and petroleum ether. From 1 kg of ground blossoms, 20 mg of pigment are obtained in this way.

Chemical Constitution

Very little is at present known regarding the structure of petaloxanthin. The molecular formula $C_{40}H_{56}O_3$ or $C_{40}H_{58}O_3$, the absorption spectrum (maxima

* None of these isomerisation products were obtained in a crystalline state.

** The formula $C_{40}H_{58}O_3$ is also a possible one for petaloxanthin.

References p. 341–343.

at 514.5 and 481 mμ in carbon disulphide), the chromatographic behaviour, the blue colouration with concentrated hydrochloric acid, and the melting point all indicate a close relationship to, or even identity with, antheraxanthin (cf. p. 191). The two pigments can, however, be chromatographically separated, antheraxanthin being found in the upper zone. This behaviour is reminiscent of the pair of isomers, flavoxanthin and chrysanthemaxanthin. These two pigments also have closely similar properties but can be separated on a zinc carbonate chromatogram, and also differ in their reaction with hydrochloric acid, only flavoxanthin giving a blue colouration (cf. p. 210). The blue colouration produced on shaking an ethereal solution of petaloxanthin with concentrated aqueous hydrochloric acid is weaker than the colouration produced with antheraxanthin.

The nature of the three oxygen atoms present in petaloxanthin is also unknown. The strong adsorption of the pigment on calcium carbonate suggests that it contains two hydroxyl groups. The presence of a third hydroxyl group is unlikely, since a compound containing three hydroxyl groups should be more strongly adsorbed than antheraxanthin which contains 2 hydroxyl and one epoxide group. A re-investigation of petaloxanthin thus appears very desirable.

Properties

Crystalline form: The pigment crystallises from a mixture of carbon disulphide and petroleum ether in long, spear-like crystals. From ethanol it is obtained in silky, lustrous, light-yellow leaflets which appear under the microscope as long, thin, straw-yellow squares. (A microphotograph will be found in the communication by ZECHMEISTER and co-workers[52]).

Solubility: Petaloxanthin is easily soluble in benzene, less easily soluble in carbon disulphide, sparingly soluble in cold ethanol and almost insoluble in petroleum ether and petrol.

Melting point: 211–212° (corr., oil-bath), 202° (BERL-BLOCK, short thermometer).

Spectral properties:

Solvent	*Absorption maxima*	
Carbon disulphide	514.5	481 mμ
Chloroform	492	460.5 mμ
Benzene	494	460.5 mμ
Ethanol	483	451.5 mμ

Optical activity: No data are available.

Partition test: Petaloxanthin is entirely hypophasic.

Chromatographic behaviour: The pigment is well adsorbed on calcium carbonate from carbon disulphide solution, or on calcium hydroxide from

benzene solution, and is found below antheraxanthin but above zeaxanthin in the chromatogram.

Colour reactions: On shaking an ethereal solution of petaloxanthin with 37 % aqueous hydrochloric acid, a pale blue colouration is observed which eventually changes to violet-blue.

Detection and estimation: The separation of petaloxanthin from other phytoxanthins is effected by adsorption on calcium carbonate or calcium hydroxide. The pigment can be identified by determining the absorption maxima and by the hydrochloric acid reaction.

TABLE 55

COMPARISON OF SOME PROPERTIES OF PETALOXANTHIN AND ANTHERAXANTHIN

	Petaloxanthin	Antheraxanthin
Empirical formula	$C_{40}H_{56}O_3$ (or $C_{40}H_{58}O_3$)	$C_{40}H_{56}O_3$
Number of hydroxyl groups	probably 2	2
Number of double bonds	?	10, all conjugated
Melting point	202° (uncorr.) (Berl-block)	205° (uncorr.)
Mixed melting point	198° (uncorr.) (Berl-block)	
Position in chromatogram	lower	higher
Colour reaction with concentrated hydrochloric acid	blue, soon changes to violet-blue	blue, soon fades
Absorption maxima in carbon disulphide	514.5 481 mμ	510 475 mμ
Absorption maxima in ethanol	483 451.5 mμ	479 449 mμ

14. SARCININ AND SARCINAXANTHIN

The pigments of *Sarcina lutea* were investigated by CHARGAFF and DIERYCK[53] and by CHARGAFF[54] who isolated a new carotenoid termed sarcinin. The constitution of this pigment is till entirely unknown. It may be a hydrocarbon, but the amount isolated was so small that no conclusive data could be obtained. In petroleum ether solution, sarcinin exhibits absorption maxima at 469, 440 and 415 mμ. Besides sarcinin, *Sarcina lutea* contains another new phytoxanthin which has the same optical properties.

In 1936, NAKAMURA[55] isolated from *Sarcina lutea* a yellow carotenoid which he regarded as a phytoxanthin ester. This pigment exhibits absorption maxima at 490, 460 and 433 mμ in carbon disulphide solution. This carotenoid is

References p. 341–343.

evidently not the hypophasic pigment of CHARGAFF, since the latter exhibits absorption maxima at longer wavelengths.

Very recently the pigments of *Sarcina lutea* have been reinvestigated by YOSHIHARU TAKEDA and TATUO OHTA[56] who described the isolation of a new carotenoid which they termed sarcinaxanthin. From 385 g of dried bacteria, 3.4 mg of sarcinaxanthin were obtained. The pigment crystallised from a mixture of benzene and petrol in tufted red spheroids, m.p. 149–150° (KOFLER-HILBCK micro-melting point apparatus). On partition between petroleum ether and methanol, sarcinaxanthin behaves like a phytoxanthin containing one hydroxyl group. It is fairly strongly adsorbed on alumina from petrol solution and can only be eluted with difficulty by means of petrol containing some ethanol.

Solvent	*Absorption maxima*		
Carbon disulphide	499	466.5	436 mμ
Chloroform	480	451	423 mμ
Petrol	469	440	(415) mμ
Benzene	481	451	424 mμ
Ethanol	469.5	441	(415) mμ

It appears that sarcinaxanthin may be identical with the hypophasic carotenoid reported — though not isolated — by CHARGAFF[53, 54].

15. TARAXANTHIN $C_{40}H_{56}O_4$

History and Occurrence

During an investigation of the carotenoids from the blossoms *Taraxacum officinale* (dandelion), KUHN and LEDERER[57] discovered a new pigment which they termed taraxanthin. Later studies by KARRER and RUTSCHMANN[58] showed that the occurrence of taraxanthin evidently depends on geographical and climatic factors since the material investigated by the last-named authors did not contain the pigment.

Numerous references to the occurrence of taraxanthin are given in the literature, but the pigment never occurs in large concentrations and its isolation is relatively tedious.

References p. 341–343.

TABLE 56

OCCURRENCE OF TARAXANTHIN *

Source	Literature references
a) In blossoms:	
Helianthus annuus	L. ZECHMEISTER and P. TUZSON, *Ber. 67* (1934) 170.
Impatiens noli tangere	R. KUHN and E. LEDERER, *Z. physiol. Chem. 213* (1932) 188.
Leontodon autumnalis	do.
Ranunculus acer	R. KUHN and H. BROCKMANN, *Z. physiol. Chem. 213* (1932) 192. — P. KARRER, E. JUCKER, J. RUTSCHMANN and K. STEINLIN, *Helv. chim. Acta 28* (1945) 1146.
Taraxacum officinale	R. KUHN and E. LEDERER, *Z. physiol. Chem. 200* (1931) 108.
Tussilago Farfara	P. KARRER and R. MORF, *Helv. chim. Acta 15* (1932) 863.
Ulex europaeus	K. SCHÖN, *Biochem. J. 30* (1936) 1960.
b) In hips:	
Rosa canina	R. KUHN and C. GRUNDMANN, *Ber. 67* (1934) 339, 1133.
Rosa damascena Mill.	do.
Rosa rubiginosa	do.
c) In algae:	
Cladophora Sauteri	I. M. HEILBRON, E. G. PARRY and R. F. PHIPERS, *Biochem. J. 29* (1935) 1376.
Nitella opaca	do.
Oedogonium	do.
Rhodymenia palmata	do.
d) In liver:	
(Lophius piscatorius)	N. A. SÖRENSEN, *Chem. Centr. 1934*, II, 682. — I. M. HEILBRON and coworkers, *Biochem. J. 29* (1935) 1379.

Preparation[57]

The dried and finely ground dandelion blossoms are extracted with a mixture of acetone and petroleum ether (1:1) at room temperature. The pigment is transferred to the petroleum ether layer by addition of water, and saponified with ethanolic potassium hydroxide. The free phytoxanthins are dissolved in methanol, the solution is covered with a layer of petroleum ether, and the pigments are precipitated by very careful addition of water. The pigments are crystallised from methanol in order to remove colourless impurities. In this way about 400 mg of fairly pure phytoxanthin are obtained from 15,000 dandelion blossoms (without cups). The phytoxanthins are separated by chromatographic adsorption on calcium carbonate from a mixture of benzene and petrol. Taraxanthin is ad-

* According to E. LEDERER, *Bull. Soc. Chim. biol. 20* (1938) 554 the skins of various species of fish contain taraxanthin. These findings require further confirmation.

References p. 341–343.

sorbed on the uppermost part of the column and can be eluted with a mixture of ether and methanol. It is purified by precipitation with water from methanol solution covered with petroleum ether and subsequent crystallisation from methanol. About 40 mg of taraxanthin are obtained from 200 mg of phytoxanthin mixture.

Taraxanthin can be prepared more simply from spring cabbage[59]. The blossoms are dried, finely ground, and extracted with petroleum ether at room temperature. The phytoxanthin esters are saponified with ethanolic potassium hydroxide and the products are separated into an epiphasic and an hypophasic fraction. The free phytoxanthins are precipitated from the latter by the addition of water under a layer of petroleum ether. The pigment thus obtained is again dissolved in methanol, precipitated with water and finally recrystallised from methanol. In this way 4 mg of pure taraxanthin are obtained from about 500 blossoms.

Chemical Constitution

The constitution of taraxanthin is still unknown. The molecular formula $C_{40}H_{56}O_4$ shows that the pigment is isomeric with violaxanthin (cf. p. 193). The similar spectral properties of the two pigments indicate that they contain similar chromophoric systems.

KUHN and LEDERER[57] obtained values corresponding to 3–4 active hydrogen atoms in ZEREWITINOFF determinations. On catalytic hydrogenation, taraxanthin absorbs 11 mols of hydrogen and the composition of the perhydro-derivatives shows that the pigment contains two isocyclic rings.

Properties

Crystalline form: Taraxanthin crystallises from methanol in fine lustrous prisms or plates.

Melting point: 185–186° (uncorr.)[60], 184–185° (corr.)[61].

Solubility: The pigment is readily soluble in benzene, ether, ethanol and methanol, but insoluble in petroleum ether.

Spectral properties:

Solvent:	*Absorption maxima:**		
Carbon disulphide	501	469	441 mμ
Petrol	472	443	mμ

(cf. Fig. 24, p. 358)

Optical activity: $[\alpha]^{22}_{643.8} = +200°$ in ethyl acetate solution.

Partition test: Taraxanthin exhibits entirely hypophasic properties.

Chromatographic behaviour: The pigment is well adsorbed on zinc carbonate from benzene solution (cf. KUHN and BROCKMANN[61]).

* Quantitative extinction measurements are reported by K. W. HAUSSER and A. SMAKULA, *Z. angew. Chem.* 47 (1934) 663; 48 (1935) 152. — K. W. HAUSSER, *Z. techn. Phys.* 15 (1934) 13.

Colour reactions: In contrast to violaxanthin, taraxanthin in ethereal solution does not give a blue colouration on shaking with concentrated aqueous hydrochloric acid.

Detection and estimation: Taraxanthin is separated from other phytoxanthins by adsorption on zinc carbonate from benzene solution. It can be identified by the determination of the absorption maxima and by the negative hydrochloric acid reaction. For the colourimetric determination, cf. KUHN and BROCKMANN[61].

Cis-trans Isomers of Taraxanthin

ZECHMEISTER and TUZSON[62] studied the behaviour of taraxanthin in the presence of iodine and found that the chromatogram of the reaction products contained several bands which were ascribed to *cis-trans* isomers of natural taraxanthin. Three neo-taraxanthins could be distinguished; they were characterised by their absorption spectra in carbon disulphide. None of these pigments has yet been obtained in the crystalline state.

