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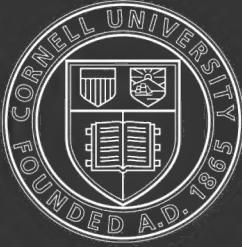
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The Occurrence of Organic Phosphorus Compounds in Plants

A THESIS

Presented to the Faculty of the Graduate School
of Cornell University for the Degree of
Doctor of Philosophy

By
RUDOLPH J. ANDERSON

May, 1919

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The Occurrence of Organic Phosphorus Compounds in Plants*

INTRODUCTION

It has been recognized for a long time that only a small part of the total phosphorus contained in plant material is present as inorganic phosphate because only very small quantities of phosphoric acid could be precipitated from solutions obtained by digesting powdered plant material, rich in phosphorus, in water or dilute acids (Hart and Andrews 1903). In order to determine total phosphorus it was necessary either to ash the material or subject it to complete hydrolysis with mineral acids.

It is evident, therefore, that the greater percentage of the phosphorus existing in plant cells is combined in some way with organic compounds. The knowledge of the various groups of organic phosphorus compounds existing in plants has been gradually developed through the efforts of numerous investigators. Although much work has already been done on this subject, the field is not exhausted; future investigations will probably reveal a number of new phosphorus compounds elaborated in plant cells.

About the middle of the last century it was found that a small percentage of the phosphorus in vegetable material, particularly in seeds, could be extracted with ether or other solvents for fats (Knop 1860 and Töpler 1861). This class of ether-soluble phosphorus compounds was shown to be closely related to lecithin and the phosphatides, if not identical with similar substances isolated from animal tissues (Hoppe-Seyler 1867; Jacobson 1889; Schulze and Likiernik 1891). The wide distribution of lecithin in the plant world was shown by Heckel and Schlagdenhauffen (1886). The plant phosphatides have been extensively investigated in Schulze's laboratory. A large number of important papers dealing with methods of estimation, composition and properties have been published by Schulze and Winterstein and their students.

*The experimental work reported in this thesis was done in the Chemical Laboratory of the New York Agricultural Experiment Station, Geneva, N. Y., in the Chemical Laboratory of the University of Berlin and in the Ludwig Mond Biochemical Research Laboratory of the Institute of Physiology, University College, London; and the series of papers dealing with this subject, as given in the bibliography, was originally published in the "Journal of Biological Chemistry."

A monograph on lecithin in which the above subject is reviewed, with a complete list of references, by Maclean (1918) has recently been published.

The first plant nucleic acid was discovered by Altmann (1889) as a constituent of yeast cells. This substance was similar to nucleic acids isolated from animal tissues, but it showed certain differences in composition, particularly in cleavage products (Kossel 1891, 1893, 1894). Osborne and Harris (1902) described the triticonucleic acid obtained from wheat embryo. This substance was found to be similar to yeast nucleic acid and the identity of the two acids was established by the investigations of Levene and La Forge (1910). It appears, therefore, that there is only one plant nucleic acid. This substance has been very extensively investigated by Levene, Levene and Jacobs and more recently by Jones and Read (1917). The structure of plant or yeast nucleic acid has not yet been fully established. It has been shown, however, by Levene and Levene and Jacobs that the molecule of yeast nucleic acid contains four nucleotides which are made up of the following constituents:

Phosphoric acid — d — ribose — guanine
Phosphoric acid — d — ribose — cytosine
Phosphoric acid — d — ribose — uracil
Phosphoric acid — d — ribose — adenine

This subject is extensively reviewed in the monograph by Jones (1914), "Nucleic Acids: Their Chemical Properties and Physiological Conduct."

The observation that fermentation is accelerated by the addition of phosphates was first made by Wroblewski (1901) and later by Buchner and Hahn (1903). Studies made since that time have shown that during fermentation inorganic phosphate disappears and that an organic phosphoric acid is produced. Phosphates are apparently essential in the process of fermentation and in the absence of phosphates no fermentation can take place (Harden 1914). The nature of the organic phosphoric acid and the conditions under which it is formed have been studied by Harden and Young (1905, 1908, 1909, 1911), by Iwanoff (1905, 1909), Young (1909, 1911), Lebedeff (1909, 1910, 1911, 1912, 1913) and by Euler (1912) and his students (1911, 1912).

There is no complete agreement among these investigators as to the constitution of this organic phosphoric acid. Iwanoff regards it as a triosemonophosphoric acid, $C_3H_5O_2 (PO_4H_2)$, while Harden and Young believe that it is a hexosediphosphoric acid, $C_6H_{10}O_4 (PO_4H_2)_2$. Euler believes that a mixture of these two acids is formed during fermentation. For further particulars regarding this interesting phosphoric acid, reference is made to the monograph by Harden (1914).

Another interesting substance, the phosphoric acid of starch, has

recently been investigated by Northrup and Nelson (1916). It has been known for some time that starch contained a small percentage of phosphorus which could not be completely removed by washing, dialysis or by repeated precipitations (Thomas 1914). Samec (1914) believed that the phosphoric acid in starch was present in an ester combination and he called it **amylophosphoric acid**. Northrup and Nelson succeeded in isolating a new organic phosphoric acid from partially hydrolyzed starch, corresponding to the formula $C_{17}H_{33}O_{19}P$. The acid is optically active, reduces Fehling's solution and on hydrolysis yields glucose and phosphoric acid.

The phosphorus compounds occurring in plant or vegetable material may be divided, on the basis of our present knowledge, into the following groups:

1. Inorganic phosphates.
2. Nucleic acid.
3. Lecithin or phosphatides.
4. Hexosephosphoric acid.
5. Starch phosphoric acid and other combinations of organic radicals with phosphoric acid.
6. Inosite phosphoric acids or phytin.

The inorganic phosphates will not be discussed in this paper.

According to Osborne (1918), there is no evidence of the occurrence of phosphoproteins in plants although references are frequently found in the literature to plant phosphoprotein.

The inosite phosphoric acid, or phytin, is quantitatively the most important phosphorus compound found in plants. The chemistry and properties of this substance, as well as closely related inosite phosphoric acids, will be particularly considered in this paper.

A large number of investigations have been made regarding the metabolism and utilization of organic phosphorus compounds in the animal organism. This subject has been exhaustively reviewed by Forbes and Keith (1914), to which reference is made.

INOSITE PHOSPHORIC ACID OR PHYTIN

In the excellent resumé of the literature on the subject of inosite phosphoric acid by Rose (1912) will be found an interesting historical sketch of the investigations which led up to the discovery of inosite phosphoric acid. It may be stated briefly here that the first chemical examination of the substance was made by Brandau under Pfeffer's direction (1872), the object being to determine the constituents of the aleuron grains which had been described and named earlier by Hartig (1855). This investigation revealed the fact that the aleuron grains from seeds, named "globoid" by Pfeffer, contained phosphorus, calcium and magnesium.

The next contributions to this subject were made from Schulze's laboratory. Palladin, Schulze and Winterstein, Winterstein and later Posternak studied the chemical properties and composition of the substance. Palladin (1894) discovered that a substance containing phosphorus, calcium and magnesium, insoluble in hot but soluble in cold water, could be obtained from the seed of *senapis niger*. The work of Palladin was later confirmed and amplified by Schulze and Winterstein (1896). These authors believed that the substance in question was identical with the globoid of Pfeffer. Winterstein (1897) described the substance more closely and also studied its cleavage products. This author was able to show that it gave inositol and phosphoric acid when hydrolyzed in a sealed tube and, therefore, suggested the name *inositol phosphoric acid*. Somewhat later Posternak took up the study of this subject (1900, 1903, 1904, 1905). This author not only studied the chemical nature of the product, preparing and analyzing many salts as well as the free acid, but he also elaborated plans and methods for the production of phytin on a commercial scale.

During the course of a study of the effect upon the metabolism of ash constituents, particularly phosphorus, inaugurated by Jordan (1906), Patten and Hart (1904) showed that phytin was present in wheat bran. The occurrence of the same substance in rice bran and other plant material was subsequently demonstrated by Suzuki, Yoshimura and Takaishi (1907). They also discovered that rice bran and wheat bran contained an enzyme capable of hydrolyzing phytin into inositol and phosphoric acid. This enzyme they named "phytase."

Notable contributions to the chemistry and distribution of phytin were made later by Vorbrodt (1910) and particularly by Starkenstein (1908, 1910, 1911). Levene (1909) also studied a preparation obtained from hemp seed. Hart and Tottingham (1909) carried out extensive investigations regarding the nature of the organic phosphoric acid occurring in a number of grains, tubers and feeding material. They concluded that phytin was present in all of them except dried alfalfa and rutabagas. Rising (1909) reported the occurrence of the same substance in barley and other grains and in flour.

More recent investigations of phytin or inositol phosphoric acid preparations have been reported by Anderson (1912, 1914, 1915), Plimmer and Page (1913), Plimmer (1913), Thompson (1915), Rather (1913, 1917, 1918), Jegorow (1913, 1914, 1915), Clarke (1914, 1915), Heubner (1914), Robinson and Mueller (1915) and Boutwell (1917). Special reference to these and other investigations will be made below.

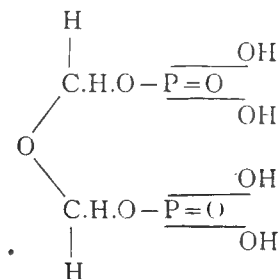
DISTRIBUTION OF INOSITOL PHOSPHORIC ACID IN THE PLANT WORLD

It is evident from the result of investigations mentioned above

that salts of inosite phosphoric acid exist in all seeds. In the cereal grains the outer layers or bran are particularly rich in organic phosphorus which is largely composed of inosite phosphoric acid. The substance is also present in various roots and tubers, such as potatoes, radishes, turnips and dahlias, also in clover leaves and in flowering millet. This list will undoubtedly be extended through further investigations, but it shows that inosite phosphoric acid is very extensively distributed in the vegetable kingdom and that it is present in practically all of the material so far examined.

AS TO THE NOMENCLATURE AND CONSTITUTION OF INOSITE PHOSPHORIC ACID

Winterstein, as mentioned above, found that inosite and phosphoric acid were produced on hydrolyzing the substance in a sealed tube. Without determining the actual relation between carbon and phosphorus, he named the acid **inosite phosphoric acid**, but suggested no constitutional formula. Posternak, as a result of his extensive investigations, concluded that the empirical formula of the acid was $C_2H_8O_9P_2$; i. e., the relation of C : P was as 2 : 2 and he believed it to have the following constitution:



This relation found expression in the name "anhydro-oxy-methylenediphosphoric acid." On hydrolysis the "anhydrooxymethylen," according to this author, combined, producing inosite by synthesis.

The same author gave the name "phytin" to the calcium salt of this acid, which was placed on the market as a pharmaceutical preparation. Since that time the organic phosphorus compounds isolated from various plant material have been described under the name of *phytin*, *phytates* or *phytic acid*, as well as *inosite phosphoric acid*. Phytin is a trade name for a commercial product and the use of this name for various organic phosphorus compounds isolated from plants which frequently differ in composition is likely to lead to confusion. The use of the terms inosite, hexa-, penta-, etc., phosphoric acid in the scientific literature to designate such substances would probably be more desirable.

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and rice polish. All of these preparations are said to be identical and all correspond to an acid having the following composition: $C_{12}H_{41}O_{42}P_9$, or else inosite pentaphosphoric acid, $C_6H_{17}O_{21}P_5$.

It is very evident from this resumé that there is still great confusion in the minds of the investigators who are working on this subject as to the actual composition of the inosite phosphoric acid. Nearly every one who embarks upon an investigation in this field reports entirely different results from any predecessor. This condition merely emphasizes the difficulty encountered in purifying the preparations which are isolated. Further complications that arise are due to the fact that the substance originally present in the plant material may undergo changes of various kinds during the process of isolation. It may suffer partial hydrolysis through the activity of the enzyme (phytase) contained in the material itself or such partial hydrolysis may be caused by the reagents or processes employed in the purification; the final product which is obtained may represent, therefore, not the substance as it existed in the plant, but a mixture of such fragments of the molecules of the inosite phosphoric acid as escaped either the action of the enzymes or the more or less drastic action of the reagents. The fact that the same investigator has produced apparently identical results from a number of different materials is not necessarily a sign of the purity of the preparations; being isolated by identical methods, the same errors are carried over. As an example of this kind of error we need only mention the fact that we (Anderson 1912, (3) 1914 (5)) were unable to obtain any inosite phosphoric acid having the composition of inosite hexaphosphoric acid from a 0.2 per cent. hydrochloric acid extract of wheat bran. Instead of inosite hexaphosphoric acid we obtained consistently an amorphous barium salt corresponding to the formula, $C_{20}H_{45}O_{49}P_9Ba_5$, which on hydrolysis gave inosite and phosphoric acid. This compound was obtained repeatedly and we were entirely unable to isolate a substance having any other composition. Continued experiments with this compound showed, however, that it was not homogeneous but that it could be separated into various fractions and finally a substance was obtained from this compound which corresponded in composition to inosite triphosphoric acid, $C_6H_{15}O_{15}P_3$. The original substance was therefore undoubtedly a mixture of inosite di- and tri-phosphoric acids with possibly some other admixtures. The extracts obtained on digesting ground seeds, bran or other plant material in dilute acid contain a large number of soluble constituents in addition to the phosphorus compounds. It contains among others, also, soluble carbohydrates, such as pentoses which are carried down in the precipitates of the inosite phosphoric acids. Levene (1909), for instance, noted a pentose in the preparation which he analyzed. In the barium salt prepared

from wheat bran we (Anderson 1912 (3)) obtained evidence of the presence of pentose and Rather (1913) also showed that such preparations contain varying amounts of pentose. Small quantities of these soluble carbohydrates adhere to the inosite phosphoric acid preparations with great tenacity. We have noticed that barium salts of inosite phosphoric acid isolated from various plant material generally show too high a percentage of carbon in the early stages of purification.