Solvent:	*Absorption maxima:*		
Neo-taraxanthin A	494.5	464	434 mμ
Neo-taraxanthin B	497	470.5	443 mμ
Neo-taraxanthin C	480	449	mμ
Taraxanthin (natural) . . .	501	469	440 mμ

16. ESCHSCHOLTZXANTHIN

In the course of investigations on the pigments from the blossoms of *Eschscholtzia californica*, STRAIN[63] discovered a previously unknown carotenoid for which he proposed the term eschscholtzxanthin.

For the isolation of the pigment, the blossoms are dried at 45–47°, finely ground and extracted with petroleum ether. The extract is concentrated and saponified with methanolic potassium hydroxide. The pigments are separated into epiphasic and hypophasic fractions, and the latter are extracted with ether. The ethereal solution is concentrated in vacuum and on cooling most of the eschscholtzxanthin crystallises out. About 4 g of crude product are obtained from 1.15 kg of dried blossoms. The pigment is purified by repeated re-crystallisation from acetone or chromatographic absorption. (Products of equal purity are obtained by the two methods).

Only a beginning has been made in the elucidation of the constitution of eschscholtzxanthin. The molecular formula is $C_{40}H_{54}O_2$ ($\pm$ H_2)*. The two oxygen atoms are present as hydroxyl groups. On microhydrogenation the pigment takes up 12 mols of hydrogen. The long-wavelength location of the

* The analytical figures are in better agreement with the formula $C_{40}H_{56}O_2$ than with the formula $C_{40}H_{54}O_2$, but the differences are too small to reach a definite conclusion.

References p. 341–343.

absorption maxima indicates that the 12 double bonds are in conjugation.

Eschscholtzxanthin is extremely unstable in air. The rate of oxygen uptake is different in different organic solvents and is not increased by haemin, in contrast to the behaviour of lycopene[64]. If solutions of eschscholtzxanthin are warmed, the absorption bands are displaced.

Melting point: 185–186° (corr., Berl-block). $[\alpha]_{6678}^{18} = +225° \pm 12°$ (in chloroform).

Solvent:	*Absorption maxima:*		
Carbon disulphide	536	502	475 mμ
Benzene	516	485	458 mμ
Chloroform	513	484	456 mμ
Ethanol	503	472	446 mμ
Pyridine	521	489	463 mμ

On adding concentrated sulphuric acid to a solution of the pigment in chloroform, the latter assumes a blue colouration. No colouration is obtained with concentrated hydrochloric acid.

Eschscholtzxanthin can be chromatographed on magnesium carbonate or calcium carbonate from carbon disulphide or benzene solution. It is found above zeaxanthin but below capsanthin in the chromatogram. The results so far obtained in these preliminary investigations suggest that eschscholtzxanthin may be a dihydroxy-γ-carotene; all its properties are in accord with such a structure.

Esters of Eschscholtzxanthin

1. *Diacetate:* Melts over the range 200–240° with decomposition. $[\alpha]_{6678}^{20}$: + 132° (in chloroform).
2. *Dipalmitate:* M.p. 100–110°.
3. *Dibenzoate:* M.p. 133°. $[\alpha]_{6678}^{20} = -142°$.
4. *Di-p-nitrobenzoate:* M.p. 260° $[\alpha]_{6678}^{20} = -234°$.
5. *Dioleate:* Could not be crystallised.

17. ECHINENONE

During investigations on the pigments from sea urchin (*Strongylocentrotus lividus**), LEDERER[65] isolated a new carotenoid, echinenone.

For the preparation of echinenone, the sexual glands of sea urchins are ground with sand and extracted at room temperature with acetone. These extracts are combined with those obtained from the back shells of the animals and concentrated

* In his first communication dealing with echinenone, E. LEDERER [*Compt. rend.* *201* (1935) 300] erroneously described the sea urchins as *Echinus esculentus*. The error was corrected in the second communication[65].

References p. 341–343.

in vacuum. The residual acetone solution is then diluted with water and the pigments are first extracted with petroleum ether and then with benzene. From the petroleum ether solution mainly polyene hydrocarbons are obtained, while the benzene extract contains mainly phytoxanthins and is worked up for pentaxanthin (p. 327). The petroleum ether solution is shaken with 90% methanol and the phytoxanthins thus extracted are worked up together with those obtained from the benzene solution.

For the isolation of echinenone, the petroleum ether extract is washed with water and then saponified with methanolic potassium hydroxide. After working up in the usual way and freezing out accompanying steroids, the pigments are adsorbed on a column of calcium carbonate from petroleum ether solution. The echinenone fraction is separated and chromatographed on alumina. α-Carotene and β-carotene are adsorbed on the lower part of the column. The echinenone fraction found in the upper part of the column is still not homogeneous, so that the pigment must be chromatographed repeatedly on alumina and on calcium carbonate. It is finally crystallised from a mixture of benzene and methanol. From 400 sea urchins about 4 mg of echinenone can be obtained in this manner.

A certain amount of information is available regarding the chemical constitution of this pigment, but the structure* has not yet been fully clarified. The elementary analysis of echinenone gives the molecular formula $C_{40}H_{58}O$ ($\pm H_2$). It produces strong vitamin A activity in rats and therefore probably contains an unsubstituted β-ionone ring. The single-banded (?) absorption spectrum** suggests the presence of a carbonyl group and the epiphasic behaviour of the pigment excludes the presence of a hydroxyl group. In its general properties echinenone is reminiscent of β-semicarotenone (p. 139). LEDERER suggested the possibility that echinenone may be identical with myxoxanthin (p. 225). Some of the properties of the two compounds are in fact in good agreement, but HEILBRON considers it unlikely that the two pigments are identical. (Private communication to LEDERER).

Echinenone crystallises from petroleum ether or from a mixture of benzene and methanol in violet needles with a metallic lustre, m.p. 178–179°. (On a single occasion LEDERER obtained crystals melting at 193°, but the m.p. 178–179° is more probably correct). The absorption maxima of the pigment in carbon disulphide solution are located at 520, 488 and 450 mμ**.

On partition between methanol and petroleum ether, echinenone exhibits purely epiphasic behaviour. By treatment with iodine the pigment can be precipitated from petroleum ether solution as the iodide from which it can be

* A possible structure for echinenone is proposed by E. LEDERER and T. MOORE, *Nature*, London, *137* (1936) 996.

** E. LEDERER observed a single band at 488 mμ on examination in a visual spectrometer. Photoelectric determinations by R. KUHN showed the presence of two additional weak bands at 520 and 450 mμ. E. LEDERER considers it possible that these weak bands belong not to echinenone itself but to an accompanying carotenoid. This question requires further investigation.

References p. 341–343.

regenerated by treatment with thiosulphate. Echinenone gives no blue colouration with concentrated aqueous hydrochloric acid in ethereal solution.

18. PECTENOXANTHIN

LEDERER[66] investigated the pigments which occur during the development of the sexual organs of St. Jacques shell (*Pecten maximus*) and discovered a new carotenoid for which he proposed the term pectenoxanthin*.

For the isolation of pectenoxanthin, the finely cut sexual organs of the shells are treated with acetone. Water is added and the pigments are extracted from the solution with petroleum ether. They are transferred to 90% methanol and the solution is strongly concentrated in vacuum. On diluting the methanolic residue with a little petroleum ether, pectenoxanthin crystallises out. For further purification the pigment is adsorbed on a column of calcium carbonate and then crystallised from a mixture of pyridine and water. From 500 sex organs about 50 mg of pure pectenoxanthin were obtained in this way.

No conclusive evidence is at present available regarding the chemical constitution of pectenoxanthin. The molecular formula appears to be $C_{40}H_{54}O_3$ ($\pm H_2$). Microhydrogenation showed the presence of 11 double bonds. ZEREWITINOFF determination yielded a quantity of methane corresponding to 2 atoms of active hydrogen. Thus it appears that 2 oxygen atoms are present in the form of hydroxyl groups. The nature of the third oxygen atom is as yet unknown. The pectenoxanthin molecule does not appear to contain an unsubstituted β-ionone ring as the compound exhibits no vitamin A activity.

Solvent:	*Absorption maxima:*		
Carbon disulphide	518	488	454 mμ
Benzene	496	464	434 mμ
Petroleum ether	488	458	mμ

The pigment crystallises from aqueous pyridine in long yellow-brown prisms, m.p. 182°. Pectenoxanthin is adsorbed above zeaxanthin on calcium carbonate. The pigment is much more readily soluble in 90% methanol than in petroleum ether. It is readily soluble in benzene, carbon disulphide and pyridine. With concentrated sulphuric acid it produces a deep blue colouration. No colouration is observed with aqueous hydrochloric acid.

19. PENTAXANTHIN

Besides echinenone, LEDERER isolated a further previously unknown pigment, pentaxanthin, from sea urchin (*Strongylocentrotus lividus*)[67].

* P. KARRER and U. SOLMSSEN, *Helv. chim. Acta 18* (1935) 915 discovered a carotenoid which exhibits similar absorption maxima as pectenoxanthin in *pecten jacobaeus*. The two pigments are possibly identical.

References p. 341–343.

For the isolation of the pigment, the phytoxanthin solutions obtained during the preparation of echinenone (p. 325) are washed with water, dried and adsorbed on alumina. After elution, pentaxanthin, which is accompanied by other phytoxanthins, is again chromatographed on calcium carbonate. This operation is repeated once again and the pigment is then recrystallised from benzene. From 400 sea urchins about 40 mg of pure pentaxanthin are obtained.

The chemical constitution of pentaxanthin is largely unknown. The molecular formula is $C_{40}H_{56}O_5$ ($\pm$ H_2). Only three of the five oxygen atoms appear to be present in the form of hydroxyl groups. Microhydrogenation indicates the presence of 12 double bonds in the molecule. LEDERER assumes that 11 double bonds are in conjugation, while the remainder of the hydrogen is used to saturate a carbonyl group or an isolated ethylenic double bond. It should be remarked, however, that the longest wavelength location of the absorption maximum in carbon disulphide solution produced by 11 conjugated double bonds would be expected to be near 520 mμ, (e.g. β-carotene), whereas the absorption maxima of pentaxanthin are located at 506, 474 and 444 mμ.

The pigment crystallises from benzene in red needles, m.p. 209–210°. Pentaxanthin is adsorbed more strongly than xanthophyll on calcium carbonate.

Solvent:	*Absorption maxima:*		
Carbon disulphide	506	474	444 mμ
Benzene	487	456	424 mμ
Methanol	(445)	475	(505) mμ

The pigment is very sparingly soluble in ether and petroleum ether, somewhat more soluble in benzene and readily soluble in carbon disulphide and chloroform.

20. SULCATOXANTHIN

HEILBRON and co-workers[68] isolated a previously unknown carotenoid, sulcatoxanthin, from *Anemonia sulcata*.

500 finely cut plants were extracted with a mixture of acetone and ether (1:1), the combined extracts were concentrated in vacuum, and the pigments were extracted with petroleum ether. The solution was then repeatedly shaken with 65 % methanol. Only a small quantity of pigment exhibiting absorption maxima at 478 and 450 mμ in carbon disulphide remained in the ether layer. The sulcatoxanthin was extracted from the methanolic solution with petroleum ether and chromatographed on alumina. It was then adsorbed on calcium carbonate from carbon disulphide solution and finally recrystallised from a mixture of ether and petroleum ether. The yield amounted to about 50 mg.

Very little is known about the chemical constitution of sulcatoxanthin. The molecular formula appears to be $C_{40}H_{52}O_8$. The high oxygen content is

References p. 341–343.

reflected in the solubility in 65 % methanol. The pigment is insoluble in petroleum ether, sparingly soluble in carbon disulphide and readily soluble in benzene and ethanol. Sulcatoxanthin crystallises from a mixture of ether and petroleum ether in deep scarlet-red needles which show no sharp melting point but sinter at 110°, soften at 125° and have completely melted at 130°. The pigment is decomposed by the action of alkali. Concentrated sulphuric acid produces a blue colouration.

Solvent:	*Absorption maxima:*		
Carbon disulphide	516	482	(450) mμ

21. GLYCYMERIN

This carotenoid was isolated by LEDERER[69] and by FABRE and LEDERER[70] from the sexual organs of *Pectunculus glycymeris* shells. On working up a larger quantity of shells, glycymerin could no longer by obtained[71], a mixture of several phytoxanthins being found instead. As the isolation of the pigment could not be repeated and as LEDERER himself obtained a very small quantity which he did not consider as pure, the existence of this pigment must be regarded as doubtful. Glycymerin crystallises in irregular brown-violet crystal aggregates, m.p. 148–153°. It is almost insoluble in petroleum ether, but fairly readily soluble in methanol. In carbon disulphide solution the pigment exhibits a single absorption band at 495 mμ. It exhibited no acidic properties, thus differing from astacene. It is believed that the pigment occurs partly esterified in the shell.

Glycymerin has also been observed in the coat of *Pectunculus glycymeris* and in the liver of *Mytilus edulis*[69, 70].

22. CYNTHIAXANTHIN

In the course of his investigations of the pigments of different species of ascidiae, LEDERER found besides astacene, a new pigment, for which he proposed the name cynthiaxanthin, in *Halocynthia pipillosa*[72]. The same source was later examined by KARRER and SOLMSSEN[73], but no cynthiaxanthin was obtained.

In some respects cynthiaxanthin possesses properties very similar to those of zeaxanthin. Chromatography of a mixture of the two pigments, however, gives rise to two zones, the lower of which contains cynthiaxanthin. It is therefore improbable that the two pigments are identical. Cynthiaxanthin also differs in chromatographic properties from pectenoxanthin, since pectenoxanthin is adsorbed above zeaxanthin on calcium carbonate.

For the isolation of cynthiaxanthin, the animals (15 specimens weighing 120 g) are dissected and extracted with acetone. The pigments are transferred to petroleum

References p. 341–343.

ether by dilution with water and the petroleum ether solution is extracted with 90 % methanol. α-Carotene, β-carotene and esterified astacene (astaxanthin ?) remain in the petroleum ether layer. The hypophasic pigments are taken up in benzene and chromatographed on calcium carbonate. Two main zones are formed, the upper yielding astacene after saponification, while the lower contains the new pigment, cynthiaxanthin. By elution with aqueous methanol and crystallisation from the same solvent under petroleum ether, the pigment was obtained in small, yellow-orange needles. After recrystallisation from aqueous ethanol the m.p. was 188–190°. The yield was 1 mg. The pigment does not give a blue colouration with concentrated aqueous hydrochloric acid.

Solvent:	*Absorption maxima:*		
Carbon disulphide	517	483	451 mμ
Petroleum ether	482	452	mμ

23. TORULIN

During investigations of the carotenoids of *Torula rubra*, LEDERER[74, 75] found besides β-carotene and two unknown pigments present in very small amounts, another new carotenoid for which he proposed the term torulin. Later KARRER and RUTSCHMANN[76] also isolated torulin besides torularhodin (p. 330) from the same source. Torulin appears to be fairly widely distributed in nature. Besides *Torula rubra*, it has been found in *Sporobolomyces roseus*, *Sporobolomyces salmonicolor*, *Lycogala epidendron*[74] and *Rhodotorula Sanniei*[77].

For the isolation of torulin, red yeast is extracted with acetone, the mixture of pigments is extracted with petroleum ether after addition of water, and the petroleum ether solution is extracted with ethanol and saponified with ethanolic potassium hydroxide. After working up in the usual way the epiphasic pigments are dissolved in a little petroleum ether and the solution is allowed to stand in the cold to allow considerable quantities of steroids to crystallise out. The mother liquors are then chromatographed on alumina, the β-carotene in the lower portion of the chromatogram is separated, and the torulin, which is adsorbed above β-carotene, is crystallised from petroleum ether in the cold. The pigment is further purified by crystallisation from benzene and methanol.

Torulin melts at 185°.

Solvent:	*Absorption maxima:*[76]		
Carbon disulphide	565	525	491 mμ*
Pyridine	545	508	475 mμ
Benzene	541	503	470 mμ
Ethanol	520	486	456 mμ
Chloroform	539	501	469 mμ

Nothing definite is known at the present time concerning the chemical constitution of the pigment. LEDERER[78] found a certain similarity between

* E. LEDERER records absorption maxima at 563, 520 and 488 mμ in carbon disulphide.

References p. 341–343.

torulin and rhodoviolascin (p. 295) and suggested that the former may be formed from rhodoviolascin by cyclisation of one half of the molecule, which would explain the shorter wavelength location of the absorption maxima. This suggestion, however, lacks experimental foundation.

24. TORULARHODIN $C_{37}H_{48}O_2$

History and Occurrence

In 1933, LEDERER[79] investigated the carotenoids of *Torula rubra*. Besides β-carotene, he found the previously unknown pigment torulin, as well as two other polyene pigments, one of which appeared to be a hydrocarbon while the other exhibited acidic properties.

FINK and ZENGER[80] later carried out similar investigations and also observed the two last-mentioned pigments. The investigation of *Torula rubra* pigments was continued by KARRER and RUTSCHMANN[81]. These authors were able to elucidate the main features of the constitution of the acidic pigment which they termed torularhodin.

Up to the present time torularhodin has only been observed in the red yeast *Torula rubra*.

Preparation[81, 82]

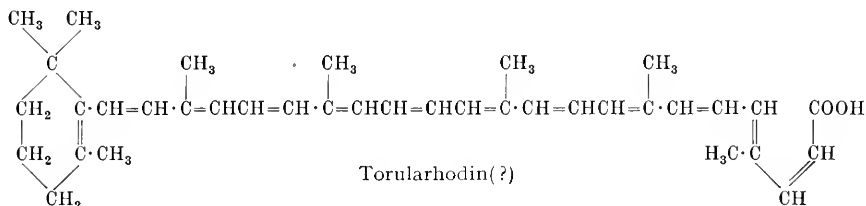
After pre-extraction with ethanol in an atmosphere of carbon dioxide, the yeast cells are ground with quartz sand in a large porcelain mortar and then extracted with acetone in vacuum The acetone solution is diluted with water and the pigments are extracted with a small quantity of petroleum ether. For the separation of the pigments this solution is adsorbed on alumina and the chromatogram is developed with a mixture of ether and methanol. Torularhodin forms an upper light-red zone in the chromatogram and is eluted with a mixture of ether and acetic acid (10:1). This solution is washed free from acid, the ether is evaporated and the oily residue is dissolved in a little methanol. The methanol solution is allowed to stand for 2–3 weeks at room temperature in an evacuated tube. During this period, torularhodin crystallises out, together with a considerable quantity of aliphatic acids, which are removed by washing with petroleum ether. The torularhodin remains undissolved and is obtained in a fairly pure condition after boiling with petroleum ether and methanol. The yield from 1 kg of yeast amounted to about 3–5 mg. For analysis, the pigment is reprecipitated several times from a mixture of benzene and methanol, but nevertheless still contains varying amounts of ash.

Chemical Constitution[76]

Torularhodin probably has the molecular formula $C_{37}H_{48}O_2$ and contains 12 conjugated double bonds. It is a monocarboxylic acid, as shown by its behaviour towards alkalis and the preparation of a well-crystallised monomethyl ester. The latter exhibits vitamin A activity which is considerably

References p. 341–343.

weaker than that of β-carotene. Since growth-promoting properties are generally associated with the presence of an unsubstituted β-ionone ring, it appears probable that such a ring is present in torularhodin. On the basis of these facts, the following possible structural formula, which is in agreement with the analytical data, the number of double bonds and the absorption spectrum, can be deduced.



Torularhodin(?)

In accordance with its acidic nature, torularhodin forms salts with alkalis. The salts exhibit the same optical properties as the free pigments, showing that salt formation does not result in any structural changes. The pigment is entirely indifferent to hydroxylamine in boiling ethanol and towards boiling alcoholic potassium hydroxide. Torularhodin is reduced by zinc dust and acetic acid in pyridine. This reaction is of considerable interest, as it has previously only been observed with carotenoids containing systems of conjugated double bonds terminated by two carbonyl or carboxyl groups (e.g. bixin, crocetin, rhodoxanthin, β-carotenone etc.). Torularhodin is rapidly reduced under the same conditions to give a light yellow product exhibiting the following absorption maxima.

Solvent:	*Absorption maxima:*	
Carbon disulphide	482	453 mμ
Petroleum ether	453	mμ

This compound shows an acidic reaction and is more hypophasic than torularhodin on partition between methanol and petroleum ether. The displacement of the absorption bands towards the violet relative to the parent pigment is extraordinarily large for a dihydroderivative.

Properties

Crystalline form: Torularhodin crystallises in fine red needles on slowly evaporating a methanol-ether solution. From a mixture of benzene and methanol, the compound is obtained as a violet-black crystalline powder.

Melting point: 201–203° (with decomposition, uncorr., in vacuum)

References p. 341–343.

Solubility: Torularhodin is easily soluble in carbon disulphide, chloroform and pyridine, less easily soluble in ether, benzene and hot ethanol, very sparingly soluble in methanol and almost insoluble in petroleum ether.

Solvent:	*Absorption maxima:*		
Carbon disulphide	582	541	502 mμ
Benzene	557	519	485 mμ
Petrol	537	501	(467) mμ
Pyridine	558	518	485 mμ
Chloroform	554	515	(483) mμ
Methanol	529	493	(460) mμ
Ethanol	532	495	463 mμ

The large difference in the location of the absorption maxima in carbon disulphide and in petrol or ethanol, is remarkable. The differences in the positions of the longest wavelength bands amount to 45–50 mμ, whereas in other carotenoids the differences are usually of the order of 30–40 mμ.

Optical activity: No data have been recorded.

Partition test: On partition between petroleum ether and 95 % methanol, the two zones are about equally coloured.

Chromatographic behaviour: Torularhodin is very strongly adsorbed on zinc carbonate from benzene solution, and forms a deep-violet zone in the uppermost part of the column. On alumina, the pigment is adsorbed with a light red colour. The chromatogram on zinc carbonate is developed with benzene, while the chromatogram on alumina is developed with a mixture of ether and methanol. The pigment is eluted with a mixture of ether and acetic acid (10:1).

Colour reactions: Torularhodin exhibits a different behaviour towards antimony trichloride and towards strong acids than any other known carotenoid. With antimony trichloride, a permanganate-red colouration is at first produced which immediately fades. After some time the solution assumes a faint blue colour. Anhydrous formic acid and concentrated sulphuric acid also decolourise the yellow-red torularhodin solution; on standing, however, a faint blue colour appears. On adding trichloracetic acid to a solution of the pigment in chloroform, a faint green colouration is observed after initial bleaching.

Detection and estimation: Torularhodin is identified by its acidic character and by means of its long-wavelength spectrum.

Torularhodin methyl ester $C_{36}H_{47}CO_2CH_3$

The methyl ester is formed by the action of diazomethane on a solution of torularhodin in benzene. It crystallises from a mixture of benzene and methanol in dark red needles, m.p. 172–173°, uncorr. The absorption spectrum of the ester differs slightly from that of the free acid.

References p. 341–343.

Solvent:	*Absorption maxima:*		
Carbon disulphide	581	541	502 mμ
Benzene	554	517	484 mμ
Petrol	533	498	468 mμ
Pyridine	560	519	485 mμ
Ethanol	533	496	464 mμ

25. ACTINIOERYTHRIN

This pigment was first obtained by LEDERER[69] from sea anemones *Actinia equina* and was shortly afterwards isolated from the same source by HEILBRON and co-workers[68].

For the isolation of the pigment[68, 70], the finely cut anemones are extracted with a mixture of ether and acetone (1:1) and the solution is concentrated under reduced pressure. The pigments are transferred to petroleum ether and the solvent is again evaporated. By dilution of the residue with acetone, part of the accompanying phosphatides and steroids can be precipitated. The remainder is frozen out. The pigments remaining in the mother liquors are taken up in petroleum ether and chromatographed on alumina. Actinioerythrin forms a violet-black zone in the upper part of the chromatogram. After elution, the pigment is again adsorbed on calcium carbonate and finally crystallised from absolute ethanol. 30 mg of actinioerythrin were obtained from 500 anemones.

Very little is yet known about the constitution of actinioerythrin. It is not even certain whether it is a carotenoid. According to LEDERER[69], actinioerythrin is the ester of a coloured acid. This is supported by the low melting point and by the good solubility in petroleum ether. On alkaline hydrolysis, however, HEILBRON and co-workers[68] obtained a compound, violerythrin, which showed no acidic properties towards dilute alkalis (cf. below). It was therefore assumed that violerythrin contains one or more enol groups which are isomerised to the stable ketoform.