In order to obtain salts having constant composition it is necessary to recrystallize them a great number of times. We have found that it is possible to obtain preparations having uniform composition after repeated, careful recrystallization of the products. After this degree of purity has been attained further crystallization does not alter the composition. All of the crystalline barium salts which we have thus obtained correspond in composition to salts of inosite hexaphosphoric acid; i. e., the relation of C : P is as 6 : 6. We believe for this reason that the inosite phosphoric acid exists in plants or in the majority of plant material at least as salts of inosite hexaphosphoric acid. In this connection it should be noted that it is very much easier to obtain preparations from plant extracts which differ in composition from those just mentioned. In order to obtain preparations corresponding in composition to salts of inosite hexaphosphoric acid the very greatest care is necessary in the manipulations connected with the isolation and purification. In isolating the material from wheat bran, for instance, the bran must not be digested in 0.2 per cent. hydrochloric acid, but in an acid solution of such strength as to inhibit the activity of the enzyme phytase. The same applies when isolating the preparation from rice bran. Unfortunately, several investigators appear to have overlooked this important fact. It might be argued that repeated recrystallizations might cause decomposition or partial hydrolysis of the product, for these purifications naturally require considerable time. Of course, that danger always exists. We have always employed as mild methods as possible, avoiding contact with strong acid and carefully avoiding heat. The various operations have been performed as rapidly as possible. But even when working with the greatest possible rapidity it is sometimes not possible to complete the isolation and crystallization of a particular preparation in less time than a week or ten days. If a notable amount of hydrolysis occurred during this time when the substances during crystallizations are in contact with dilute acid for varying periods, the resulting product should show a steady increase in carbon content and a decrease in the percentage of phosphorus because the hydrolysis is progressive, the molecules of phosphoric acid splitting off one by one. This is not the case, however, for, as mentioned above, such carefully recrystallized barium

salts contain carbon and phosphorus in the ratio of 6 : 6, whereas in less carefully purified products the percentage of carbon is generally higher.

The contradictory results in ultimate composition obtained by various investigators of the inosite phosphoric acids from seeds or other plant material can be explained, it seems to us, only as being due to any one of the following three causes; viz:

1. Insufficient purification. The preparations which have been analyzed have either contained unknown quantities of inorganic impurities or else organic complexes, such as pentoses or other carbohydrates, have been carried down by absorption with the salts of inosite phosphoric acid.

2. Partial hydrolysis during isolation due to enzyme action or partial hydrolysis during the process of purification due to repeated boiling of the solutions of salts of the inosite phosphoric acid or the repeated evaporation of aqueous or alcoholic solutions of the acid itself.

3. The existence in plant material of different inosite phosphoric acids.

This last cause might be quite important in connection with certain seed cakes, such as cottonseed meal. Varying degrees of heat are applied to the crushed seed during the expression of the oil. This process might affect the composition of the resulting residue or the meal might be stored under conditions of heat and moisture which may favor enzymatic processes. In one report Rather (1917 (1)) states that he obtained an inosite phosphoric acid from cottonseed meal which correspond in composition to inosite triphosphoric acid, but he was never able to isolate this acid from any other sample of the material.

PROPERTIES OF INOSITE PHOSPHORIC ACID

The inosite hexaphosphoric acid, when prepared from pure salts, is obtained as a colorless syrup, the consistency, of course, depending upon the amount of water which is present. When the acid has been dried in a desiccator over sulfuric acid until it is practically water free it forms a solid, glassy, brittle mass which is very hygroscopic. The acid, which is colorless when freshly prepared, assumes gradually at room temperature a light yellow, yellowish, straw and finally, after many months, a very dark color. The color change, which is very gradual at room temperature, is very much hastened by heat. After drying at 100° for a few hours to constant weight the acid is practically black in color. Drying at the temperature of boiling chloroform or alcohol gives a product which is light brown or brown in color. This change in color is associated with some decomposition and sep-

aration of inorganic phosphoric acid. Concentrated solutions of the acid undergo gradual spontaneous decomposition at room temperature, but much more rapidly when heated. The decomposition is only partial; i. e., there is formed inorganic phosphoric acid but no inosite. After standing for 18 months a sample of the acid contained considerable free phosphoric acid but no inosite. Heating this same acid for 48 hours to 100° caused some of the acid to decompose completely because inosite was found as one of the products of decomposition, but only to a small extent. Dilute solutions of the freshly prepared acid give no precipitate with ammonium molybdate either in the cold or on heating for half an hour at 60°. Continued heating at 60°, however, causes a gradual separation of a small amount of yellow precipitate due to hydrolysis of the acid with formation of phosphoric acid. In concentrated solutions ammonium molybdate produces a white crystalline precipitate. The acid is soluble in all proportions in alcohol, but insoluble in other organic solvents. The addition of ether to an alcoholic solution of the acid causes a cloudiness and the acid gradually separates out in minute oily drops. The acid coagulates egg albumin immediately, even in dilute solutions. The alkali salts are very soluble in water. Posternak states that the acid alkali salts are also soluble in alcohol and water. Aqueous solutions of the acid are not precipitated by the chlorides of the alkaline earths, but the acetates and hydroxides of these metals give white amorphous precipitates. Magnesium acetate, however, precipitates the acid only from neutral solutions. Alcohol produces in solutions containing the acid and the above chlorides white amorphous precipitates, but the salts of magnesium and calcium are less completely precipitated than those of strontium and barium. While barium chloride and the acid give no precipitate, yet on allowing such a solution to stand a few hours, needle-shaped crystals of the barium salt separate. Silver nitrate in excess produces a heavy, white, amorphous precipitate. Precipitates are also produced with other heavy metals. Copper acetate in excess produces green colored precipitates, even in the presence of dilute acetic, hydrochloric or sulfuric acids. Lead acetate gives white precipitates in solutions of the free acid or in solutions of its salts in acetic acid. The copper and lead salts of inosite phosphoric acid are particularly suitable for isolating the acid because these metals can be easily removed by hydrogen sulfide. All of the salts of inosite hexaphosphoric acid except the iron salt are more or less easily soluble in dilute mineral acids. The iron salt is very sparingly soluble, even in strong hydrochloric acid. The great insolubility of this salt in dilute acids has led to the development of a volumetric method by Heubner and Stadler (1914) for determining inosite phosphoric acid by titrating with a standardized solution of iron chloride, using ammo-

niium thiocyanate as indicator. The barium salt can be obtained in the form of spherical masses consisting of microscopic needles; other salts with inorganic bases crystallize much less readily or not at all. Posternak describes, however, several double salts which were crystalline. The only organic base found so far that forms readily crystalline salts with inosite hexaphosphoric acid is strychnine, as was first shown by Clarke (1914).

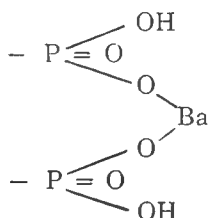
PROPERTIES OF THE SALTS OF INOSITE HEXAPHOSPHORIC ACID

The alkali salts of the acid have not been obtained in crystalline form. The magnesium and calcium salts have no definite crystalline structure, but are obtained under certain conditions as small spherules or globules of uniform size. Posternak obtained double salts of the acid with alkali and alkaline earths which crystallized in small needles. Of the salts with inorganic bases the acid barium salts crystallize most readily. If the barium salt dissolved in dilute hydrochloric acid is precipitated with alcohol an amorphous precipitate is formed which, however, on standing in contact with the aqueous alcoholic mother-liquor, usually assumes the crystalline form after a few hours. These crystals are minute rosettes consisting of microscopic needles. The same-shaped crystals may be obtained by carefully adding alcohol to the dilute hydrochloric acid solution of the barium salt until a slight cloudiness appears. The solution is now filtered and allowed to stand, when the salt separates out in the above-mentioned crystalline form. These salts are crystalline, but they do not contain a constant quantity of barium unless an excess of barium chloride is added to the solution before crystallization begins. It is also necessary to provide as nearly identical conditions as possible as to concentration of the salt and acid; slight variation in the amount of free hydrochloric acid present will cause a difference of 1.5 to 2 per cent. in the amount of barium in the crystalline salt. Similar crystalline barium salts are obtained when solutions of the barium salt in dilute hydrochloric acid are heated nearly to boiling, only in this case the salt contains about 4 per cent. more barium than when separated from the acid alcohol mixture mentioned above. A salt of similar crystal form and composition is also formed when the dilute hydrochloric acid solution of the salt is nearly neutralized with dilute barium hydrate, filtered and allowed to stand. The first salts mentioned above correspond to tribarium inosite hexaphosphate, $C_6H_{12}O_{24}P_6Ba_3$, and the last mentioned salts correspond to heptabarium inosite hexaphosphates, $(C_6H_{11}O_{24}P_6)_2 Ba_7$, or else to equimolecular mixtures of tribarium and tetrabarium inosite hexaphosphates, $C_6H_{12}O_{24}P_6Ba_3 + C_6H_{10}O_{24}P_6Ba_4$. The tribarium ino-

site hexaphosphate contains water of crystallization corresponding to 8 molecules H_2O and the water of crystallization in the heptabarium salt corresponds to 14 molecules H_2O . These crystalline barium salts are free from inorganic phosphoric acid. However, after standing for several months at room temperature in a well closed bottle a slight reaction for inorganic phosphoric acid will be obtained with ammonium molybdate. Drying these salts at 100° likewise causes a slight liberation of free phosphoric acid.

As intimated above, inosite phosphoric acid generally forms acid salts; in fact, it is very difficult to obtain any salt of this acid, even from neutral solutions, which does not show an acid reaction on moist litmus paper. The silver salt, for instance, obtained by precipitating a solution of the acid neutralized with ammonia contains only about 8 or 7 equivalents of silver and has a strong acid reaction.

It has been mentioned previously that only one-half of the acid hydroxyls enter readily into reaction with bases. Salts with binary bases, such as barium, contain therefore only 3 atoms of the base when separated from slightly acid solutions. Starkenstein (1911) also mentions this fact. In titrating the acid with standard uranium acetate he observed that he obtained only one-half the value for phosphorus. These reactive acid hydroxyls are probably adjacent, but linked to different phosphoric acid residue, giving salts with binary bases of the following type:



The salts of inosite phosphoric acid are more soluble in cold than in hot water. This fact was first observed by Palladin (1894) and led to the discovery of the substance in an aqueous extract of *senapis niger*. Advantage has frequently been taken of this property in isolating crude salts of the acid from acidulated aqueous extracts of plant material. The dilute acid extract is heated to boiling. This coagulates the proteins and the alkaline earth salts of inosite phosphoric acid are also precipitated, but they redissolve on cooling the liquid. After cooling, the permanent precipitate, consisting of proteins, etc., is filtered off. The filtrate is again heated to boiling and the precipitate which then forms represents the crude phytin preparation mixed with some inorganic phosphate.

Pure salts of inosite phosphoric acid are generally white or color-

less. The copper salt is bluish green and the iron salt is yellowish brown in color. Various salts of inosite phosphoric acid which we have prepared and analyzed will be described in the experimental part.

SYNTHETIC ESTERS OF INOSITE AND PHOSPHORIC ACID

Several unsuccessful attempts have been made to synthesize phytic acid or the inosite phosphoric acid. Neuberg and Kretschmer (1911) report obtaining a polyphosphoric acid ester of inosite by their method of preparing phosphoric acid esters of carbohydrates and glycerin; that is, by the action of phosphorus oxychloride. They could not obtain any pure product, however, as it was found impossible to separate the new compound from inorganic phosphates.

Contardi (1910) claims to have succeeded in preparing inosite hexaphosphoric acid by heating inosite with an excess of phosphoric acid at a temperature of 160 to 165° in an atmosphere of carbon dioxide. The product was purified as the barium salt and the free ester which was finally obtained he described as identical with "phytic acid" which was prepared from rice bran. The same author claims to have prepared polyphosphoric acid esters of mannite quercite and glucose by the same methods.

Carré (1911) was unable to substantiate the results of Contardi and stated that the reaction mixtures in question consisted only of free phosphoric acid and the poly-hydric alcohols, together with some decomposition products.

Contardi (1912) answered this criticism by repeating his work and claimed to show that his original conclusions were correct.

The results of our experiments showed that inosite and phosphoric acid react when heated to 140 to 160° in vacuum and that water is given off. The reaction product after purifying as a barium salt and removing inorganic phosphoric acid gave results on analysis corresponding to inosite tetraphosphoric acid, $C_6H_8(OH)_2(PO_4H_2)_4$, (Anderson 1912 (1)). It was also found that inosite and pyrophosphoric acid react at a temperature of about 200° with the production of esters (Anderson 1912 (2)).

Jegorow (1914 (1)) has more recently reported some experiments along the same line. Inosite and phosphoric acid were heated in a flask in an atmosphere of hydrogen or nitrogen. He noted that the final reaction product contained 22.6 per cent of organic phosphorus.