Actinioerythrin crystallises from ethanol in brown-violet rhombs, m.p. 85°. It is sparingly soluble in ethanol, but readily soluble in petroleum ether, carbon disulphide, chloroform and pyridine.

Solvent:	*Absorption maxima:*		
Carbon disulphide	574	533	495 mμ
Petroleum ether	534	497	470 mμ
Ethanol		577–518 mμ (broad band)	

26. VIOLERYTHRIN

Violerythrin was obtained by HEILBRON, JACKSON and JONES[68] by the alkaline hydrolysis of actinioerythrin (cf above). The fact that hydrolysis

References p. 341–343.

occurs on shaking actinioerythrin with alkalis was previously observed by FABRE and LEDERER[70] who were unable, however, to isolate a homogeneous derivative and therefore assumed that decomposition of the pigment had taken place. Violerythrin can only be obtained in relatively good yields by carrying out the hydrolysis under very carefully controlled conditions.

According to HEILBRON and co-workers, a solution of actinioerythrin in petroleum ether is shaken at room temperature with 2.5% methanolic sodium hydroxide until the pigment has been entirely transferred to the lower layer (about 1 hour). The reaction mixture is then diluted with water, acidified with acetic acid and extracted with ether. The ethereal solution is washed and dried, the solvent is evaporated and the residue is crystallised from aqueous pyridine. 1 mg of violerythrin was obtained in this way from 5 mg of actinioerythrin.

The structure of this pigment is still unknown. It is not certain whether it belongs to the carotenoid series.

Violerythrin crystallises from aqueous pyridine in dark-violet micro-crystals, m.p. 191–192°. It dissolves in carbon disulphide with a purple-red colour, in alcohol and ether with a violet-red colour, in acetone and pyridine with a blue colour, and in benzene with a deep-blue colour. The absorption maxima in carbon disulphide are located at 625, 576 and 540 mμ.

27. δ-CAROTENE

WINTERSTEIN[83] examined the shells of *Gonocaryum pyriforme* for carotenoids and found besides lycopene, α-carotene, β-carotene and γ-carotene, a new pigment for which he proposed the term δ-carotene. In the chromatogram, δ-carotene is found between γ-carotene and β-carotene. It could not be obtained in a crystalline state so that its existence cannot be regarded as proven.

Solvent:	*Absorption maxima:*		
Carbon disulphide	526	490	457 mμ
Chloroform	503	470	440 mμ
Hexane	490	458	428 mμ

WINTERSTEIN[83] regards δ-carotene as the hitherto unknown carotene-isomer derived from one half-molecule of α-carotene and one half-molecule of γ-carotene.

28. FENICOTTERIN

During an investigation of the pigments responsible for the red colour of the fat of flamingo, C. MANUNTA[84] discovered a carotenoid which is similar to astacene but differs from the latter by a shorter-wavelength absorption maximum (487 mμ in carbon disulphide). As this compound was not obtained in a pure state or analysed, its existence cannot be regarded as certain.

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29. OSCILLAXANTHIN

In the course of an investigation of the carotenoids of *Oscillatoria rubescens*, KARRER and RUTSCHMANN[85] found, besides myxoxanthin, myxoxanthophyll, zeaxanthin* and β-carotene, a hitherto unknown pigment for which they proposed the term oscillaxanthin.

For the isolation of this pigment, the algae are dehydrated with ethanol, dried and extracted with warm methanol. This extract is combined with that obtained from the dehydration and the solutions are strongly concentrated. They are then saponified with aqueous potassium hydroxide and extracted with a large volume of ether, and the aqueous phase is almost neutralised with dilute sulphuric acid. From this solution the methanol is removed by distillation and the remaining aqueous solution is slightly acidified with dilute sulphuric acid (to pH4–5). The solution is then again extracted with ether whereby most of the saponification products of chlorophyll are removed. From the mother liquors, oscillaxanthin can then be extracted with ethyl acetate. After evaporation of the solvent the pigment is taken up in acetone and chromatographed on zinc carbonate. After elution, the oscillaxanthin is further purified by precipitation from ethyl acetate by means of ether. The pigment could not be obtained in a pure state because of the small amount of material available.

Oscillaxanthin exhibits acidic properties. It is readily soluble in alcohol, pyridine and acetone, but almost insoluble in ether, benzene, carbon disulphide and petroleum ether. Oscillaxanthin esters are readily soluble in ether, benzene, pyridine and chloroform, but almost insoluble in ethanol. The constitution of oscillaxanthin is still entirely unknown.

Solvent:	*Absorption maxima:*		
Carbon disulphide	568	528	494 mμ**
Methanol	531	496	464 mμ
Pyridine	552	514	483 mμ

Antimony trichloride produces a blue-green, concentrated sulphuric acid a blue, and concentrated hydrochloric acid an unstable blue colouration.

30. TROLLIXANTHIN AND TROLLICHROME $C_{40}H_{56}O_4$

During investigations of the carotenoids of *Trollius europaeus*, KARRER and JUCKER[86] discovered a new pigment which had previously escaped notice and for which they proposed the name trollixanthin[87]. It posseses the properties of an epoxide. In addition to trollixanthin, the flowers also contained β-carotene,

* I. M. HEILBRON and B. LYTHGOE, *J. Chem. Soc. 1936*, 1376 found myxoxanthin, myxoxanthophyll, β-carotene and xanthophyll, but no zeaxanthin in *Oscillatoria rubescens*.

** Absorption maxima in carbon disulphide refer to solutions prepared by diluting one drop of an alcoholic solution of the pigment with a large volume of carbon disulphide.

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xanthophyll, xanthophyll epoxide and another new carotenoid, also of epoxide character. The latter was not obtained in sufficient quantity for more detailed investigation.

For the preparation of trollixanthin, the blossoms are dried at about 40° and are exhaustively extracted with petroleum ether at room temperature. The combined extracts are concentrated to a small volume in vacuum and the residue is saponified with methanolic potassium hydroxide at room temperature. The pigments are then separated into an epiphasic and a hypophasic fraction. The latter is repeatedly boiled with ligroin to remove colourless products and the undissolved portion is chromatographed on zinc carbonate. Trollixanthin is adsorbed on the upper part of the column and can be separated from the less strongly adsorbed xanthophyll epoxide. It is eluted with methanol-containing ether and is recrystallised from benzene.

The constitution of the pigment is still unknown. The molecular formula of trollixanthin is $C_{40}H_{56}O_4$[88]. Trollixanthin is a monoepoxide; by the action of dilute mineral acid it is converted into a furanoid derivative, trollichrome, the absorption maxima of which are displaced by 22 mμ towards shorter wavelengths (in carbon disulphide solution). The remaining oxygen atoms are present in the form of hydroxyl groups; the latter are responsible for the stronger adsorption of trollixanthin as compared with xanthophyll epoxide on zinc carbonate. In view of the close similarity in the spectral properties of trollixanthin and xanthophyll epoxide, and of trollichrome, flavoxanthin and chrysanthemaxanthin, it is probable that similar chromophoric systems are present. Trollixanthin crystallises from benzene in thin, clustered light-yellow leaflets, m.p. 199° (uncorr., in vacuum). On shaking an ethereal solution of the pigment with concentrated aqueous hydrochloric acid, the latter assumes a weak blue colouration, as in the case of most carotenoid monoepoxides,

Solvent:	*Absorption maxima:*	
Carbon disulphide	501	473 mμ
Ethanol	474	447 mμ
Benzene	483	457 mμ
Chloroform	482	455 mμ

On partition between methanol and petroleum ether, trollixanthin is entirely hypophasic.

Trollichrome separates from benzene in light yellow crystals, m.p. 206–208° (uncorr., in vacuum). The hydrochloric acid reaction and partition test are the same as with trollixanthin,

Solvent:	*Absorption maxima:*	
Carbon disulphide	479	450 mμ
Ethanol	451	424 mμ
Benzene	459	432 mμ
Chloroform	458	430 mμ

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31. HAEMATOXANTHIN

During investigations of the spores of *Haematococcus pluvialis*, TISCHER[89] found, besides β-carotene, α-carotene*, xanthophyll, zeaxanthin and astacene**, a previously unknown carotenoid for which he proposed the name haematoxanthin.

About 6 g of the red spores of *Haematococcus pluvialis* were available for the isolation of the carotenoid. The spores were ground with quartz sand under acetone and exhaustively extracted with this solvent at room temperature. The extract was diluted with water and the pigments were extracted with petroleum ether. After removing the solvent by distillation, a red resinous residue remained which was dissolved in a little petrol and saponified with methanolic potassium hydroxide. The pigments were then divided in the usual way into epiphasic and hypophasic fractions and the epiphasic fraction was adsorbed on a column of calcium hydroxide. A very small quantity of haematoxanthin was eluted from the upper part of the chromatogram with alcohol-containing petrol and recrystallised from petrol. The pigment could not be obtained entirely pure because of lack of material.

It is not yet known whether haematoxanthin occurs in algae as an ester[90]. The constitution of the pigment is unknown.

Haematoxanthin is entirely epiphasic on partition between petroleum ether and 90% methanol. If 95% methanol is employed, the lower layer is also weakly coloured. The crude material melts at 205°. The pigment crystallises from petrol in brown-violet leaflets.

Solvent:	*Absorption range*	*Absorption maxima*
Carbon disulphide	463–563	513 mμ
Petrol (b.p. 70–80°)	450–515	478 mμ
Ether	445–515	480 mμ

32. CANARYXANTHOPHYLL AND PICOFULVIN

In the course of their investigations of the yellow pigments of different birds, BROCKMANN and VÖLKER[91] found that the carotenoids present were derived mostly from xanthophyll or, occasionally from zeaxanthin***. By means of feeding tests, these authors were able to prove that the feathers of canaries only attain a yellow colour if the diet contains xanthophyll or zeaxanthin†. In the digestive tract, the xanthophyll is converted into canary-

* J. TISCHER, *Z. physiol. Chem.* 252 (1938) 225.

** In his paper, J. TISCHER reports the isolation of "Euglenarhodon". This carotenoid later proved to be identical with astacene and thus does not require a special name.

*** The yellow pigment of the budgerigar (*Melopsittacus undulatus*) is not a carotenoid.

† Polyene pigments such as β-carotene, lycopene, violaxanthin and taraxanthin are not assimilated by birds. In canaries which have turned white by being kept on a suitable diet, the yellow colour is only restored on feeding xanthophyll or zeaxanthin, but not on feeding a carotenoid hydrocarbon, or violaxanthin or taraxanthin.

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xanthophyll and probably also into picofulvin, previously observed by KRUKENBERG.

Neither canaryxanthophyll nor picofulvin could be obtained in the crystalline state or investigated in detail, so that their constitutions are still unknown. If the diet contains zeaxanthin, the feathers also assume a yellow colour, but the pigments formed are not identical with those formed from a xanthophyll-containing diet. In this case the pigments appear to be decomposition products of zeaxanthin which do not exhibit sharp absorption maxima.

The following is a brief summary of the pigments of the feathers of some birds*.

Bird	*Pigment*
Bullfinch	Red decomposition products
Canary	Canaryxanthophyll
Green finch	Canaryxanthophyll, xanthophyll
Grey wagtail	Xanthophyll
Oriole	Xanthophyll, canaryxanthophyll
Weaver	Xanthophyll, canaryxanthophyll, decomposition products
Woodpecker	Picofulvin, xanthophyll

Canaryxanthophyll exhibits absorption maxima at 472, 443 and 418 mμ in ethanol. Aqueous 25 % hydrochloric acid produces no displacement of the absorption maxima towards shorter wavelengths in an ethereal solution of the pigment. Picofulvin exhibits absorption maxima at 450 and 424 mμ in ethanol. This pigment differs from flavoxanthin in its negative hydrochloric acid reaction. Canaryxanthophyll is adsorbed more strongly than xanthophyll on calcium carbonate.

33. LEPROTIN $C_{40}H_{54}$

Leprotin was isolated by GRUNDMANN and TAKEDA[92] from a pure strain of acid-resisting bacteria of a leper. The same pigment was later found by TAKEDA and OHTA[93] in *Mycobacterium phlei*.