There is no doubt but that esters of phosphoric acid are produced when inosite and phosphoric acid are heated together, but we do not believe that inosite hexaphosphoric acid or phytic acid can ever be prepared in this way. Contardi, for the reasons mentioned below, must have been in error when he claimed to have synthesized the substance by this method. Numerous experiments have shown that phytic

acid can not be dried even at a temperature under 100° in vacuum over phosphorus pentoxide without undergoing a very considerable decomposition with liberation of free phosphoric acid. Attempts to synthesize the substance by subjecting mixtures of inosite and phosphoric acid to a high temperature would appear, therefore, to be doomed to failure.

METHODS FOR ESTIMATING INOSITE PHOSPHORIC ACID

Inosite phosphoric acid or its salts can not be extracted from plant material and quantitatively determined by any direct method. Indirect methods of estimation, therefore, were developed and used. More recently efforts have been made to perfect direct volumetric methods for the determination of the acid. The earlier methods consisted in extracting the plant material with dilute hydrochloric or acetic acids and determining the inorganic phosphoric acid and the total soluble phosphorus in the resulting extract. The difference between the two values thus obtained was regarded as phytin phosphorus. Ammonium molybdate precipitates the inorganic phosphoric acid but not organic phosphoric acids, hence it was possible to effect an approximately accurate separation of inorganic phosphate in the presence of inosite phosphoric acid. The total soluble phosphorus in the extract was determined after destroying organic matter by the Neumann method.

Hart and Andrews (1903) worked out the first method along the line mentioned above for determining organic and inorganic phosphorus in plant material. They employed a practically neutral solution of ammonium molybdate. These authors believed that it was necessary to restrict as much as possible the amount of free acid in the precipitating mixture in order to prevent hydrolysis of the organic phosphorus compound by the nitric acid. They examined a large number of plant products and found that practically all of the phosphorus was present in organic combination. The method which they employed has been criticised by several authors, but their results have in general been confirmed by all other investigators. Various modifications have been proposed for the indirect estimation of organic or phytin phosphorus in plant material by several investigators, among whom may be mentioned Schulze and Castoro (1904), Stutzer (1908), Vorbrodts (1910) and Rising (1911) in Europe and particularly Forbes and his co-workers (1914, 1915, 1916) in this country. It was shown by Suzuki and Yoshimura (1907) that the ordinary nitric acid solution of ammonium molybdate could be used for precipitating inorganic phosphate in the presence of organic or phytin phosphorus without effecting a notable cleavage of the organic phosphorus compound. Plimmer (1913) (2) has also shown that it is necessary to heat phytin dissolved in $N/1$ or $2N/1$ nitric acid for several hours to 75° or higher

before appreciable quantities of inorganic phosphoric acid are split off. In view of these facts, it appears to be unnecessary to determine inorganic phosphate in plant material in the presence of a minimum quantity of acid. If the usual quantity of nitric acid is present the precipitation of the phosphomolybdate is more rapid and complete. Many determinations of inorganic phosphate in plant material have been made by using the ordinary ammonium molybdate solution and there appears to be no reason to believe that these results are very far from the truth.

Starkenstein (1911) studied the direct determination of inosite phosphoric acid by titrating with uranylacetate, using cochineal or ferrocyanide as indicator. The most promising method for the direct determination of inosite phosphoric acid is undoubtedly that proposed by Heubner and Stadler (1914). This method is based upon the fact that the iron salt of inosite phosphoric acid is very sparingly soluble in dilute acid. It is possible, therefore, to determine the amount of inosite phosphoric acid in the presence of phosphoric acid or other phosphoric acid esters whose salts are soluble in dilute hydrochloric acid by titrating with ferric chloride, using ammonium thiocyanate as indicator. The method has recently been used by Rather (1917) in determining the percentage of inosite phosphoric acid present in a number of feeding materials.

OTHER PHOSPHORIC ACID ESTERS OF INOSITE OCCURRING IN PLANT MATERIAL

Evidence of the existence of various phosphoric acid esters of inosite formed by hydrolysis from phytic acid or inosite hexaphosphoric acid was obtained in our study of the properties of phytic acid (Anderson 1914 (4)). At that time, however, no definitely homogeneous compound could be isolated. Clarke (1914) reported that he had obtained from wild Indian mustard compounds which corresponded to inosite tetra- and di-phosphoric acid.

Probably the most interesting substance of this kind that has been obtained from plant material is the inosite monophosphoric acid which was isolated from wheat bran (Anderson 1914 (6)). The properties of this acid differ from those of all other inosite phosphoric acids so far described. When pure it crystallizes very easily and may be purified by recrystallization from dilute alcohol or water. All other inosite phosphoric acids have been obtained only as non-crystallizable syrups. Inosite monophosphoric acid separates in beautiful, colorless crystals which melt under decomposition at 190°. All of the salts of this acid, with the exception of the lead salt, are easily soluble in water, whereas other inosite phosphoric acids are precipitated by the alkaline earth and heavy metals. In addition to obtaining this acid from wheat bran

we have isolated the same substance from a mixture of phytin after this has been acted upon by the enzyme phytase from wheat bran (Anderson 1915 (2)). A good yield has been obtained on the partial hydrolysis of phytin with dilute sulfuric acid in an autoclave. At first we believed that inosite monophosphoric acid occurred naturally in the wheat bran. But since the substance is produced through the action of phytase on phytin and also by the partial hydrolysis of this substance with sulfuric acid, it seems more reasonable to believe that it is produced during the extraction by the action of the phytase upon inosite hexaphosphoric acid which is present in the wheat bran.

As a result of the action of phytase upon phytin certain compounds were also produced which appeared to consist of a mixture of inosite tri- and di-phosphoric acid. These substances, however, could not be separated into definitely homogeneous salts (Anderson 1915 (2)).

We have separated from the mixture of inosite phosphoric acids obtained from wheat bran on digesting this material in 0.2 per cent. hydrochloric acid a substance which on analysis gave results corresponding to the composition of inosite triphosphoric acid (Anderson 1915 (1)). This compound had been purified by means of the crystalline strychnine salt and then converted into the barium salt. The barium salt was amorphous and the free acid was a non-crystallizable syrup. The only claim to homogeneity for this substance, therefore, is based upon a crystalline strychnine salt and, in view of this fact, we would not emphasize this claim too strongly. The molecular weight of such strychnine salt is so high that the acid part of the molecule is very small in comparison with the strychnine and it is quite impossible to determine accurately from the analysis of these salts the composition of the acid.

The reactions of the free inosite triphosphoric acid prepared from the barium salt differed in several particulars from the reactions given by inosite hexaphosphoric acid. The concentrated aqueous solution gave no precipitate with ammonium molybdate, while inosite hexaphosphoric acid gives a white, crystalline precipitate. Silver nitrate in excess gave no reaction, while this reagent causes a white precipitate with inosite hexaphosphoric acid. The aqueous solution of the acid gave only a slight turbidity when added to egg albumin and only on longer standing was a white precipitate produced.

Rather (1917 (1)) described a preparation obtained from cottonseed meal which corresponded in composition to inosite triphosphoric acid. This substance had also been separated as a crystalline strychnine salt. This author was never able to duplicate this result with any other sample of cottonseed meal.

Boutwell (1917), in working on the inosite phosphoric acids in

wheat bran, believed that a part of it existed as inosite tetraphosphoric acid, but no definite compound of this composition was isolated.

As previously mentioned, Rather (1918) in an extensive series of investigations disputes the existence of inosite hexaphosphoric acid in any plant material. All of the preparations which he obtained were regarded as salts of inosite pentaphosphoric acid, $C_6H_6(OH)(PO_4H_2)_5$, or of a hypothetical acid having the formula $C_{12}H_{41}O_{42}P_9$.

As a result of our studies we believe that the whole series of possible esters of inosite and phosphoric acid exists; viz., inosite hexa-, penta-, tetra-, tri-, di- and monophosphoric acid. We believe, however, that the lower esters are formed by hydrolysis through enzymes, acids or through spontaneous decomposition of a mother substance which is inosite hexaphosphoric acid. Judging by our experience, the lower esters are always obtained as mixtures and it is extremely difficult to separate them. The amorphous barium salts of such lower esters as the inosite di- and triphosphoric acids are comparatively soluble in cold water and may, therefore, be removed from the higher inosite tetra-, penta- or hexaphosphoric acids with a fair degree of completeness by repeating the operation of extraction with cold water several times. In like manner, it is possible to effect a partial separation of inosite di- and triphosphoric acids from a mixture of these two compounds, but it is practically impossible to separate completely the two substances by this method.

When these esters occur in conjunction with inosite hexaphosphoric acid, as they may frequently do when isolating the latter substance from plant material in which the enzyme phytase exists, it is very difficult to remove the lower esters completely and obtain compounds which give results on analysis corresponding to salts of inosite hexaphosphoric acid.

While we do not doubt the existence of all of these esters, as stated above, we do not believe that experimental evidence has been presented sufficient to prove the existence of more than the following:

Inosite hexaphosphoric acid.

Inosite tetraphosphoric acid (synthetic).

Inosite triphosphoric acid.

Inosite monophosphoric acid.

As has already been mentioned, it has been observed that very carefully purified preparations of inosite phosphoric acid from different sources, either the crystalline barium salt or the free acid, undergo spontaneous decomposition with the gradual liberation of inorganic phosphoric acid and the production of lower phosphoric acid esters of inosite. This process is very much hastened by the enzyme phytase in aqueous solutions under proper conditions of temperature and acidity. Evidently the same reactions do not occur in the dry plant material.

Apparently the cleavage of the inosite phosphoric acid in the plant occurs only during germination or when the material is digested in aqueous or faintly acid solutions favorable to enzyme activity.

THE EFFECT OF ENZYMES UPON INOSITE PHOSPHORIC ACID

Suzuki, Yoshimura and Takaishi (1907) observed that the amount of inorganic phosphoric acid increased when wheat or rice bran was allowed to stand in contact with water. These investigators were also able to separate a preparation from rice bran which, when added to a solution of phytin, caused a rapid formation of inorganic phosphoric acid and led finally to a complete hydrolysis of the phytin molecule into inosite and phosphoric acid. This enzyme was termed *phytase*. The existence of phytase has been demonstrated in other plant material and in animal tissues by several investigators. McCollum and Hart (1908) showed that enzymes extracted from various animal tissues were capable of liberating inorganic phosphoric acid from phytin. Vorbrodt (1910) showed that it was present in wheat, rye, barley, etc. Investigations by Dox and Golden (1911) and by Jegorow (1913) demonstrated that certain fungi cause a cleavage of the phytin molecule with production of inorganic phosphoric acid. Plimmer (1913) also found that certain enzymes of animal tissue liberated inorganic phosphoric acid from phytin but the most active phytase was contained in wheat bran.

We have studied quantitatively the activity of the wheat bran phytase by determining the amounts of inorganic phosphoric acid formed on digesting wheat bran in water or dilute acids (Anderson 1915 (3)). The enzyme is very active in aqueous solutions and also in 0.1 per cent. or 0.2 per cent. hydrochloric or acetic acid. The hydrolysis of the inosite phosphoric acid begins at once and proceeds with great rapidity. After digesting wheat bran in water for thirty minutes about 45 per cent. of the soluble phosphorus is inorganic and after 24 hours about 88 per cent. of the total soluble phosphorus is present as inorganic phosphoric acid. The enzyme is very sensitive to certain concentrations of hydrochloric acid. Greater amounts of free phosphoric acid are liberated when the bran is digested in 0.1 or 0.2 per cent. hydrochloric acid than when distilled water is used, but when the bran is digested in 0.3 per cent. hydrochloric acid the activity of the enzyme is almost entirely inhibited. With increasing strength of hydrochloric acid the amount of inorganic phosphoric acid in the extract decreases, the minimum amount being obtained when 1.0 per cent. hydrochloric acid is used. The activity of the enzyme is likewise inhibited or destroyed by 0.25 per cent. ammonia or by the action of boiling water, as well as by exposing the bran for a short time to the action of 0.5 per cent. hydrochloric acid.

Boutwell (1917) has also studied the activity of the wheat bran

phytase and his results confirm in the main those mentioned above. This author found that heating the dry bran to temperatures much above boiling water apparently did not destroy the enzyme.

It is evident that certain plant materials contain an enzyme, phytase, which under proper conditions of moisture, temperature and acidity rapidly hydrolyzes inosite phosphoric acid into inorganic phosphoric acid and inosite. This hydrolysis occurs in stages with the intermediate formation of certain lower phosphoric acid esters of inosite (Anderson 1915 (2)). These lower esters must consist of inosite penta-, tetra-, tri-, di- and monophosphoric acids. Since there is a rapid formation of inorganic phosphoric acid when certain plant material is digested in water or dilute acids, it is self-evident that it is necessary to choose such solvents as will inhibit the activity of the phytase while the inosite phosphoric acid is extracted from the plant substance. In the case of wheat bran 1.0 per cent. hydrochloric acid may be safely used for this purpose. It is likely that the same strength acid would also destroy or inhibit the activity of the phytase during the extraction of the organic phosphorus compound from other seeds or plant material containing this enzyme.

METHODS FOR ISOLATING AND PURIFYING INOSITE HEXAPHOSPHORIC ACID AS BARIUM SALT

The barium salt of inosite hexaphosphoric acid has been isolated from cottonseed meal by the following method:

The cottonseed meal, 25 pounds, was digested over night in 0.2 per cent. hydrochloric acid in porcelain percolators covered on the inside with a double layer of cheesecloth. It was then percolated using 0.2 per cent. hydrochloric acid until about 20 liters of extract were obtained. The extract was of a dirty, dark color and contained some suspended particles from which it was freed as much as possible by centrifugalizing the solution. A concentrated solution of 300 grams of barium chloride was then added and the precipitate allowed to settle. The precipitate was centrifugalized and finally brought upon a Buchner funnel and freed as far as possible from the mother-liquor. It was then digested in several liters of about 5 per cent. hydrochloric acid until no further solution took place. The insoluble residue was removed by centrifugalizing and the still very dirty colored solution precipitated by adding barium hydroxide until the free acid was neutralized. The barium hydroxide was added slowly, with constant shaking, when the precipitate separated in crystalline form. It was then filtered and washed thoroughly in water and again dissolved in dilute hydrochloric acid, filtered and reprecipitated with barium hydroxide. These operations were repeated three times. The hydrochloric acid solution was then precipitated by gradually adding an equal volume of alcohol when the substance again separated in crystalline form consisting of globular masses of microscopic needles. It was

then precipitated a fourth time with barium hydroxide and after that two more times with alcohol. It was then filtered, washed free of chlorides with dilute alcohol and then in alcohol and ether and dried in vacuum over sulfuric acid. The product was then a nearly white, crystalline powder and it weighed 94 grams.

The dry substance was shaken up with about 1.5 liters of cold water, allowed to stand for several hours and then filtered and washed in water. The aqueous solution contained very little substance precipitable with alcohol and it was therefore discarded.

The washed precipitate was dissolved in dilute hydrochloric acid and precipitated a fifth time by the very gradual addition of barium hydroxide; after filtering and washing, this operation was repeated a sixth time. After again dissolving in dilute hydrochloric acid, nearly neutralizing the free acid with barium hydroxide and filtering, the substance was brought to crystallization by the gradual addition of an equal volume of alcohol. After standing for several hours the substance was filtered and washed in dilute alcohol, alcohol and ether and dried in vacuum over sulfuric acid. It was then a voluminous snow-white crystalline powder.

The dry substance was again dissolved in dilute hydrochloric acid, the free acid nearly neutralized with barium hydroxide and the solution filtered and allowed to stand over night. The substance soon began to crystallize. Under the microscope it appeared perfectly homogeneous and consisted as before of globular masses of microscopic needles. The substance was filtered, washed free of chlorides with water and then in alcohol and ether and dried in vacuum over sulfuric acid. The dry, snow-white, crystalline powder weighed 69 grams.

Qualitative analysis failed to reveal any heavy metals other than barium and from 0.5 gram of the salt no weighable residue of alkali was obtained. It gave no reaction with ammonium molybdate in nitric acid solution. It was free from sulfur and nitrogen.

It was analyzed after drying at 105° in vacuum over phosphorus pentoxide.

0.4641 gram substance gave 0.0556 gram H_2O and 0.1125 gram CO_2 .

0.1982 gram substance gave 0.1333 gram $BaSO_4$ and 0.1203 gram $Mg_2P_2O_7$.

Found: C = 6.61; H = 1.34; P = 16.91; Ba = 39.57 per cent

For tribarium inosite hexaphosphate:

$C_8H_{12}O_{24}P_6Ba_3 = 1066$.

Calculated: C = 6.75; H = 1.12; P = 17.44; Ba = 38.65 per cent.

A portion of this salt was re-crystallized as follows: 5 grams were dissolved in a small quantity of 5 per cent. hydrochloric acid and the free acid nearly neutralized with barium hydroxide, the solution was then filtered and 2 grams of barium chloride dissolved in a little water added

and the solution allowed to stand. The substance separated slowly in the usual crystal form. After two days it was filtered, washed free of chlorides with water, again dissolved in the dilute hydrochloric acid, the solution filtered and alcohol added gradually until a slight cloudiness remained. After standing for 24 hours at room temperature the substance had crystallized in the usual form. It was filtered, washed free of chlorides in dilute alcohol and then in alcohol and ether, and dried in vacuum over sulfuric acid. The dilute nitric acid solution of the substance gave no reaction with ammonium molybdate. The snow-white crystalline powder was analyzed after drying at 105° in vacuum over phosphorus pentoxide.

0.3588 gram substance gave 0.0464 gram H_2O and 0.0867 gram CO_2 .

0.1726 gram substance 0.1138 gram $BaSO_4$ and 0.1058 gram $Mg_2P_2O_7$.

Found: C = 6.59; H = 1.44; P = 17.08; Ba = 38.79 per cent.

Another portion of the substance was re-crystallized as follows: 2 grams were dissolved in a small amount of the dilute hydrochloric acid, barium hydroxide added, with constant shaking, until a faint permanent precipitate remained, and the solution filtered. The filtrate was then heated to boiling and allowed to stand for a few minutes. As the temperature rose the solution began to turn cloudy and finally a heavy precipitate separated which appeared to be amorphous at first but it soon changed into the same crystal form as previously described. This was filtered and washed free of chlorides in boiling water and then in alcohol and ether and allowed to dry in the air. The dry substance weighed 1.6 grams. The snow-white, crystalline powder was free from inorganic phosphate. It was analyzed after drying at 105° in vacuum over phosphorus pentoxide.

0.4695 gram substance lost 0.0443 gram H_2O .

0.4252 gram substance gave 0.0423 gram H_2O and 0.0981 gram CO_2 .

0.1238 gram substance gave 0.0885 gram $BaSO_4$ and 0.0735 gram $Mg_2P_2O_7$.

Found: C = 6.29; H = 1.11; P = 16.54; Ba = 42.06; H_2O 9.43 per cent.

For heptabarium inosite hexaphosphate $(C_6H_{11}O_{24}P_6)_2Ba_7 = 2267$

Calculated: C = 6.35; H = 0.97; P = 16.40; Ba = 42.39 per cent.

For 14 H_2O calculated, 10.00 per cent.

Still another portion of the substance was re-crystallized in the following manner: 2 grams were dissolved in the dilute hydrochloric acid and then nearly neutralized with barium hydroxide as before. The solution was filtered and 10 cc. N/1 barium chloride added and allowed to stand over night. The substance had then separated as a heavy crystalline powder of the same form as before except that the individual

crystals were much larger. The crystals were filtered, washed free of chlorides with water and finally in alcohol and ether and allowed to dry in the air. It was analyzed after drying as above.

0.6430 gram substance lost 0.0745 gram H_2O .

0.5685 gram substance gave 0.0603 gram H_2O and 0.1258 gram CO_2 .

0.2208 gram substance gave 0.1608 gram BaSO_4 and 0.1252 gram $\text{Mg}_2\text{P}_2\text{O}_7$.

Found: C = 6.03; H = 1.18; P = 15.80; Ba = 42.85; H_2O = 11.58 per cent.

For heptabarium inosite hexaphosphate $(\text{C}_6\text{H}_{11}\text{O}_{24}\text{P}_6)_2\text{Ba}_7 = 2267$.

Calculated: C = 6.35; H = 0.97; P = 16.40; Ba = 42.39 per cent.

For 16 H_2O calculated: 11.27 per cent.

With very slight modifications in the method of isolation identical preparations were obtained from oats, corn and from commercial phytin (Anderson 1914).

In preparing the substance from wheat bran the following method was used:

The bran, 700 grams, was digested in 5 liters of 1 per cent. hydrochloric acid for 5 hours. It was then strained through cheesecloth and the liquid filtered. Barium hydroxide (Kahlbaum) was added to the filtrate until the reaction was alkaline. The precipitate was filtered and washed with water and then dissolved in about 3 per cent. hydrochloric acid. The opalescent solution was filtered through charcoal and the filtrate precipitated by adding about an equal volume of alcohol. After standing over night the precipitate was filtered, washed in dilute alcohol and again dissolved in 3 per cent. hydrochloric acid and filtered through charcoal. A dilute solution of barium hydroxide was gradually added to the filtrate until a precipitate began to form. After standing over night the substance had separated out in semi-crystalline form. It was filtered and washed in water, dissolved in 3 per cent. hydrochloric acid and precipitated by alcohol. After filtering and washing with dilute alcohol it was again dissolved in the dilute hydrochloric acid and precipitated by barium hydroxide.

The precipitate was still dark colored and it contained some impurities, not completely soluble in the dilute hydrochloric acid, apparently of colloidal nature, which could not be removed by filtration. In order to eliminate these impurities the barium precipitate was suspended in water and the barium removed with a slight excess of dilute sulfuric acid. The barium sulfate was filtered off and the filtrate precipitated with excess of copper acetate. The copper precipitate was filtered and washed free of sulfates with water. It was then suspended in water and decomposed with hydrogen sulfide. After filtering off the copper sulfide a clear and colorless solution of the free acid was obtained. By these various operations the oxalic acid had also been removed, as, after nearly neu-

tralizing with barium hydroxide and adding barium chloride, no precipitate or turbidity occurred. The solution was precipitated with barium hydroxide, filtered and washed in water. The substance was again twice precipitated with barium hydroxide from 3 per cent. hydrochloric acid and finally twice precipitated with alcohol from the same strength hydrochloric acid. After finally filtering, the substance was washed free of chlorides with dilute alcohol, alcohol and ether, and dried in vacuum over sulfuric acid. It was a snow-white, semi-crystalline powder which weighed 11 grams. It was free from chlorides and inorganic phosphate and did not contain any bases except barium.

For analysis it was re-crystallized as follows: 2 grams of the substance were dissolved in the minimum quantity of 2 per cent. hydrochloric acid and the free acid nearly neutralized with barium hydroxide and the solution filtered. The clear filtrate was heated to boiling when the substance separated as a heavy crystalline powder. This was filtered and washed in boiling hot water and finally in alcohol and ether and dried in the air. Yield, 1.5 grams. It was re-crystallized a second time in the same manner except that 20 cc. of N/1 barium chloride were added to the solution before boiling. After filtering, washing and drying, as before, about 1.3 grams substance were obtained. It consisted of fine, microscopic, needle-shaped crystals. It was free from chlorides and the nitric acid solution gave no precipitate with ammonium molybdate, showing that inorganic phosphate was absent.

It was analyzed after drying at 105° in vacuum over phosphorus pentoxide.

0.3771 gram substance lost 0.0385 gram H_2O .

0.1917 gram substance lost 0.0196 gram H_2O .

0.3386 gram substance gave 0.0361 gram H_2O and 0.0794 gram CO_2 .

0.2571 gram substance gave 0.0269 gram H_2O and 0.0617 gram CO_2 .

0.1715 gram substance gave 0.1218 gram $BaSO_4$ and 0.1026 gram $Mg_2P_2O_7$.

Found: C = 6.39; H = 1.19; P = 16.67; Ba = 41.79 per cent.

C = 6.54; H = 1.17.

H_2O , 10.20 and 10.22 per cent.

For heptabarium inosite hexaphosphate $(C_6H_{11}O_{24}P_6)_2Ba_7 = 2267$.

Calculated: C = 6.35; H = 0.97; P = 16.40; Ba = 42.39 per cent.

For 14 H_2O calculated, 10.00 per cent.

PREPARATION OF THE CRYSTALLINE TRIBARIUM INOSITE HEXAPHOSPHATE

This was prepared by dissolving 5 grams of the original substance in 2 per cent. hydrochloric acid, nearly neutralizing with dilute barium hydroxide, filtering and adding alcohol gradually until the solution turned

cloudy. It was then allowed to stand for two days at room temperature. The substance separated slowly in the form of globular masses or rosettes of microscopic needles. The crystal form was identical with that previously described for the tribarium inosite hexaphosphate. The substance was filtered and washed in dilute alcohol, alcohol and ether, and dried in the air. It was re-crystallized a second time by dissolving in the minimum quantity of 2 per cent. hydrochloric acid, filtering, and adding alcohol until a slight permanent cloudiness remained. After standing for two days the substance had separated in the same form as before. It was filtered, washed free of chlorides with dilute alcohol, and then in alcohol and ether and dried in the air. It was obtained as a snow-white, crystalline powder. It was free from chlorides and inorganic phosphates.

For analysis it was dried at 105° in vacuum over phosphorus pentoxide.

0.2922 gram substance lost 0.0334 gram H_2O .

0.2588 gram substance gave 0.0291 gram H_2O and 0.0648 gram CO_2 .

0.1421 gram substance gave 0.0944 gram $BaSO_4$ and 0.0867 gram

$Mg_2P_2O_7$.

Found: C = 6.82; H = 1.25; P = 17.00; Ba = 39.09 per cent

H_2O = 11.43 per cent.

For tribarium inosite hexaphosphate, $C_6H_{12}O_{24}P_6Ba_3$ = 1066.

Calculated: C = 6.75; H = 1.12; P = 17.44; Ba = 38.65 per cent.

For $_8H_{12}O$ calculated 11.90 per cent.

PREPARATION OF THE FREE INOSITE

HEXAPHOSPHORIC ACID

Inosite hexaphosphoric acid is easily prepared from the purified barium salt by the method of Patten and Hart (1904). The barium salt is suspended in water and decomposed with a slight excess of sulfuric acid. After removing the barium sulfate, the filtrate is precipitated with a hot, concentrated solution of copper acetate. The precipitate is filtered on a Buchner funnel and washed thoroughly in water. It is then suspended in water and decomposed with hydrogen sulfide. The copper sulfide is filtered off and the filtrate is concentrated in vacuum at a temperature of about 45° until a thick syrup remains. This is transferred to an evaporating dish and dried in a vacuum desiccator over sulfuric acid.

METHOD OF ANALYSIS OF THE BARIUM

INOSITE HEXAPHOSPHATE

Most authors working with organic phosphorus compounds report much difficulty in obtaining proper values for the carbon. This difficulty is particularly great in burning compounds such as those reported above

acid and, consequently, an increase in weight. The actual composition can therefore never be determined by analysis of the free acid itself.