For the isolation of leprotin, the bacteria are dried and extracted with acetone. The extracts are saponified and the pigments of the epiphasic fraction are chromatographed on alumina. After elution and evaporation of the solvent, leprotin is crystallised from a mixture of benzene and methanol. The pigment is very similar to β-carotene but differs from the latter in melting point, in the stronger adsorption on alumina, and in the absence of vitamin A activity[94]. The molecular formula of leprotin appears to be $C_{40}H_{54}$[93]. On microhydrogenation 12 mols of hydrogen are absorbed[93].

Leprotin crystallises from a mixture of benzene and methanol in fine,

* Further information will be found on pp. 92 and 93–95.

felted, copper-red needles which melt at 198–200°. (KOFLER block). On treating a chloroform solution of the pigment with antimony trichloride, a stable blue colouration is produced.

Solvent:	*Absorption maxima:*		
Carbon disulphide	517	479	447 mμ
Chloroform	495	460	428 mμ
Petrol	484	452	425 mμ

34. SALMON ACID

The pigments responsible for the red colouration of the flesh of salmon (*Salmo salar*) have been the subject of a number of investigations with partly contradictory results. In 1885, KRUKENBERG and WAGNER[94] established the presence of three carotenoids, namely xanthophyll, carotene and an unknown pigment. In 1933, the latter was obtained in a crystalline state by VON EULER, HELLSTRÖM and MALMBERG[96] and termed salmon acid. Salmon acid was later studied by EMMERIE, VAN EEKELEN, JOSEPHI and WOLFF[97].

The chemical nature of salmon acid is still unknown. According to VON EULER and co-workers[96], salmon acid is readily soluble in acetic acid and can be precipitated from the solution with alkali. It forms blue-black crystals and shows a single broad absorption maximum at 485 mμ in pyridine solution. A very weak subsidiary maximum at 525 mμ is also observed. EMMERIE and co-workers, on the other hand, report that the pigment exhibits an absorption band near 500 mμ in pyridine solution. In the partition test salmon acid is found entirely in the 90 % methanol layer,

35. ASTERIN ACID

During an investigation of the carotenoids of the back skin of *Asterias rubens* and of the eggs of *Coregonus albula*[98], VON EULER and co-workers[99] discovered a previously unknown polyene pigment which they termed asterin acid. The compound possesses properties similar to those of astacene (or astaxanthin). In a later investigation of *Asterias rubens*, KARRER and RÜBEL[100] found that astacene was present. Asterin acid is therefore probably identical with astacene.

36. MYTILOXANTHIN

In the course of extensive investigations concerning the part played by carotenoids in the metabolism of *Mytilus californianus* shells, SCHEER[101] established the presence of zeaxanthin and of another new pigment, mytiloxanthin. The latter occurs as such in sea shells and appears to play a part in their metabolism. The structure of mytiloxanthin is still unknown. It appears to

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be an acid or an enol, as its colour is reversibly changed on addition of alkalis. This behaviour and the single-banded absorption spectrum (maximum at 500 mμ in carbon disulphide) are reminiscent of astacene, but mytiloxanthin has a much lower melting point (140–144°). (The melting point of astacene is 228°).

37. CAROTENOID FROM THE BLOSSOMS OF SATIN OAK (*Grevillea robusta*)

Zechmeister and Polgár[102] found, besides β-carotene, cryptoxanthin and xanthophyll, a previously unknown hypophasic carotenoid in the blossoms of satin oak (*Grevillea robusta*). The pigment is only present in very small amounts, so that neither its physical properties nor its constitution could be determined. It crystallises in long plates.

Solvent:	*Absorption maxima:*		
Carbon disulphide	490.5	457	mμ
Benzene	479.5	440.5	mμ
Petrol	457.5	430	mμ

38. CAROTENOIDS FROM DIATOMS, BROWN ALGAE AND DINOFLAGELLATES

According to Strain, Manning and Hardin[103], certain species of diatoms and brown algae contain, besides fucoxanthin, a number of other carotenoids which were named diatoxanthin, diadinoxanthin, neofucoxanthin A and neofucoxanthin B. The chemical nature of these carotenoids is still entirely unknown.

According to the same authors, dinoflagellates *Peridinium cinctum* contain pigments which were named dinoxanthin, neodinoxanthin, diadinoxanthin, neodiadinoxanthin and peridinin. The chemical nature of these compounds is also unknown.

39. CAROTENOID FROM NEUROSPORA

A new carotenoid named neurosporen has been isolated by Haxo[104] from the fungus *Neurospora crassa*. Neurosporen occurs together with lycopene, γ-carotene and rhodoviolascin (spirilloxanthin) and smaller quantities of β-carotene, and of four other pigments resembling δ-carotene, rhodopurpurin, lycoxanthin and rhodopin.

Neurosporen was obtained crystalline, m.p. 124°. It is a hydrocarbon and has the molecular formula $C_{40}H_{58}$ ($\pm$ 2H).

Solvent:	*Absorption maxima:*			
Carbon disulphide	502.5	470.5	439.5	mμ
Petroleum ether	470	441.5		mμ

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REFERENCES

1. E. R. Lankester, *Quart. J. Mikroscop. Science 13* (1873) 408; *16* (1876) 27.
2. E. Warming, cited by H. Molisch, *Die Purpurbakterien*, Jena 1907, p. 2. — T. W. Engelmann, *Bot. Ztg. 46* (1888) 661, 667, 693, 709. — S. Winogradsky, *Bot. Ztg. 45* (1887) 489, 513, 529, 545, 569, 585, 606. — O. Bütschli, *Über den Bau der Bakterien und verwandter Organismen*, Leipzig 1890. — G. A. Nadson, *Bull. Jard. bot. St. Pétersbourg 3* (1903) 109.
3. V. Archichovskji, *Bot. Zentr. 99* (1905) 25.
4. H. Molisch, *Die Purpurbakterien*, Jena 1907.
5. Cf. P. Karrer and U. Solmssen, *Helv. chim. Acta 18* (1935) 1306.
6. P. Karrer and U. Solmssen, *Helv. chim. Acta 18* (1935) 25; *18* (1935) 1306; *19* (1936) 3; *19* (1936) 1019; P. Karrer, U. Solmssen and H. Koenig, *Helv. chim. Acta 21* (1938) 454; P. Karrer and H. Koenig, *Helv. chim. Acta 23* (1940) 460.
7. A. Polgár, C. B. van Niel and L. Zechmeister, *Arch. Biochem. 5* (1944) 243.
8. C. B. van Niel and J. H. C. Smith, *Arch. Microbiol. 6* (1935) 219.
9. P. Karrer and U. Solmssen, *Helv. chim. Acta 18* (1935) 25, 1306; *19* (1936) 3, 1019; P. Karrer, U. Solmssen and H. Koenig, *Helv. chim. Acta 21* (1938) 454; P. Karrer and H. Koenig, *Helv. chim. Acta 23* (1940) 460.
10. Cf. H. Koenig, *Dissertation*, Zürich, 1940, p. 54.
11. P. Karrer and U. Solmssen, *Helv. chim. Acta 19* (1936) 1019.
12. P. Karrer and H. Koenig, *Helv. chim. Acta 23* (1940) 460.
13. P. Karrer and U. Solmssen, *Helv. chim. Acta 18* (1935) 25, 1306; *19* (1936) 3, 1019; P. Karrer, U. Solmssen and H. Koenig, *Helv. chim. Acta 21* (1938) 454.
14. P. Karrer and U. Solmssen, *Helv. chim. Acta 19* (1936) 1019. — H. Koenig, *Dissertation*, Univ. Zürich 1940.
15. H. Kylin, *Z. physiol. Chem. 166* (1927) 50. — Cf. *Kungl. Fysiogr. Sällskid Lund Föhr. 7* No. 12 (1937) 1.
16. I. M. Heilbron and B. Lithgoe, *J. Chem. Soc. 1936*, 1376.
17. P. Karrer and J. Rutschmann, *Helv. chim. Acta 27* (1944) 1691. — J. Rutschmann, *Dissertation*, Zürich, 1946.
18. J. Tischer, *Z. physiol. Chem. 251* (1938) 109; *260* (1939) 257.
19. J. Tischer, *Z. physiol. Chem. 251* (1938) 127.
20. J. Tischer, *Z. physiol. Chem. 260* (1939) 257.
21. R. Kuhn and C. Grundmann, *Ber. 66* (1933) 1746.
22. J. Tischer, *Z. physiol. Chem. 251* (1938) 109.
23. A. Scheunert and K. H. Wagner, *Z. physiol. Chem. 260* (1939) 272.
24. J. Tischer, *Z. physiol. Chem. 260* (1939) 269, 270.
25. P. Karrer and E. Jucker, *Helv. chim. Acta 28* (1945) 427. — Cf. p. 147.
26. *Z. physiol. Chem. 260* (1939) 267.
27. S. Rosanoff, *Mém. Soc. Sci. nat. Cherbourg 13* (1867) 195.
28. G. Kraus and A. Millardet, *Compt. rend. 66* (1868) 505; *Compt. rend. 68* (1869) 462.
29. H. C. Sorby, *Proc. Roy. Soc. London 21* (1873) 474.
30. M. Tswett, *Ber. dtsch. bot. Ges. 24* (1906) 234.
31. R. Willstätter and H. J. Page, *Ann. 404* (1914) 237.
32. P. Karrer and co-workers, *Helv. chim. Acta 14* (1931) 623; *Z. angew. Chem. 12* (1929) 918.
33. R. Kuhn and A. Winterstein, *Ber. 64* (1931) 326.
34. I. M. Heilbron and R. F. Phipers, *Biochem. J. 29* (1935) 1373.

34*a*. P. W. Carter, L. C. Cross, I. M. Heilbron and E. R. H. Jones, *Biochem. J. 43* (1948) 349.

35. I. M. Heilbron and co-workers, *Proc. Roy. Soc.* (London), B *128* (1939) 82.
36. H. H. Strain and W. M. Manning, *J. Am. Chem. Soc. 64* (1942) 1235. — H. H. Strain, W. M. Manning and G. Hardin, *J. biol. Chem. 148* (1943) 655. *Biochem. Bull. 86* (1944) 169.