The inosite phosphoric acid preparations which have been isolated and analyzed recently by Rather (1918) were obtained by the following method:

"Digest the ground material in 0.2 per cent. hydrochloric acid for 3 hours with frequent stirring. Pass the digestion mixture through a double cloth and filter the resulting extract. Add copper acetate in excess immediately, filter and wash the precipitate with water on a Buchner funnel. Suspend the washed copper precipitate in water and decompose it with hydrogen sulfide. Filter off the copper sulfide and wash with water. Evaporate the filtrate to small volume at a temperature of 55° to 60° under a strong current of air. Take up the residue in six volumes of 95 per cent. alcohol. Filter off any insoluble matter. Dissolve the insoluble matter, consisting of acid salts, in dilute acid and precipitate this solution with copper acetate. Decompose the copper precipitate with hydrogen sulfide and evaporate the filtrate to small volume as before and take up in alcohol. If any alcohol-insoluble matter results repeat the copper acetate precipitation as before. Evaporate the combined alcoholic solutions to a syrup at 50° under a strong current of air, take up the residue again in alcohol, filter if necessary, and evaporate as before. This process removes all inorganic bases. The inosite phosphoric acid is then separated as follows: Weigh the crude acid and dissolve it in about 3 liters of water, filter off the insoluble matter, if any, and add to the warm solution an amount of reprecipitated strychnine equal in weight to the acid syrup. It is necessary to use this ratio of strychnine to acid in order to get a salt of the inosite phosphoric acid which will not give equivocal results on analysis. All of the strychnine should go into solution. Filter if necessary and concentrate at a temperature of 55° to 60° under a strong current of air until crystals begin to form. Then allow to cool. Filter off the crystals and concentrate the mother-liquor as above until crystals begin to appear, cool and filter off the crystals. Concentrate the mother-liquor again for another crop of crystals. Purify the fractions by re-crystallization in the usual manner. This process separates the inosite phosphoric acid from orthophosphoric acid, oxalic acid and other acids. . . .

"The silver salts are prepared from the strychnine salts as follows: Three grams of the purified strychnine salt are dissolved in warm water and the strychnine precipitated by excess of ammonia. The mixture is cooled rapidly and the strychnine is filtered off. The filtrate is washed with ether to remove any strychnine which may remain in solution. The filtrate is then boiled to expell the excess of ammonia. A solution of 3 grams of silver nitrate is then added to the hot solution of the ammonium salt and the resulting silver salt is filtered off and washed with water and

where the percentage of carbon is so low. Under ordinary conditions it is impossible to obtain a complete combustion—the ash is usually more or less dark colored. Some authors recommend mixing the substance intimately with fine copper oxide. This procedure is very serviceable when burning salts of these organic phosphoric acids with organic bases, like the strychnine salts which we have previously reported, but with barium salts we have not found copper oxide to be of much use. In the analyses reported above we have used the following method, for the suggestion of which we are indebted to Prof. E. Fischer of Berlin.

The substance is first burned in the usual way in a current of oxygen, the combustion lasting about an hour. The calcium chloride tube and the potash bulb are then weighed. The increase in weight of the calcium chloride tube is taken as the correct weight of the water. The residue in the boat, which is dark colored from particles of unburned carbon, is powdered in an agate mortar with some recently fused potassium bichromate and again placed in the boat, the mortar being rinsed out with some more powdered bichromate. The whole is again burned in the usual way. The potassium bichromate fuses and oxidizes all the carbon in the residue. The increase in weight in the potash bulb is added to the first, giving the total carbon dioxide.

The analytical data of these various crystalline barium salts, as well as of the free acids from them, is reproduced below in tabular form. These compounds represent the purest preparations that we have been able to produce.

The relation of carbon to phosphorus in all of these salts is very closely that of 1 : 1 or 6 : 6, and the composition agrees fairly closely with calculated percentages for salts of inosite hexaphosphoric acid. We were forced to the conclusion, therefore, that phytin or inosite phosphoric acid existing in the natural plant material which we have examined is represented by the formula $C_6H_{18}O_{24}P_6$ or inosite hexaphosphoric acid. In the beginning of our study we assumed that the old formula of Posternak, $C_2H_8O_6P_2$, or as formulated by Neuberg, $C_6H_{24}O_{27}P_6$, was correct. The analysis of amorphous and less carefully purified material gives results in close agreement with this formula. In the carefully purified re-crystallized barium salts, which we have since prepared and which have been described above, the composition is slightly different and the results agree more closely with the phytic acid formula of Neuberg minus 3 molecules of water; viz., $C_6H_{24}O_{27}P_6 - 3H_2O = C_6H_{18}O_{24}P_6$. In the analysis of the free acid which we have made we have always found that the hydrogen is too high and the phosphorus too low, in some cases one per cent, too low. The free acid is not suitable for analytical determinations for, as we have shown, it can not be dried for analysis without undergoing considerable hydrolysis. With the hydrolysis is associated the absorption of water and production of free phosphoric

BARIUM SALTS CRYSTALLIZED FROM DILUTE HYDROCHLORIC ACID
BY THE ADDITION OF ALCOHOL

TABLE I

Composition Found by Analysis						
Calculated for	Cottonseed	Oats	Corn	Commercial Phytin	Wheat Bran	Hexaphosphate Inosite $C_6H_{12}O_8P_6Ba_3$
C	6.61, 6.59	6.50	6.50	6.64	6.82	6.75
H	1.34, 1.44	1.56	1.40	1.39	1.25	1.12
P	16.91, 17.08	17.00	17.08	17.15	17.00	17.44
Ba	39.57, 38.79	38.01	39.14	38.62	39.09	38.65

BARIUM SALTS CRYSTALLIZED FROM DILUTE HYDROCHLORIC ACID
IN THE PRESENCE OF BARIUM CHLORIDE

Composition Found by Analysis						
Calculated for	Cottonseed	Oats	Corn	Commercial Phytin	Wheat Bran	Hexaphosphate Inosite $(C_6H_{11}O_8P_6)_2Ba_7$
C	6.29	6.23	6.27	6.27	6.39	6.35
H	1.11	1.27	1.32	1.19	1.19	0.97
P	16.54	16.17	16.28	16.22	16.67	16.40
Ba	42.06	41.48	42.06	42.03	41.79	42.39

THE FREE INOSITE PHOSPHORIC ACIDS PREPARED
FROM THE CRYSTALLINE BARIUM SALTS

Composition Found by Analysis						
Calculated for	Cottonseed	Oats	Corn	Commercial Phytin	Hexaphosphate Inosite $C_6H_{18}O_{12}P_6$	
C	10.68	10.82	10.86	10.80	10.90	
H	3.09	3.09	3.02	3.23	2.72	
P	27.66	27.12	27.22	27.31	28.18	

dried in vacuo over phosphorus pentoxide. When the silver salt is protected from light it shows only a slight change in color."

Using this method of isolation and purification, Rather has analyzed the strychnine and silver salts of the inosite phosphoric acid obtained from a large number of feeding materials. He believes, as previously mentioned, that all of his preparations represent salts of inosite pentaphosphoric acid and that this is the only inosite phosphoric acid which exists in natural plant material.

Clarke, (1914, 1915), in his examination of the inosite phosphoric acid existing in wild Indian mustard and in wheat bran, isolated the acid from 0.2 per cent. hydrochloric acid extracts of the material by a method similar to that first used by Palladin. The crude phytin thus obtained was purified by means of the crystalline strychnine salts. Judging by the analytical results reported, these salts were evidently mixtures of various inosite phosphoric acids.

Boutwell (1917), who has recently reported an investigation of the inosite phosphoric acid of wheat bran, also followed in principal the method used by Clarke. The different steps in his process were as follows: The bran was macerated in 2.0 per cent. hydrochloric acid at a temperature of about 20° for five hours. The mixture was then pressed through cheesecloth and allowed to stand over night. The clear extract was then heated to boiling to precipitate the protein and allowed to cool. After filtering off the insoluble matter the clear filtrate was heated to boiling and neutralized with ammonia. The sticky, brown precipitate which formed was filtered from the hot solution and washed with boiling water. The precipitate was then extracted with 8.0 per cent. acetic acid, filtered and the filtrate heated to boiling and, after cooling, the insoluble matter was filtered off. The clear filtrate was diluted with an equal volume of water, heated to boiling and made alkaline with ammonia. The white precipitate which formed was filtered off from the hot mother liquor and washed in boiling water. This precipitate was extracted with 0.8 per cent. acetic acid and the insoluble matter filtered off. The clear solution was heated to boiling, when the phytin separated out as a heavy, powdery precipitate which was filtered off, washed with boiling water and dried.

The product thus obtained consisted of spheroidal crystals which had the following composition:

C=7.99; H=2.49; P=20.42; Ca=3.65; Mg=10.81 per cent. From this material crystalline barium salts were prepared which, however, did not correspond closely in composition to salts of inosite hexaphosphoric acid. This author expresses the opinion that the inosite phosphoric acid existing in bran is probably inosite hexaphosphoric acid and that his preparation may have undergone some change during the repeated heating of the solutions in the purification process.

PREPARATION OF INOSITE MONOPHOSPHORIC ACID.

Wheat bran is digested in 0.2 per cent. hydrochloric acid over night. Strain through cheesecloth and filter the extract. Add about 4 volumes of 95 per cent. alcohol, allow the precipitate to settle and then filter on a Buchner funnel and wash with dilute alcohol. Dissolve the precipitate in 0.2 per cent. hydrochloric acid, filter through a heavy layer of paper pulp on a Buchner funnel and precipitate with 4 volumes of alcohol. These operations are repeated six times or until a nearly white product is obtained which dissolves in the dilute acid, giving a perfectly clear solution. The substance is then dried in vacuum over sulfuric acid. Dissolve it in water with the aid of a few drops of hydrochloric acid and precipitate the solution with a concentrated solution of pure barium hydroxide. Filter off the precipitate and wash with cold water. Evaporate the *filtrate* to about one-fourth its volume, remove the excess of barium hydroxide with carbon dioxide, filter and evaporate the filtrate to a thin syrup on the water bath. Pour the syrupy residue into a large volume of 95 per cent. alcohol. Filter off the precipitate and dry it in vacuum over sulfuric acid. Dissolve the dry substance in water and reprecipitate with a large volume of alcohol. Filter, wash with alcohol and dry as before. There is then obtained a nearly white amorphous powder. One preparation obtained in this way had the following composition:

C = 39.22; H = 5.43; N = 14.26; Amino N (by Van Slyke method) = 0.99; organic Phosphorus = 0.87; Ba = 10.43 per cent.

The crude nitrogen-containing substance was dissolved in water and a concentrated solution of lead acetate added in excess. The resulting precipitate was filtered, washed thoroughly in cold water and then suspended in hot water and decomposed with hydrogen sulfide. The lead sulfide was filtered off and the filtrate boiled to expel hydrogen sulfide. The solution was then strongly acid to litmus and it had a sharp acid taste. It was again precipitated as above three times with lead acetate. The pure white colored lead precipitate which was finally obtained was decomposed with hydrogen sulfide. The filtrate was concentrated in vacuum at a temperature of 40 to 45° and then dried in vacuum over sulfuric acid until a thick, practically colorless syrup remained. On scratching with a glass rod this immediately began to crystallize, forming a white, solid mass. It was very soluble in water, but insoluble in alcohol. It was extracted several times with 95 per cent. alcohol, filtered and washed in absolute alcohol and ether and allowed to dry in the air. For recrystallization it was dissolved in a small quantity of water and absolute alcohol added until the solution turned slightly cloudy. On scratching, the substance began to crystallize. After standing in the ice chest over night it had separated in large, colorless plates or prisms arranged in star-shaped aggregates. It was recrystallized a second time in the same manner.

The substance was free from bases and also free from nitrogen and sulfur, but it contained organically bound phosphorus. The aqueous solution gave no precipitate with ammonium molybdate on being kept at a temperature of 65° C. for some time but after decomposing by the Neumann method it gave an immediate precipitate of ammonium phosphomolybdate with this reagent.

The substance has no sharp melting point. When rapidly heated in a capillary tube it softens at 200° C. and decomposes under effervescence at 201 to 202°; when slowly heated it begins to soften at 188° and melts under decomposition at 190° to 191° (uncorrected).

It is optically inactive. A 10 per cent. solution in a 1 dcm. tube shows no rotation.

For analysis it was dried at 100° in vacuum over phosphorus pentoxide but it did not lose in weight.

0.1550 gram substance gave 0.0749 gram H_2O and 0.1566 gram CO_2 .

0.0766 gram substance gave 0.0325 gram $Mg_2P_2O_7$.

Found: C = 27.55; H = 5.40; P = 11.82 per cent.

For inosite monophosphate, $C_6H_{13}O_9P = 260$.

Calculated: C = 27.69; H = 5.00; P = 11.92 per cent.

Titrated against barium hydroxide, using phenolphthalein as indicator, it forms a neutral barium salt, $C_6H_{11}O_9P Ba$.

0.1985 gram substance required 7.6 cc., N/5 $Ba(OH)_2$.

For $C_6H_{11}O_9P Ba$, calculated: 7.6 cc., N/5 $Ba(OH)_2$.

PROPERTIES OF INOSITE MONOPHOSPHORIC ACID.

The acid is very soluble in water. The aqueous solution shows a strong acid reaction to litmus and it has a sharp, somewhat astringent, acid taste. It is insoluble in alcohol, ether and the other usual organic solvents.

Its aqueous solution gives no precipitate with barium hydroxide or with calcium or barium chloride; ammonia produces no precipitate in these solutions but the addition of alcohol causes white amorphous precipitates. Silver nitrate produces no precipitate even in a solution neutralized with ammonia. When alcohol is added to the solution containing silver nitrate a white amorphous precipitate is produced which dissolves on warming; on cooling the silver salt separates in small, round crystal aggregates. It gives no precipitate with ferric chloride or mercuric chloride nor with copper sulfate. In the cold no precipitate is produced with copper acetate, but on warming this solution a bluish-white precipitate separates which again dissolves completely on cooling.

With excess of lead acetate a white, heavy amorphous precipitate is formed which is but slightly soluble in dilute acetic acid, but readily soluble in dilute hydrochloric or nitric acid. Ammonium molybdate produces no precipitate in either dilute or concentrated aqueous solutions.

The acid crystallizes without water of crystallization from either water or dilute alcohol.

The aqueous solution of inosite monophosphate does not precipitate egg albumin, differing in this respect from phytic acid.

CLEAVAGE OF INOSITE MONOPHOSPHORIC ACID INTO INOSITE AND PHOSPHORIC ACID

I. Acid Hydrolysis.

The acid, 0.35 gram, was heated in a sealed tube with 15 cc. of 3 per cent. sulfuric acid to 120—125° for about 3 1/2 hours. After cooling, the liquid was of a pale straw color. The sulfuric and phosphoric acids were precipitated with barium hydroxide and the excess of barium hydroxide removed with carbon dioxide. The filtrate was evaporated to dryness on the water-bath. The residue gave no precipitate with ammonium molybdate, but after decomposing by the Neumann method, a heavy precipitate of ammonium phosphomolybdate was obtained, showing that only a portion of the acid had been hydrolyzed under the above conditions. The residue, however, contained some inosite which was isolated as follows: The substance was taken up in a few cubic centimeters of hot water, a little more than an equal volume of alcohol was added which caused a voluminous, white amorphous precipitate consisting of the barium salt of the unchanged inosite monophosphate. After filtering, the precipitate was again treated with water, again precipitated with alcohol and filtered. The filtrates were evaporated on the water-bath, taken up in a little water and the inosite brought to crystallization by the addition of alcohol and ether. It crystallized in the usual needle-shaped crystals. After standing several hours in the ice chest the crystals were filtered, washed in alcohol and ether and dried in the air. Yield, 0.06 gram. It gave the reaction of Scherer and melted at 224° C. (uncorrected).

II. Alkaline Hydrolysis.

Another portion of the acid, 0.4 gram, was heated in a sealed tube with 10 cc. of 10 per cent. ammonia for six hours to 120°. The solution then contained some free phosphoric acid as it gave a precipitate with ammonium molybdate but the greater portion of the acid remained unchanged. It was found impossible to isolate any inosite from this reaction mixture.

The residue was therefore again heated in a sealed tube with 10 per cent. ammonia for about 4½ hours to 150°. In this case complete hydrolysis had taken place, and after isolating the inosite in the usual way 0.15 gram was obtained. This was recrystallized three times from dilute alcohol with addition of ether and was then obtained in colorless needles free from water of crystallization. It then melted at 224° C.

(uncorrected), and it gave the reaction of Scherer. The identity of the substance was further confirmed by the analysis.

0.1206 gram substance gave 0.755 gm. H_2O and 0.1761 gm. CO_2 .

Found: C = 39.82; H = 6.97 per cent.

For $\text{C}_6\text{H}_{12}\text{O}_6 = 180$.

Calculated: C = 40.00; H = 6.66 per cent.

The yield of inosite monophosphoric acid by this method of preparation is naturally very small and its isolation is a very tedious and expensive operation. The substance can be obtained more readily and in larger quantity on partially hydrolyzing phytin in an autoclave. From 500 grams of phytin after heating with 25 per cent. sulfuric acid in an autoclave to about 150° for 45 minutes we obtained nearly 10 grams of inosite monophosphoric acid in beautiful crystalline form. The reaction mixture is diluted with water, precipitated with barium hydroxide and filtered. The filtrate which contains inosite and the barium inosite monophosphate is evaporated to small bulk on the water-bath. About 2 volumes of alcohol are added to the concentrated solution. The precipitate which forms consists of barium inosite monophosphate. This is filtered off and the inosite monophosphoric acid is isolated by means of the lead salt and finally crystallized as described above.

PREPARATION OF INOSITE TRIPHOSPHORIC ACID

The wheat bran was digested over night in 0.2 per cent. hydrochloric acid. It was then strained through cheesecloth and filtered. The free acid in the extract was nearly neutralized by adding a dilute solution of barium hydroxide until a slight permanent precipitate remained. A concentrated solution of barium chloride was added and the precipitate allowed to settle over night. The supernatant liquid was syphoned off and the residue centrifugalized. The precipitate was finally brought upon a Buchner funnel and freed as much as possible from the mother-liquor and then washed in 30 per cent. alcohol. For further purification the substance was precipitated alternately six times from about 1 per cent. hydrochloric acid with barium hydroxide (Kahlbaum, alkali free) and six times with about an equal volume of alcohol.

After this treatment the substance was obtained as a snow-white, amorphous powder. It was free from inorganic phosphate, and bases other than barium could not be detected. But it still contained oxalates. The oxalates were removed as follows: The barium salt was dissolved in the least possible amount of 0.5 per cent. hydrochloric acid; 5 grams of barium chloride dissolved in a little water was added and the solution allowed to stand for 24 hours. A heavy, white crystalline powder, consisting principally of barium oxalate, separated slowly. This was filtered off and the solution precipitated with alcohol, filtered, washed with alcohol and ether and dried in vacuum over sulfuric acid. The above

operation was repeated a second time when a further slight precipitation occurred, but on repeating a third time the solution remained perfectly clear. The solution was precipitated with alcohol, washed free of chlorides with dilute alcohol, alcohol and ether and dried in vacuum over sulfuric acid. The substance was finally precipitated from about 1 per cent. hydrochloric acid with alcohol, filtered and washed free of chlorides with dilute alcohol and then in alcohol and ether and dried in vacuum over sulfuric acid. The dry preparation weighed about 66 grams.

This crude acid salt was rubbed up in a mortar with about 500 cc. of cold water, allowed to stand a few hours and then filtered and washed with water, alcohol, and ether and dried in vacuum over sulfuric acid. The water-insoluble residue weighed about 30 grams.

The water-soluble portion contained in the filtrate was precipitated with barium hydroxide and reserved for a future investigation.

PREPARATION OF THE STRYCHNINE SALT

The water-insoluble portion of the barium salt mentioned above was suspended in water and the barium precipitated with slight excess of dilute sulfuric acid. The barium sulfate was removed and the filtrate precipitated with excess of copper acetate. The copper precipitate was filtered and washed with water until the washings gave no reaction with barium chloride. It was then suspended in water and the copper precipitated with hydrogen sulfide and the copper sulfide filtered off.

The solution containing the free organic phosphoric acid was diluted to about 2 liters with water and heated on the water-bath and 44 grams of powdered strychnine added. After heating for a few minutes the strychnine was dissolved. The solution was filtered and concentrated in vacuum at a temperature of 45 to 50° to about one-half of the volume. The strychnine salt soon began to separate in long needle-shaped crystals. After standing in the ice chest over night the crystals were filtered off and washed in ice cold water and finally in absolute alcohol and ether and dried in the air. Yield 45.8 grams.

For further purification the substance was twice recrystallized from hot water. It was then obtained in pure white, needle-shaped crystals which looked perfectly homogeneous.

From more concentrated solutions the substance sometimes separates in the form of colorless plates which differ from the needle-shaped crystals in that they contain about 4 per cent. more of water of crystallization.

The strychnine salt has no sharp or definite melting point. Heated in a capillary tube it softens at about 200° C. but it does not melt completely even at a much higher temperature. On moist litmus paper it

shows an acid reaction. The substance does not change in color on drying at 105° C.

For combustion, the substance was mixed with fine copper oxide. After drying at 105° C. in vacuum over phosphorus pentoxide it was analyzed.

The needle-shaped crystals gave the following:

0.1534 gm. substance gave 0.0901 gm. H_2O and 0.3329 gm. CO_2 .

0.2944 gm. substance gave 0.0702 gm. $Mg_2P_2O_7$.

0.1175 gm. substance gave 6.75 cc. nitrogen at 22° C. and 763 mm.

0.1688 gm. substance lost 0.0154 gm. H_2O on drying.

0.1982 gm. substance lost 0.0183 gm. H_2O on drying.

Found: C=59.18; H=6.57; P=6.64; N=6.56; H_2O =9.12 and 9.23 per cent.

The plate-shaped crystals gave the following:

0.1786 gm. substance gave 0.0993 gm. H_2O and 0.3909 gm. CO_2 .

0.4972 gm. substance gave 0.1089 gm. $Mg_2P_2O_7$.

0.1955 gm. substance lost 0.0256 gm. H_2O on drying.

0.5997 gm. substance lost 0.0783 gm. H_2O on drying.

Found: C=59.60; H=6.22; P=6.10; H_2O =13.09 and 13.05 per cent.

The compounds apparently do not represent any definite strychnine salt of inosite triphosphate but it would seem as if they were mixtures of the tri- and tetrastrychnine salts.

For tri-strychnine inosite triphosphate, $C_6H_{15}O_{15}P_3(C_{21}H_{22}N_2O_2)_3$
=1422.

Calculated: C=58.23; H=5.70; P=6.54; N=5.90 per cent.

For tetra-strychnine inosite triphosphate, $C_6H_{15}O_{15}P_3(C_{21}H_{22}N_2O_2)_4$
=1756.

Calculated: C=61.50; H=5.86; P=5.30; N=6.37 per cent.

PREPARATION OF THE BARIUM SALT FROM THE STRYCHNINE SALT

The recrystallized strychnine salt, 27 grams, was dissolved in about 750 cc. of hot water and the solution rendered alkaline with ammonia. After standing in ice water for some time the strychnine was filtered off. The filtrate was shaken with several portions of chloroform to remove the last trace of strychnine.

The solution, which contained the ammonium salt of the organic phosphoric acid, was precipitated by adding a solution of barium chloride in excess. After settling over night, the precipitate was filtered and washed several times with water. It was then dissolved in 1 per cent. hydrochloric acid, filtered and precipitated with barium hydroxide in excess. The precipitate was filtered and washed with

water until free from chlorides. It was again dissolved in 1 per cent. hydrochloric acid, filtered, and the solution rendered neutral to litmus with barium hydroxide. The precipitate was filtered and washed free of chlorides with water and then in alcohol and ether and dried in vacuum over sulfuric acid. The substance was a pure white, amorphous powder. On moist litmus paper it showed a very faint acid reaction. It was free from nitrogen.

For analysis it was dried at 100° in vacuum over phosphorus pentoxide.

0.2323 gm. substance gave 0.0370 gm. H_2O and 0.0758 gm. CO_2 .

0.2085 gm. substance gave 0.1668 gm. $BaSO_4$ and 0.0868 gm. $Mg_2P_2O_7$.

Found: C=8.89; H=1.78; P=11.60; Ba=47.07 per cent.

The substance was again dissolved in 1 per cent. hydrochloric acid and the solution neutralized to litmus with barium hydroxide. It was filtered, washed free of chlorides with water and then in alcohol and ether and dried in vacuum over sulfuric acid.

It was analyzed after drying as before and the following results obtained:

0.3168 gm. substance gave 0.0481 gm. H_2O and 0.0972 gm. CO_2 .

0.2366 gm. substance gave 0.1968 gm. $BaSO_4$ and 0.0960 gm. $Mg_2P_2O_7$.

Found: C=8.36; H=1.69; P=11.31; Ba=48.94 per cent.

For the neutral barium salt of inosite triphosphate,
 $C_6H_9O_{15}P_3Ba_3=826$.

Calculated: C=8.71; H=1.08; P=11.25; Ba=49.88 per cent.

In the two analyses reported above the carbon is somewhat low. It must be noted, however, that these barium salts burned with extreme difficulty. Traces of carbon remained after prolonged heating in a current of oxygen. The residues were mixed with fine copper oxide and re-burned when one or two milligrams of carbon dioxide were obtained, but we believe that the combustion even under these conditions was incomplete.

PREPARATION OF THE ACID BARIUM SALT

The above neutral barium salt was dissolved in the minimum quantity of 1 per cent. hydrochloric acid, the solution was filtered, and precipitated by adding about an equal volume of alcohol. The resulting precipitate was filtered, washed free of chlorides with dilute alcohol and then in absolute alcohol and ether and dried in vacuum over sulfuric acid. The substance was then a pure white, amorphous powder which showed a strong acid reaction on moist litmus paper.

It was analyzed after drying at 105° in vacuum over phosphorus pentoxide.

0.2588 gm. substance gave 0.0538 gm. H_2O and 0.1046 gm. CO_2 .

0.1691 gm. substance gave 0.1027 gm. $BaSO_4$ and 0.0890 gm.

$Mg_2P_2O_7$.

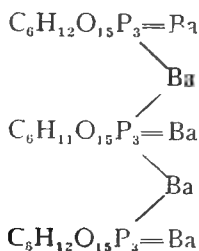
Found: C=11.02; H=2.32; P=14.67; Ba=35.74 per cent.

In the combustion of this substance a practically white ash was obtained.