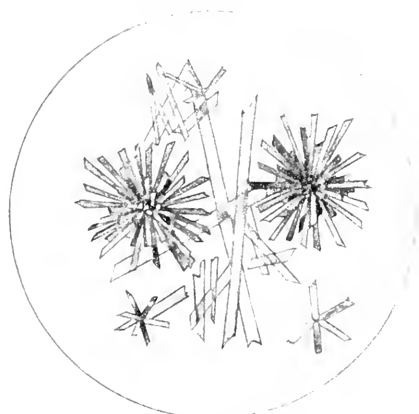
37. R. Řetovský, *Collection of Czechoslovak. chem. Communications 13* (1948) 631.
38. P. Karrer and co-workers, *Helv. chim. Acta 14* (1931) 628.
39. R. Willstätter and H. J. Page, *Ann. 404* (1914) 253.
40. I. M. Heilbron and R. F. Phipers, *Biochem. J. 29* (1935) 1369.
41. P. Karrer and co-workers, *Helv. chim. Acta 14* (1931) 623.
42. H. v. Euler, P. Karrer, E. Klussmann and R. Morf, *Helv. chim. Acta 15* (1932) 502.
43. M. Tswett, *Ber. dtsch. bot. Ges. 24* (1906) 234. — I. M. Heilbron and R. F. Phipers, *Biochem. J. 29* (1935) 1373.
44. H. H. Strain, W. M. Manning, *J. Am. Chem. Soc. 64* (1942) 1235.
45. H. H. Strain, W. M. Manning, G. Hardin, *Biochem. Bull. 86* (1944). — *J. biol. Chem. 148* (1943) 655.
46. K. Schön, *Biochem. J. 32* (1938) 1566.
47. L. Zechmeister and W. A. Schroeder, *J. Am. Chem. Soc. 65* (1943) 1535.
48. A. L. Le Rosen and L. Zechmeister, *Arch. Biochem. 1* (1943) 17.
49. G. Michaud and J. F. Tristan, *Arch. Sci. phys. nat. Genève 37* (1914) 47.
50. L. Zechmeister, T. Béres and E. Ujhelyi, *Ber. 69* (1936) 573. — Cf. also *Ber. 68* (1935) 1321.
51. H. Suginome and K. Ueno, *Bull. Soc. chem. Jap. 6* (1931) 221.
52. L. Zechmeister and co-workers, *Ber. 69* (1936) 573.
53. E. Chargaff and J. Dieryck, *Naturwissenschaften 20* (1932) 872.
54. E. Chargaff, *Compt. rend. 197* (1933) 946.
55. T. Nakamura, *Bull. chem. Soc. Japan 11* (1936) 176.
56. Yoshiharu Takeda and Tatuo Ohta, *Z. physiol. Chem. 268* (1941) I–II.
57. R. Kuhn and E. Lederer, *Z. physiol. Chem. 200* (1931) 108.
58. P. Karrer and J. Rutschmann, *Helv. chim. Acta 25* (1942) 1144.
59. R. Kuhn and E. Lederer, *Z. physiol. Chem. 213* (1932) 188.
60. K. Schön, *Biochem. J. 30* (1936) 1960.
61. R. Kuhn and H. Brockmann, *Z. physiol. Chem. 206* (1932) 41.
62. L. Zechmeister and P. Tuzson, *Ber. 72* (1939) 1340.
63. H. H. Strain, *J. biol. Chem. 123* (1938) 425.
64. Cf. W. Franke, *Z. physiol. Chem. 212* (1932) 234.
65. E. Lederer, *Recherches sur les Caroténoides des Animaux*, Thèse, Paris, 1938, p. 40.
66. R. Lederer, *Compt. rend. Soc. Biol. 116* (1934) 150; *117* (1934) 411.
67. E. Lederer, *Compt. rend. 201* (1934) 300; Thèse, Paris 1938.
68. I. M. Heilbron, H. Jackson and R. N. Jones, *Biochem. J. 29* (1935) 1384.
69. E. Lederer, *Compt. rend. Soc. Biol. 113* (1933) 1391.
70. R. Fabre and E. Lederer, *Bull. Soc. Chim. Biol. 16* (1934) 105.
71. E. Lederer, *Thèse*, Paris 1938, p. 27.
72. E. Lederer, *Compt. rend. Soc. Biol. 116* (1934) 150; *117* (1934) 1086.
73. P. Karrer and U. Solmssen, *Helv. chim. Acta 18* (1935) 915.
74. E. Lederer, *Compt. rend. 197* (1933) 1694; *Compt. rend. Soc. Biol. 117*, 1083; *Thèse*, Paris 1938, p. 66; *Bull. Soc. Chim. Biol. 20* (1938) 554.
75. Cf. H. Fink and E. Zenger, *Wschr. Brauerei 51* (1934) 89.
76. P. Karrer and J. Rutschmann, *Helv. chim. Acta 29* (1946) 355.
77. C. Fromageot, Joué Léon Tchang, *Arch. Mikrobiol. 2* (1938) 424.
78. E. Lederer, *Thèse*, Paris 1938, p. 68.
79. E. Lederer, *Compt. rend. 197* (1933) 1694.
80. H. Fink and E. Zenger, *Wschr. Brauerei, 51* (1934) 89.
81. P. Karrer and J. Rutschmann, *Helv. chim. Acta 26* (1943) 2109; *28* (1945) 795; *29* (1946) 355.
82. J. Rutschmann, *Dissertation*, Zürich, 1946.
83. A. Winterstein, *Z. physiol. Chem. 219* (1933) 249.
84. Carmela Manunta, *Helv. chim. Acta 22* (1939) 1153.
85. P. Karrer and J. Rutschmann, *Helv. chim. Acta 27* (1944) 1691.
86. P. Karrer and E. Jucker, *Helv. chim. Acta 29* (1946) 1539.

87. P. KARRER and A. NOTTHAFFT, *Helv. chim. Acta 15* (1932) 1195.
88. P. KARRER and E. KRAUSE-VOITH, *Helv. chim. Acta 30* (1947) 1772.
89. J. TISCHER, *Z. physiol. Chem. 250* (1937) 147.
90. J. TISCHER, *Z. physiol. Chem. 252* (1937) 225.
91. H. BROCKMANN and O. VÖLKER, *Z. physiol. Chem. 224* (1934) 193.
92. C. GRUNDMANN and Y. TAKEDA, *Naturwissenschaften 25* (1937) 27.
93. Y. TAKEDA and T. OHTA, *Z. physiol. Chem. 258* (1939) 6.
94. Y. TAKEDA and T. OHTA, *Z. physiol. Chem. 267* (1941) 171.
95. C. F. W. KRUKENBERG and H. WAGNER, *Z. Biol. 21* (1885) 25. — Cf. NEWBIGIN, *Colour in nature, a study in biology*, London 1898 p. 1–344.
96. H. v. EULER, H. HELLSTRÖM and M. MALMBERG, *Svensk Kem. Tidskr. 45* (1933) 151.
97. A. EMMERIE, M. VAN EEKELEN, B. JOSEPHI and L. K. WOLFF, *Acta. Brev. neerl. Physiol. Pharm. Microbiol. 4* (1934) 139.
98. H. v. EULER and co-workers, *Z. physiol. Chem. 228* (1934) 77.
99. H. v. EULER and co-workers, *Z. physiol. Chem. 223* (1934) 89.
100. F. RÜBEL, *Dissertation*, Zürich 1936.
101. B. T. SCHEER, *J. biol. Chem. 136* (1940) 275.
102. L. ZECHMEISTER and A. POLGÁR, *J. biol. Chem. 140* (1941) 1.
103. H. H. STRAIN, W. M. MANNING, G. HARDIN, *Biochem. Bull. 86* (1944) 169.
104. F. HAXO, *Arch. Biochem. 20* (1949) 400.

Plate I. Crystalline forms of some carotenoids

β-Carotene from petrol

α-Carotene from petrol



Lycopene from petrol

Xanthophyll from methanol-ether

Zeaxanthin from methanol-ether

Violaxanthin from methanol-ether

Plate II. Crystalline forms of some carotenoids

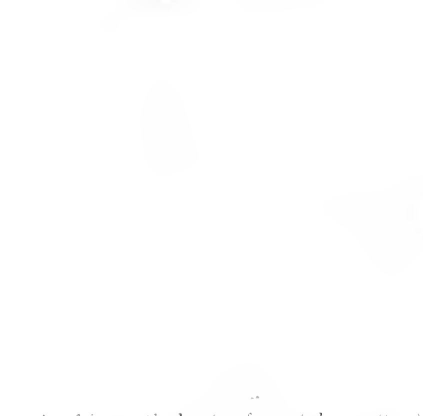
Taraxanthin from methanol-ether

Fucoxanthin from methanol-ether

Capsanthin from carbon disulphide-petrol

Crocetin dimethyl ester from chloroform-ethanol (top)
Crocetin from pyridine (bottom)

Bixin from ethyl acetate



Azafrin methyl ester from toluene (top)
Azafrin from toluene (bottom)

Light absorption curves of some carotenoids

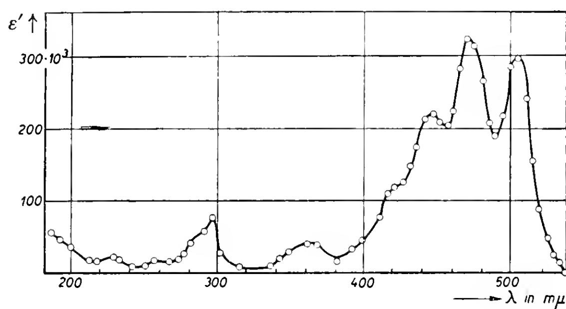


Fig. 4. Lycopene in hexane*
Z. angew. Chem. 47 (1934) 664

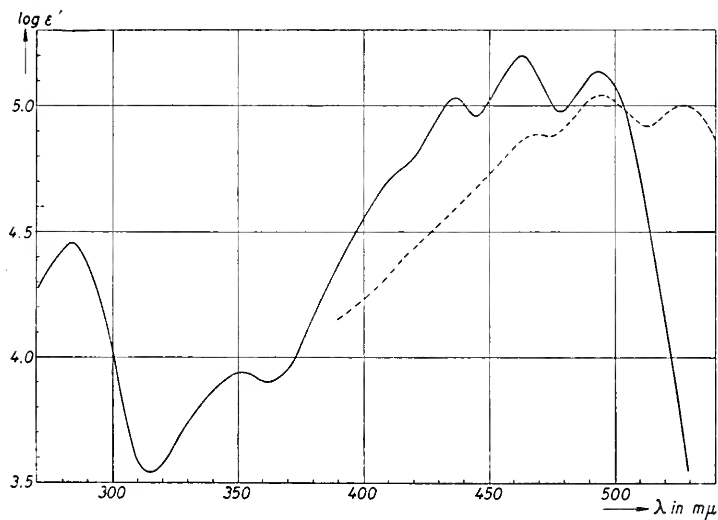


Fig. 5. γ-Carotene in hexane (————) and carbon disulphide (– – – –)
Ber. 66 (1933) 408

* ε' is the *molar extinction coefficient* given by $\varepsilon' = 2.3/cl \times \log (I_0/I)$, where I_0 = intensity of incident light, I = intensity of transmitted light, c = concentration in gm.-mols/l, and l = cell length in cm.

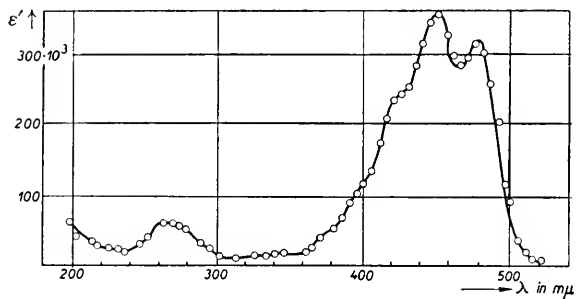


Fig. 6. β-Carotene in hexane
Z. angew. Chem. 47 (1934) 664

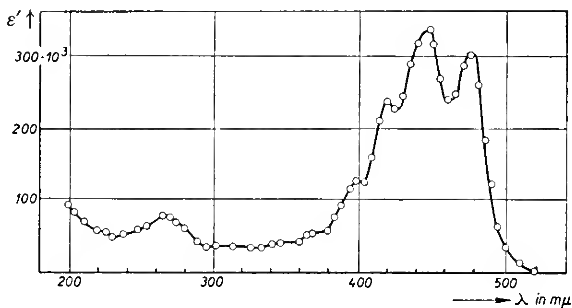


Fig. 7. α-Carotene in hexane
Z. angew. Chem. 47 (1934) 664

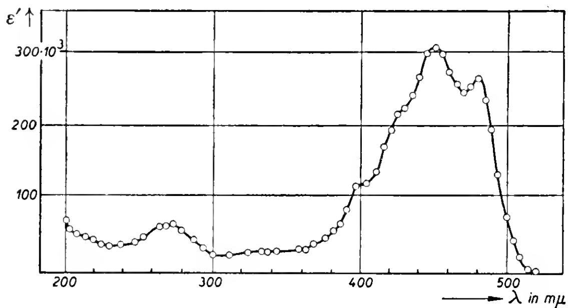


Fig. 8. Zeaxanthin in ethanol
Z. angew. Chem. 47 (1934) 664

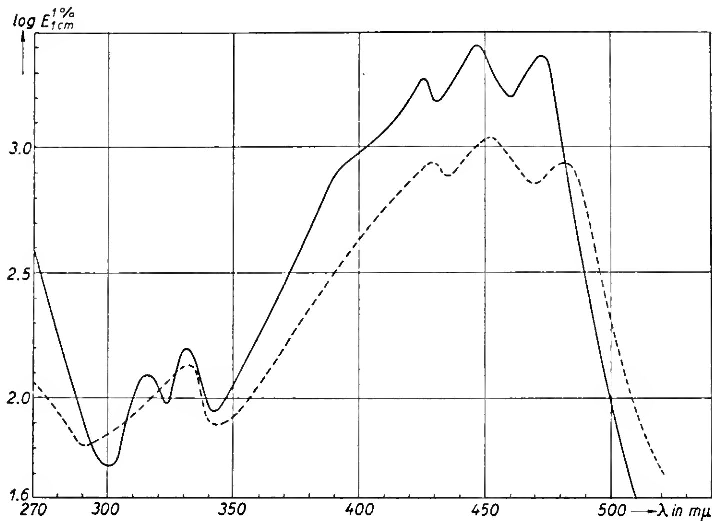


Fig. 9. ———— Violaxanthin in ethanol; —·—·—·— Fucoxanthin in hexane*
Helv. chim. Acta 26 (1943) 117

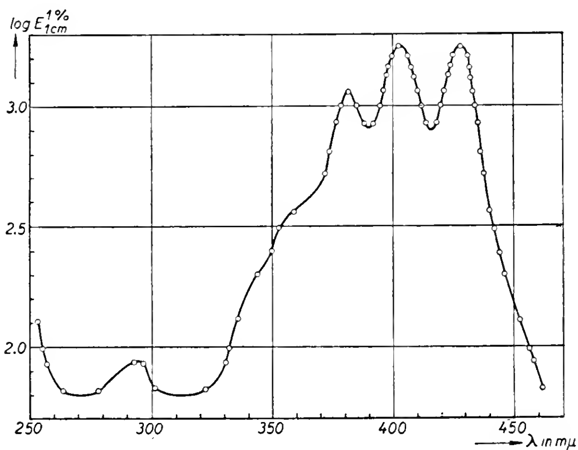


Fig. 10. Auroxanthin in ethanol
Helv. chim Acta 25 (1942) 1624

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* $E^{1\%}_{1cm}$ is the *extinction, one per cent, one centimetre,* given by $E^{1\%}_{1cm} = 1/cl \times \log (I_0/I)$, where I_0 and I are the intensities of the incident and transmitted light, respectively, c= concentration in gms/100 ml, and l= cell length in cms.