This compound is evidently a complex acid salt of inosite tri-phosphate and agrees with the following formula:



(Calculated: C=11.15; H=1.80; P=14.40; Ba=35.46 per cent.) which may be graphically represented as follows:



That is, three molecules of mono-barium inosite triphosphate joined by two atoms of barium. Whether it is a compound as represented above or a mixture of various acid salts of inosite triphosphate can hardly be determined.

PREPARATION OF THE FREE ACID

The barium salt from above was suspended in water and the barium precipitated with a slight excess of dilute sulfuric acid.

The barium sulfate was filtered off and the filtrate precipitated with excess of copper acetate. The copper precipitate was filtered and washed with water until it gave no reaction with barium chloride. It was then suspended in water and decomposed with hydrogen sulfide. The copper sulfide was filtered off and the filtrate evaporated in vacuum at a temperature of 40 to 45° to small bulk and then dried in vacuum over sulfuric acid. The substance was then obtained as a practically colorless syrup. After continued drying it forms a hard, sticky hygroscopic mass. It is extremely soluble in water and also readily soluble in alcohol and in absolute alcohol. Much time was consumed in endeavoring to obtain it in crystalline form but without success. The syrupy substance was therefore analyzed after drying first for several days in vacuum over sulfuric acid at room temperature and finally at 100° C. in vacuum over phosphorus pentoxide. On drying at this temperature it turned quite dark in color.

0.1685 gm. substance gave 0.0557 gm. H_2O and 0.1054 gm. CO_2 .

0.1693 gm. substance gave 0.1363 gm. $\text{Mg}_2\text{P}_2\text{O}_7$.

Found: C=17.06; H=3.69; P=22.44 per cent.

For inosite triphosphoric acid $\text{C}_6\text{H}_{15}\text{O}_{15}\text{P}_3=420$.

Calculated: C=17.14; H=3.57; P=22.14 per cent.

In the above combustion a slight residue of unburned carbon remained enclosed in the fused metaphosphoric acid. It was mixed with some fine copper oxide and again burned when a few additional milligrams of carbon dioxide was obtained.

PROPERTIES OF INOSITE TRIPHOSPHORIC ACID

The reactions of this acid differ in several particulars from phytic acid or inosite hexaphosphoric acid.

The concentrated aqueous solution gives no precipitate with ammonium molybdate either in the cold or on warming. The cold aqueous solution of inosite hexaphosphoric acid gives a white crystalline precipitate with ammonium molybdate.

The aqueous solution of the acid is not precipitated with silver nitrate. Inosite hexaphosphoric acid gives a white amorphous precipitate with silver nitrate in excess. However, a solution of inosite triphosphate neutralized with ammonia gives a white amorphous precipitate with silver nitrate.

An aqueous solution of inosite triphosphoric acid when added to a solution of egg albumin causes only a slight turbidity; on longer standing a white precipitate separates slowly. Inosite hexaphosphoric acid precipitates egg albumin immediately.

The acid is very soluble in water and readily soluble in alcohol and absolute alcohol; from the latter solution it is precipitated by ether in small, oily drops.

The acid is not precipitated by barium or calcium chlorides but alcohol produces in these solutions white amorphous precipitates; these salts are likewise precipitated with ammonia.

CLEAVAGE OF INOSITE TRIPHOSPHORIC ACID INTO INOSITE AND PHOSPHORIC ACID

One gram of the acid, dissolved in a little water, was heated with 10 cc. of 5N/1 sulfuric acid in a sealed tube for three hours to 150 to 155°. The contents of the tube were then slightly yellowish brown in color. The sulfuric and phosphoric acids were precipitated with barium hydroxide and the inosite isolated in the usual way. Unfortunately a portion of the solution was lost but from what remained 0.15 gram inosite was obtained. After twice recrystallizing from dilute alcohol with addition of ether, 0.12 gram of inosite in the characteristic

needle-shaped crystals was obtained. It gave the reaction of Scherer and melted at 222° C. (uncorrected). After mixing with some previously isolated and analyzed inosite the melting point did not change. The substance was therefore undoubtedly inosite and the analysis was omitted.

THE HYDROLYSIS OF PHYTIN BY THE ENZYME PHYTASE CONTAINED IN WHEAT BRAN

It has been shown by the investigations of Suzuki, Yoshimura and Takaishi that rice bran contains an enzyme which rapidly hydrolyzes phytin with formation of inosite and inorganic phosphoric acid. These authors concluded that wheat bran likewise contained a similar enzyme because the inorganic phosphorus increased in wheat bran extracts on standing.

Plimmer examined a large number of extracts prepared from the intestines, liver, pancreas, castor beans, etc., as to their action on organic phosphorus compounds. While some of these showed a slight cleavage action on phytin, none could be compared in activity to an aqueous extract of wheat bran. The hydrolytic action of these extracts was determined by estimating from time to time the amount of inorganic phosphorus split off from phytin solutions of known concentration.

Since the above experiments clearly demonstrated that large quantities of inorganic phosphate were liberated from phytin by wheat bran extracts, it appeared of interest to determine what products, in addition to inosite and inorganic phosphoric acid, were formed under these conditions. For this purpose wheat bran extract was allowed to act upon a dilute solution of phytin at a temperature of 37°. Inorganic phosphoric acid was determined in the solution from time to time.

It was found that about two-thirds of the total phosphorus was split off during the first 16 days. Afterwards there was no appreciable change even on standing for about two years.

The solution had been prepared and the original determinations made by Dr. Plimmer. At his suggestion the writer undertook to examine the final reaction mixture for such products as had been formed.

These products were separated into two portions by precipitating the original solution with barium hydroxide. The precipitate contained inorganic barium phosphate, and also those barium salts of organic phosphoric acids that were insoluble in the dilute alkaline solution. The filtrate, on the other hand, was found to contain inosite monophosphoric acid and free inosite.

The inorganic phosphate and other impurities were removed from the crude barium hydroxide precipitate, as will be described in the

experimental part. The organic phosphoric acids which remained were obtained as amorphous barium salts. It was impossible to isolate any unchanged barium phytate. It is evident, then, that all of the phytin had been partially hydrolyzed.

The above amorphous substance appeared to consist mainly of barium inosite triphosphate, but probably mixed with some barium inosite diphosphate. Owing to the difficulty by separating these compounds, their isolation was not attempted.

Among the soluble substances which had been formed we were able to isolate and identify inosite monophosphoric acid, a substance which we have previously isolated from wheat bran. In addition to this, the solution also contained some free inosite which was isolated by means of its lead compound.

The action of this enzyme phytase upon phytin appears to proceed in several stages. Only a portion of the phytin is completely decomposed into inosite and phosphoric acid, but all of the phytin is partially hydrolyzed with formation of certain lower phosphoric acid esters of inosite; viz., inosite tri-, di- and monophosphoric acid and inorganic phosphoric acid. The formation of these intermediate products is possible only through the destruction or inhibition of the enzyme before the hydrolysis is complete. The reason for this inhibition is not clear but it may be due to the excess of phosphoric acid which is liberated.

It is interesting to note, and we call particular attention to the fact, that the organic phosphoric acids which remain as intermediate products of the action of the enzyme upon phytin; viz., inosite triphosphoric acid and inosite monophosphoric acid, are identical with the substances which we have isolated previously from wheat bran after it has been digested in 0.2 per cent. hydrochloric acid.

EXPERIMENTAL PART

Commercial phytin, 100 grams, was dissolved in 500 c.c. of water and filtered from the insoluble matter which weighed 3.5 grams when dried at 100°. The pale yellow solution was treated with 38 grams of oxalic acid dissolved in about 250 cc. of water. The calcium oxalate was filtered off, washed and dried. It weighed 48 grams. The solution was diluted to 6000 cc. with water and was then found to contain 40 grams of phosphorus pentoxide. To it were added 800 cc. of an aqueous extract of wheat bran which contained 2.2 grams of P_2O_5 . The solution was kept under toluol at a temperature of 37°. No hydrolysis occurred in a week. This was evidently due to the strong acid reaction of the solution: It was nearly neutralized with ammonia and 735 cc. of bran extract containing 1.53 grams of P_2O_5 were added. It was again kept at a temperature of 37° under toluol. In 9 days one-half of the total

phosphorus was hydrolyzed; in 16 days two-thirds was hydrolyzed. In 35 days the amount of hydrolysis had not altered and after about two years it was again the same. The total and inorganic phosphorus was determined as described by Plimmer and Page.

The dark-colored solution was filtered and barium hydroxide (Kahlbaum) added in slight excess. After standing over night the precipitate was filtered and washed in water. The filtrate and washings were evaporated on the water-bath and the residue was examined as will be described later.

The barium precipitate was dissolved in about 2.5 per cent. hydrochloric acid, filtered, and precipitated by adding about an equal volume of alcohol. The precipitate was filtered and washed in dilute alcohol. The substance was again precipitated four times in the same way. It was then precipitated by barium hydroxide three times from about 2 per cent, hydrochloric acid and finally two times more with alcohol from the same strength hydrochloric acid. After the final filtering it was washed free of chlorides in dilute alcohol and then in alcohol and ether and dried in vacuum over sulfuric acid. The substance was then a snow-white, amorphous powder. It weighed 28.4 grams. It was free from chlorides and inorganic phosphate, and bases other than barium could not be detected.

The substance was then rubbed up in a mortar with 300 cc. of cold water and allowed to stand with occasional shaking for a few hours. It was then filtered and washed in water, alcohol and ether, and dried in vacuum over sulfuric acid. The dry water-insoluble portion weighed 5.4 grams.

The filtrate from above was neutralized to litmus with barium hydroxide. The precipitate was filtered and washed in water, alcohol and ether, and dried in vacuum over sulfuric acid. It weighed 23.6 grams.

These precipitates were analyzed after drying at 105° in vacuum over phosphorus pentoxide.

The first, water-insoluble portion gave the following result:

Found: C=11.46; H=1.93; P=11.59; Ba=39.94 per cent.

This substance is apparently largely composed of the di-barium inosite triphosphate; calculated for this, $C_6H_{11}O_{15}P_3Ba_2=690$.

C=10.43; H=1.59; P=13.47; Ba=39.71 per cent. It is, however, not pure but apparently contains some barium inosite di-phosphate because the carbon is high and the phosphorus is low.

The water-soluble substance which was precipitated with barium hydroxide gave the following:

Found: C=9.63; H=1.63; P=10.91; Ba=47.41 per cent.

This substance also appears to consist largely of the neutral barium salt of inosite triphosphate. Calculated for the latter

$C_6H_9O_{15}P_3Ba_3=826$. C=8.71; H=1.08; P=11.25; Ba=49.88 per cent. The carbon, however, is high and the phosphorus as well as barium are low, which points to the presence of barium inosite di-phosphate.

In the hope of approximately separating these barium inosite tri- and diphosphates, the substance, 23.6 grams, was digested in dilute acetic acid for several hours with occasional shaking. It was then filtered and washed in water and the insoluble portion dried in vacuum over sulfuric acid. It weighed 10 grams.

The filtrate and washings containing the soluble portion of the substance was precipitated by adding lead acetate in excess. After standing over night the white, amorphous precipitate was filtered and washed in water. It was suspended in water and decomposed by hydrogen sulfide, filtered, and the excess of hydrogen sulfide boiled off. It was again precipitated in the same manner with lead acetate and decomposed with hydrogen sulfide. The solution still contained a considerable quantity of barium. The barium was therefore removed with a slight excess of dilute sulfuric acid. After filtering off the barium sulfate the solution was precipitated by adding copper acetate in excess. The copper precipitate was filtered, washed and suspended in water and decomposed with hydrogen sulfide. After removing the copper sulfide, the filtrate was evaporated in vacuum to small bulk and finally dried in vacuum over sulfuric acid. There remained a thick, nearly colorless syrup. It was readily soluble in alcohol. The addition of chloroform to this solution caused the substance to separate in small oily drops; the addition of ether produced a cloudiness and, on standing, a flocculent, amorphous precipitate separated. These solutions could not be brought to crystallize. The acid preparation itself was kept for several weeks in the desiccator over sulfuric acid. It became a hard but sticky mass but showed absolutely no tendency to crystallize. Kept in this manner the color of the preparation gradually darkened.

Since the acid would not crystallize, the syrupy substance was analyzed after drying at 105° in vacuum over phosphorus pentoxide.

Found: C=18.58; H=3.82; P=20.38 per cent.

For inosite triphosphoric acid, $C_6H_{15}O_{15}P_3=420$.

Calculated: C=17.14; H=3.57; P=22.14 per cent.

For inosite diphosphoric acid, $C_6H_{14}O_{12}P_2=340$.

Calculated: C=21.17; H=4.11; P=18.23 per cent.

This acid preparation is evidently also a mixture of the inosite tri- and diphosphoric acids.

EXAMINATION OF THE FILTRATE AFTER THE WATER-INSOLUBLE BARIUM SALTS HAD BEEN PRECIPITATED

The filtrate was evaporated, as mentioned above, and the residue

taken up in hot water. It was decolorized with animal charcoal. The solution was neutral in reaction. It strongly reduced Fehling's solution on boiling, possibly due to sugars introduced with the bran extract. The solution was found to contain barium and also phosphorus in organic combination, evidently inosite monophosphate. The aqueous solution was precipitated by adding about an equal volume of alcohol and the white, amorphous precipitate filtered off, the filtrate being reserved for further examination.