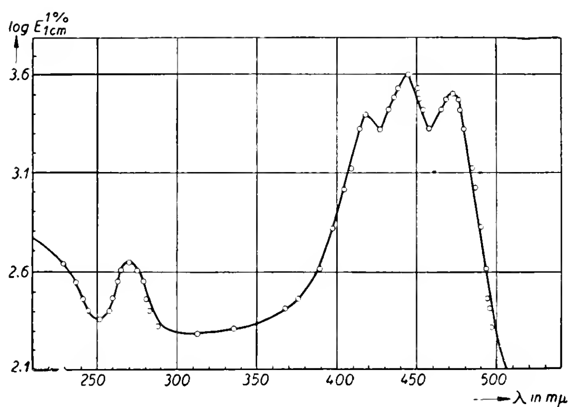

Fig. 11. Xanthophyll in hexane
Helv. chim. Acta 26 (1943) 117

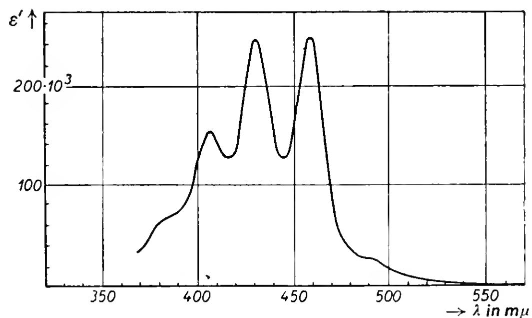

Fig. 12. Flavoxanthin in benzene
Z. physiol. Chem. 213 (1932) 194

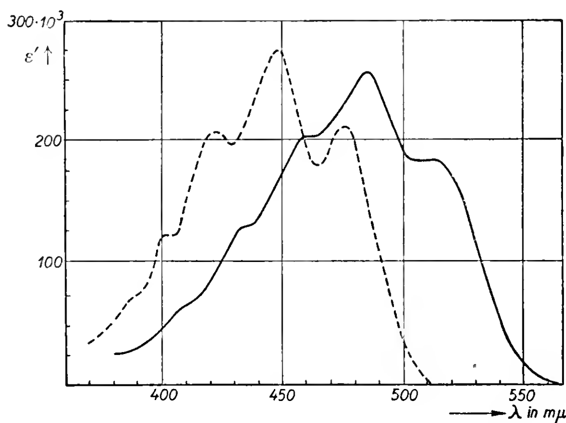

Fig. 13. Rhodoxanthin (————) and dihydrorhodoxanthin (– – – –) in hexane
Ber. 66 (1933) 828

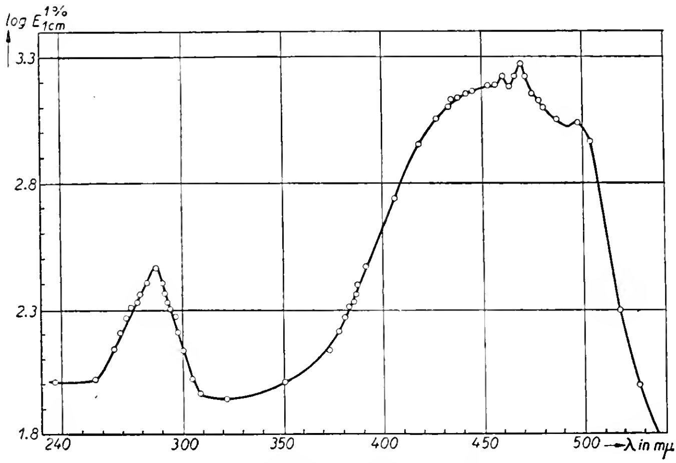

Fig. 14. Capsanthin in hexane
Helv. chim. Acta 26 (1943) 117

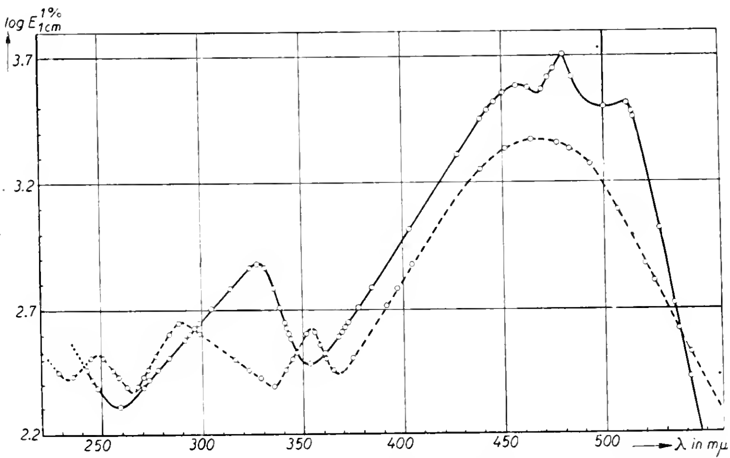

Fig. 15. Rhodoxanthin (————) and astaxanthin (- - - -) in hexane
Helv. chim. Acta 26 (1943) 118

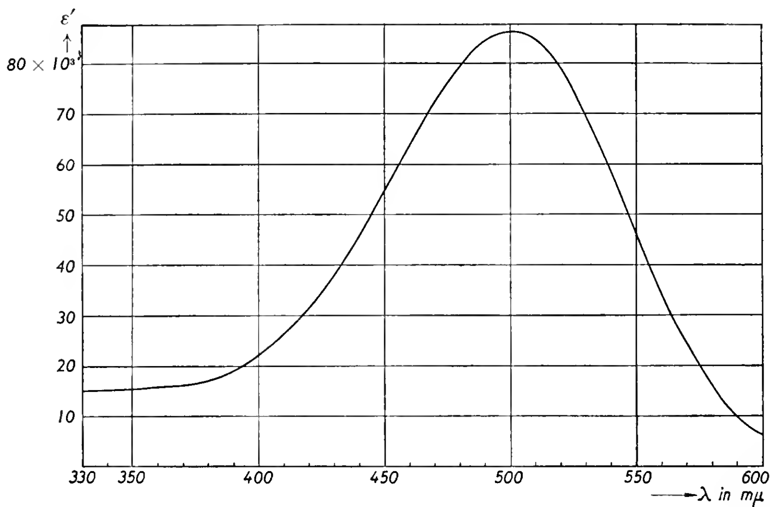

Fig. 16. Astacene in pyridine
Ber. 66 (1933) 492

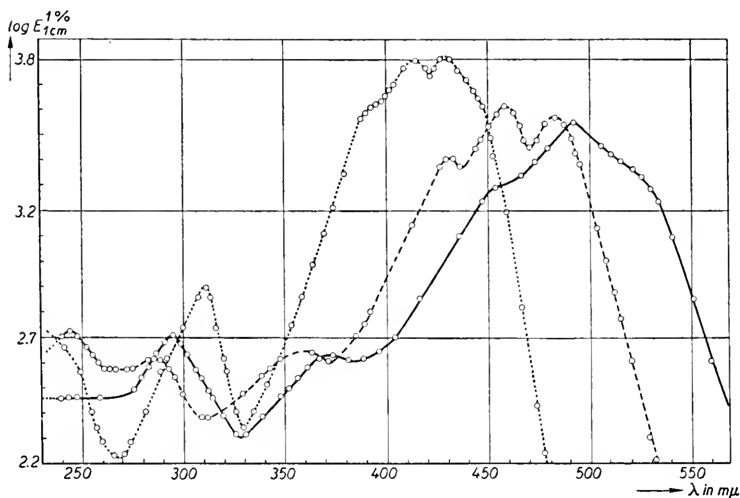

Fig. 17. - - - - Bixin; ———— Bixindialdehyde; .... Apo-3-norbixinal methyl ester from stable bixin, all in ethanol
Helv. chim. Acta 26 (1943) 120

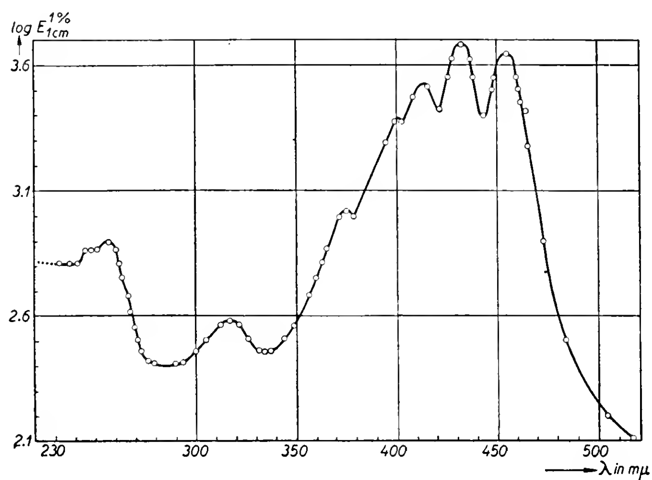

Fig. 18. Crocetin dimethyl ester in ethanol
Helv. chim. Acta 26 (1943) 120

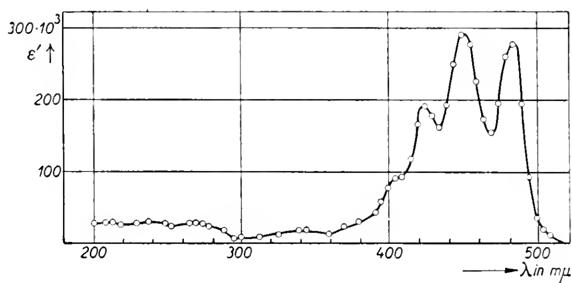

Fig. 19. Methylbixin in hexane
Z. angew. Chem. 47 (1934) 662

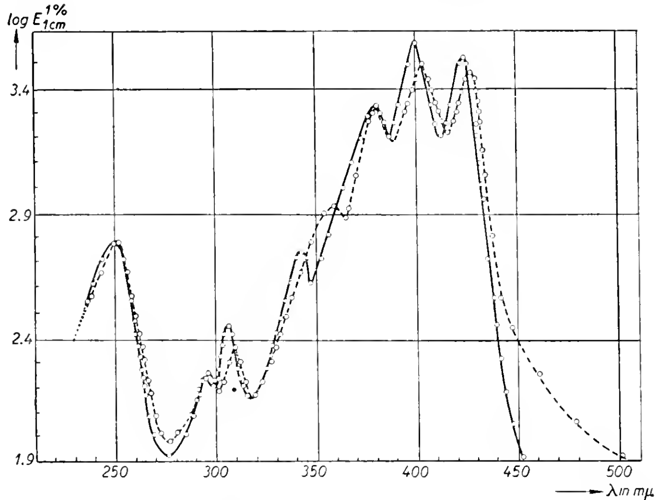

Fig. 20. – – – – Dihydrobixin; ———— Dihydromethylbixin in ethanol
Helv. chim. Acta 26 (1943) 120

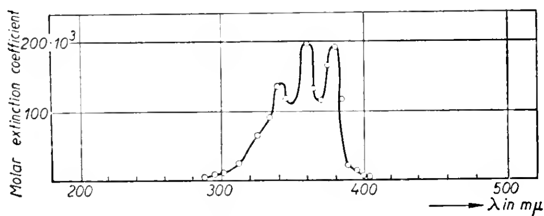

Fig. 21. Dihydrocrocetin in hexane
Z. angew. Chem. 47 (1934) 662

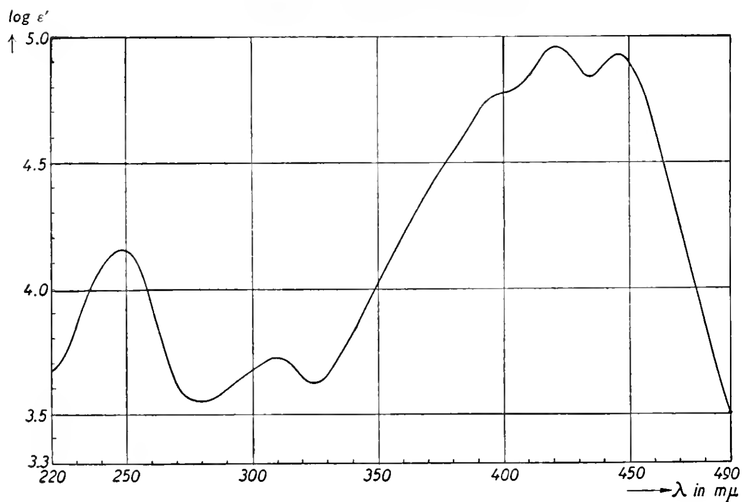

Fig. 22. Crocetin in ethanol
Z. angew. Chem. 47 (1934) 662

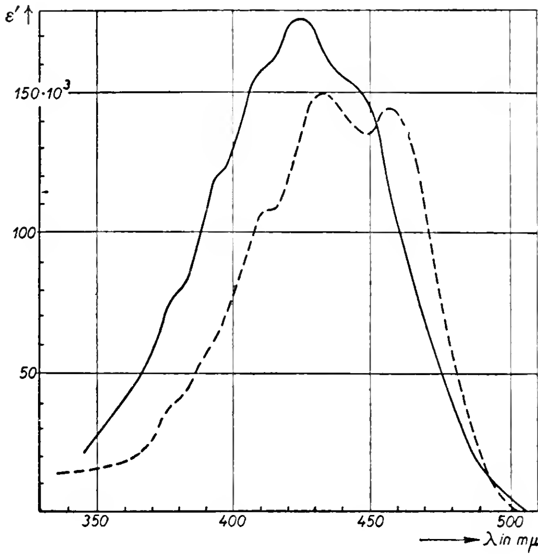

Fig. 23. ——— Methylazafrin; —·—·—·— Methylazafrinone, in ethanol
Ber. 66 (1933) 890

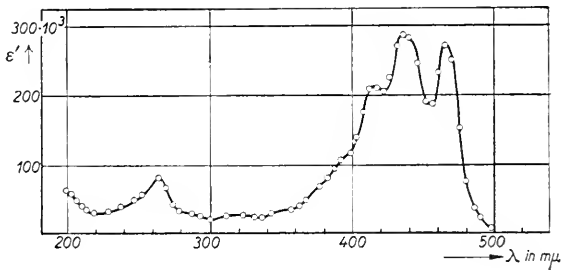

Fig. 24. Taraxanthin in ethanol
Z. angew. Chem. 47 (1934) 664

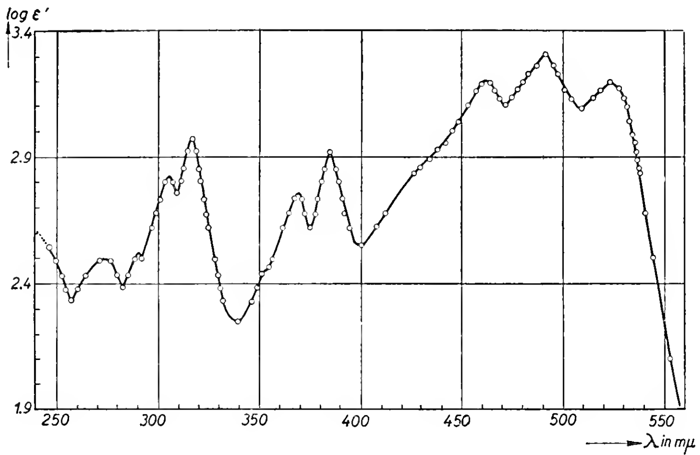

Fig. 25. Rhodoviolascin in hexane
Helv. chim. Acta 26 (1943) 118

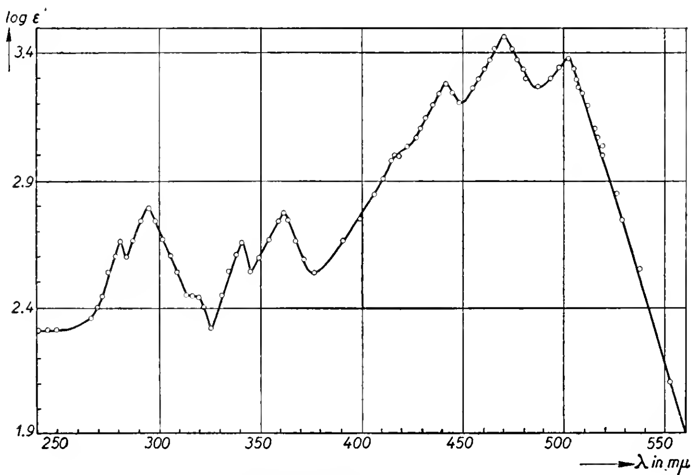

Fig. 26. Rhodopin in ethanol
Helv. chim. Acta 26 (1943) 118

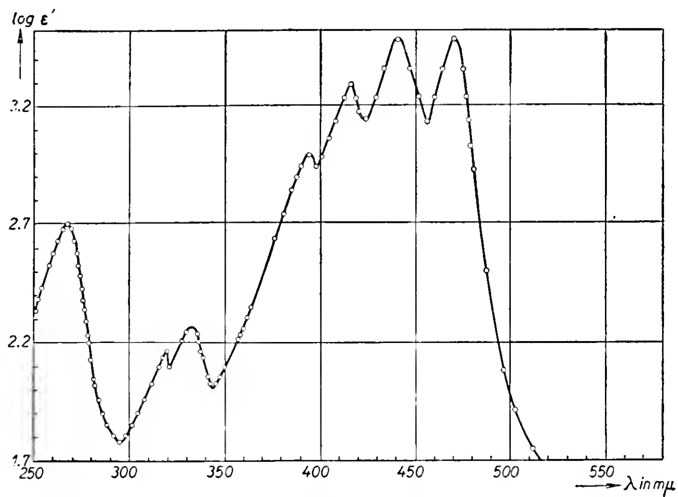

Fig. 27. Flavorhodin in hexane
Helv. chim. Acta 26 (1943) 119

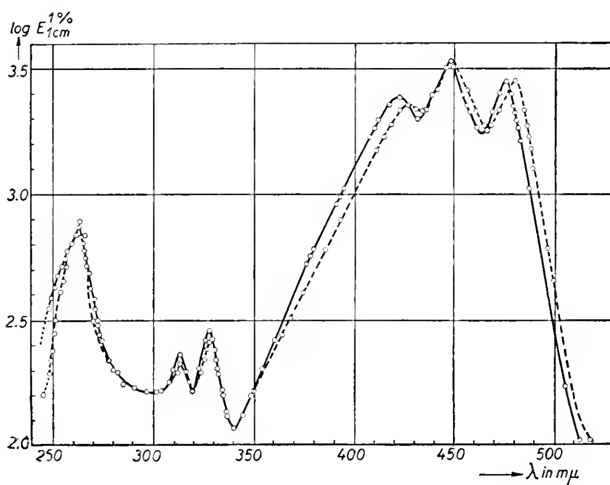

Fig. 28. – – – – α-Citraurin; ———— α-Apo-2-Carotenal, in hexane
Helv. chim. Acta 26 (1943) 119

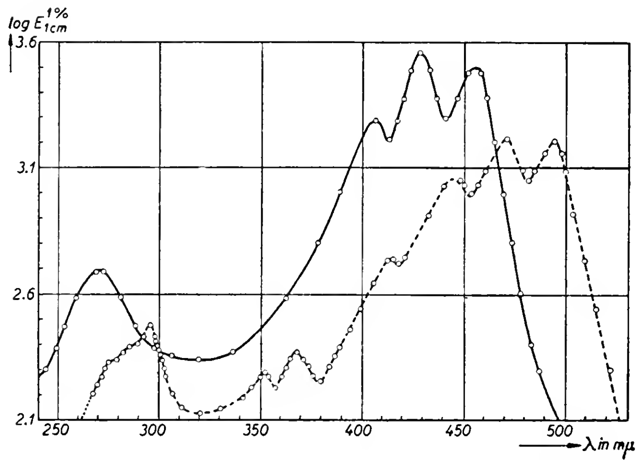

Fig. 29. ———— β-Apo-2-Carotenal; – – – – Carotenone, in hexane
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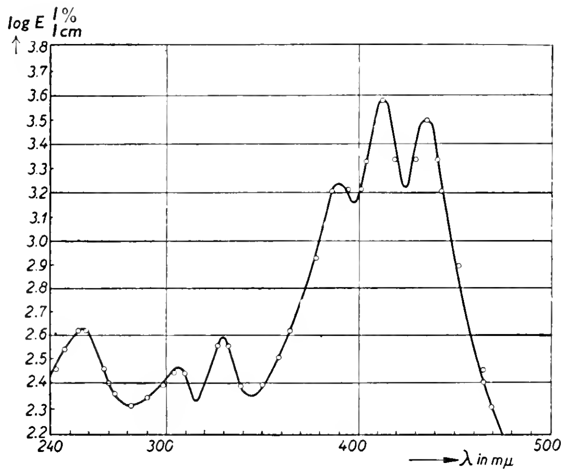

Fig. 30. Dihydro-β-carotene in hexane
Helv. chim. Acta 23 (1940) 958

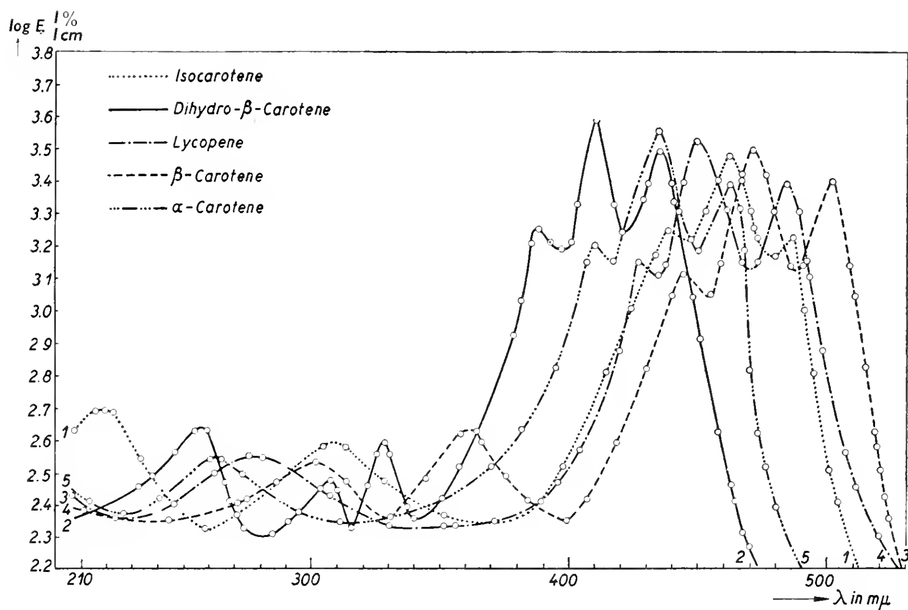

Fig. 31. Light absorption of α-Carotene, β-Carotene, Lycopene, Dihydro-β-carotene, and Isocarotene in hexane
Helv. chim. Acta 23 (1940) 956

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