ISOLATION OF INOSITE MONOPHOSPHORIC ACID

The above precipitate, which formed on the addition of alcohol, was dissolved in water, slightly acidified with acetic acid, and then precipitated with lead acetate in excess. After settling, this was filtered, washed in water and suspended in hot water and decomposed with hydrogen sulfide. It was then filtered and the filtrate boiled to expel excess of hydrogen sulfide. It was re-precipitated several times with lead acetate in the same manner until a white lead precipitate was obtained. This was finally decomposed with hydrogen sulfide, filtered, and evaporated to small bulk in vacuum and then dried in vacuum over sulfuric acid until a thick syrup remained. On scratching with a glass rod, this crystallized to a white, solid mass. It was digested in alcohol and filtered, washed in alcohol and ether, and dried in the air. It weighed 1.6 grams. It had all the properties of inosite monophosphoric acid. For further purification it was dissolved in a few cubic centimeters of water and filtered. Alcohol was then added until the solution turned cloudy; it was heated until it cleared up and more alcohol was added until a faint permanent cloudiness remained. It was allowed to stand for about 48 hours at room temperature, when the substance had separated in massive, practically colorless crystals. After filtering, washing in alcohol and ether, and drying in the air, one gram substance was obtained. When heated in a capillary tube it began to soften at 188 to 189° and melted under decomposition and effervescence at 190° (uncorrected). The appearance and properties of the substance corresponded exactly with those described for inosite monophosphoric acid and the analysis was therefore omitted.

Isolation of Inosite.

The filtrate, after precipitating the above barium salt of inosite monophosphate with alcohol, was evaporated on the water-bath until the alcohol was removed. It still contained barium, chlorides, etc. The barium was quantitatively precipitated with dilute sulfuric acid and the solution again concentrated on the water-bath.

The addition of lead acetate caused no precipitate. Basic lead acetate was then added so long as any precipitate formed. This pre-

precipitate was filtered off and discarded. The solution was then heated to boiling and more basic lead acetate added and the solution was finally made strongly alkaline with ammonia and allowed to stand overnight. This precipitate was filtered and washed in water and then decomposed in aqueous suspension with hydrogen sulfide. The filtrate was concentrated on the water-bath and the inosite brought to crystallization by the addition of alcohol. After recrystallizing several times 0.5 gram of pure inosite was obtained in the characteristic needle-shaped crystals. It gave the reaction of Scherer and melted at 218° C. (uncorrected).

THE HYDROLYSIS OF THE ORGANIC PHOSPHORUS COMPOUND OF WHEAT BRAN BY THE ENZYME PHYTASE

Total and inorganic phosphorus were determined in extracts prepared from wheat bran. The extracts were prepared by digesting wheat bran in water or dilute acid for varying lengths of time, the particulars and details being given at the beginning of each table. Total phosphorus was determined after decomposing by the Neumann method. Inorganic phosphorus was determined as follows: The extract was diluted with some 50 cc. of water, 15 grams of ammonium nitrate were added and then warmed to 65° on the water-bath. It was then strongly acidified with nitric acid and the phosphorus precipitated with ammonium molybdate and the solution kept at the above temperature for half an hour. Under these conditions there is no danger of cleavage of the organic phosphorus, at least weighable quantities of phosphorus are not precipitated during this time from preparations which are free from inorganic phosphate. Plimmer has also shown that it is necessary to heat phytin solutions with N/1 or 2N/1

TABLE 2
CHANGES IN CONDITION OF PHOSPHORUS IN WHEAT BRAN
BY WATER DIGESTION

Time	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
½ hour.....	0.362	0.165	45.77
1 "	0.420	0.239	56.95
4 hours.....	0.508	0.383	75.34
20 "	0.655	0.576	88.00
24 "	0.747	0.660	88.43

nitric acid for several hours to a temperature of 75° or higher before appreciable quantities of inorganic phosphorus are split off.

The phosphomolybdate precipitate was then filtered off and the phosphorus determined as magnesium pyrophosphate in the usual way.

The digestions were made throughout these experiments at room temperature, about 16° C.

Ten grams of the bran were digested in 100 cc. of water for the time mentioned and then filtered and 20 cc. used for each determination. The same quantities were used throughout.

It will be noticed that the hydrolysis is greatest during the first four hours. The percentage of phosphorus represents only that amount which was soluble in the water.

After digesting in water for 24 hours and then acidifying with hydrochloric acid and shaking for half an hour the following results were obtained:

TABLE 3
CHANGES IN CONDITION OF PHOSPHORUS IN WHEAT BRAN
AFTER WATER DIGESTION AND ACIDIFYING

Time	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
24 hours.....	1.06	0.649	60.85

After digesting the bran for 48 hours in water and then adding 100 cc. of 2 per cent. hydrochloric acid and allowing to stand for another 24 hours, the following results were obtained:

TABLE 4
CHANGES IN CONDITION OF PHOSPHORUS IN WHEAT BRAN
AFTER WATER DIGESTION, ACIDIFYING AND STANDING

Time Hours	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
48 in water; 24 in weak acid.	1.27	0.803	62.79
48 in water; 24 in weak acid.	1.28	0.796	62.13

The addition of toluol to the water appears to diminish the amount of phosphorus dissolved without affecting the degree of hydrolysis.

TABLE 5
EFFECT OF TOLUOL ON HYDROLYSIS OF PHOSPHORUS
COMPOUNDS IN WHEAT BRAN

Time With Toluol	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
24 hours.....	0.518	0.462	89.25

Using hydrochloric acid as the extracting medium, the strength varying from 0.2 to 2.0 per cent., the following results were obtained: 10 grams of bran were digested in 100 cc. of the acid for the length of time mentioned in the table. It was then filtered and 20 cc. of the filtrate used for each determination.

TABLE 6
EFFECT OF CONCENTRATION OF ACID ON HYDROLYSIS OF PHOSPHORIC
ACID COMPOUND IN WHEAT BRAN

Solvent HCl %	Time, Hours	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
0.2.....	3	1.130	0.415	36.52
0.2.....	24	1.200	0.925	76.87
0.3.....	24	0.999	0.203	20.22
0.4.....	24	0.939	0.146	15.61
0.5.....	20	0.922	0.124	13.44
1.0.....	5	0.894	0.081	9.13
2.0.....	30	1.080	0.117	10.83

The presence of toluol did not materially alter these results. Hydrochloric acid, 0.5 per cent. after 20 hours, with toluol gave the following:

TABLE 7
EFFECT OF TOLUOL ON HYDROLYSIS OF PHOSPHORUS
COMPOUNDS IN WHEAT BRAN

Time With HCl and Toluol	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
20 hours.....	0.890	0.117	13.14

When the bran is digested in dilute acetic acid the hydrolysis is greater than with the same strength hydrochloric acid, as shown by the following results :

TABLE 8
EFFECT OF ACETIC ACID ON HYDROLYSIS OF PHOSPHORIC ACID COM-
POUND IN WHEAT BRAN

Solvent, Acetic Acid, %	Time Hours	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
0.2.....	3	1.21	0.695	57.03
0.2.....	24	1.23	1.050	85.54

The maximum activity of the enzyme, as shown by the greatest hydrolysis, was obtained by digesting bran for 48 hours in water and then adding 0.1 per cent. hydrochloric acid and allowing to stand for another 24 hours. Ten grams of bran were digested in 100 cc. of water for 48 hours and then 100 cc. of 0.2 per cent. hydrochloric acid added. After standing for 24 hours more it was filtered and 20 cc. of the filtrate used for each determination.

TABLE 9
MAXIMUM HYDROLYSIS OF PHOSPHORIC ACID COMPOUND
IN WHEAT BRAN

Time	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
72 hours.....	1.24	1.14	92.37

DESTRUCTION OF THE ENZYME "PHYTASE" BY HEAT

As is shown by the following experiments, the hydrolytic action of the enzyme is completely destroyed when bran is exposed to the action of boiling water or boiling dilute hydrochloric acid for a short time. Ten grams of bran were placed in an Erlenmeyer flask and 100 cc. of boiling water poured over it. It was then heated for a few minutes until the water boiled. It was allowed to digest for 24 hours at room temperature. Another lot of bran was treated in the same way but using 0.2 per cent. hydrochloric acid and allowed to stand a few minutes to settle. Lots of 20 cc. of the liquid were then taken for each determination.

TABLE 10

EFFECT OF BOILING WATER OR BOILING ACID ON HYDROLYSIS OF
PHOSPHORIC ACID COMPOUND IN WHEAT BRAN

Solvent	Time, Hours	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
Boiling water.....	24	0.886	0.108	12.66
Boiling 0.2% HCl..	24	1.140	0.105	9.22

The hydrolytic action of the enzyme is likewise destroyed by exposing bran for a short time to 0.5 per cent. hydrochloric acid or by digesting bran in dilute ammonia. Ten grams of bran were digested in 100 cc. of 0.5 per cent. hydrochloric acid for 11½ hours. The acid was then nearly neutralized with ammonia, leaving the solution faintly acid, and then standing for 24 hours. Ten grams of bran were digested in 100 cc. of 0.25 per cent. ammonia for 24 hours. It was then acidified with dilute hydrochloric acid.

TABLE 11

EFFECT OF STRONG ACID AND OF AMMONIA ON HYDROLYSIS OF
PHOSPHORIC ACID COMPOUND IN WHEAT BRAN

Solvent	Time Hours	Total Phosphorus in Extract %	Inorganic Phosphorus in Extract %	Part of Total Phosphorus in Inorganic Form %
0.5% HCl.....	24	0.661	0.115	17.46
0.25% NH ₃	24	0.922	0.087	9.51

CONCERNING SOME CHEMICAL PROPERTIES OF INOSITE
HEXAPHOSPHORIC ACID**Spontaneous Liberation of Inorganic Phosphoric Acid at Ordinary
Temperature and On Drying**

The freshly prepared acid is a practically colorless syrup, especially when, in the concentration of its aqueous solution, the temperature is not allowed to rise above 50°. When the acid has been prepared from a pure salt, free from inorganic phosphate, the free acid does not give any reaction with ammonium molybdate for inorganic phosphoric acid. Whenever such colorless specimens of the acid are preserved for any length of time the color always darkens. The

change in color is more rapid when the concentrated aqueous solution is allowed to stand exposed to the air or preserved in a well stoppered bottle, than when the acid is kept in the desiccator; but even under the latter condition the color gradually deepens to light yellow, deep yellow, light brown and finally, after several months, to dark brown or black. When the acid is dried for analysis either in vacuum or in an air bath the color darkens very materially in a short time, especially when dried at 100° or higher. When dried at a temperature of 60 or 70° in vacuum the color darkens somewhat, but very slightly in comparison with that produced at higher temperatures.

Patten and Hart asserted that the acid turned dark in color on drying at 110° without undergoing any decomposition. As mentioned by Vorbrodts the grounds for this statement are not quite clear. A striking change in color, such as inosite hexaphosphoric acid suffers in drying or on mere keeping either in the desiccator or under ordinary conditions, would very likely indicate a more or less serious decomposition.

In order to determine to what extent decomposition occurs it was decided to make a series of inorganic phosphoric acid determinations by the usual molybdate method on inosite hexaphosphoric acid preparations before and after drying. While absolute accuracy could hardly be expected or claimed for this method, at least comparable results would be obtained when the precipitations were done under similar conditions.

One portion of the acid was dried at 105° in vacuum over phosphorus pentoxide to constant weight. It was then dissolved in water, neutralized with ammonia, acidified with nitric acid, ammonium nitrate added and heated to 65° . Ammonium molybdate was then added and kept at above temperature for one hour. The precipitate was then determined as magnesium pyrophosphate in the usual way.

Another portion was treated in the same manner without drying, the amount of moisture found on drying as above being deducted from the weight taken.

The fresh acid preparation, dried in vacuum over sulfuric acid as described, contained about 15 per cent. of water and it gave no reaction with ammonium molybdate. It was allowed to stand in the laboratory at summer temperature (about 80 or 90° F.) in a loosely covered dish for three or four weeks. The color had then changed to light brown. On drying at 105° in vacuum over phosphorus pentoxide for about 24 hours to constant weight it lost about 22 per cent. of its weight, showing that it had absorbed about 7 per cent. of water during this time. The acid contained 27.31 per cent. of phosphorus. The dried preparation gave the following as inorganic phosphate:

0.2508 gram dry substance gave 0.0066 gram $\text{Mg}_2\text{P}_2\text{O}_7$, equivalent to 7.73 per cent. phosphorus or 28.30 per cent. of the total phosphorus was precipitated as inorganic phosphoric acid.

Before drying:

0.1889 gram (dry substance calculated) gave 0.0039 gram $\text{Mg}_2\text{P}_2\text{O}_7$, equivalent to 0.57 per cent. of phosphorus or 2.08 per cent. of total phosphorus.

As will be noticed from the above figures, 26.2 per cent. of the total phosphorus had been hydrolyzed by drying at 105° for about 24 hours.

An old sample of inosite hexaphosphoric acid, which had been kept in the laboratory for about 18 months, was examined in the same manner. It was practically black in color. It lost about 22 per cent. of its weight on drying as above for about 20 hours. After decomposing by the Neumann method it was found to contain 27.68 per cent. of phosphorus.

The dry preparation gave the following:

0.2348 gram dry substance gave 0.0733 gram $\text{Mg}_2\text{P}_2\text{O}_7$, equivalent to 8.70 per cent. of phosphorus, or 31.43 per cent. of the total phosphorus was present as inorganic phosphoric acid.

Before drying:

0.2651 gram (dry substance calculated) gave 0.0295 gram $\text{Mg}_2\text{P}_2\text{O}_7$, equivalent to 3.10 per cent. of phosphorus or 11.19 per cent. of the total phosphorus had been hydrolyzed in about 18 months under ordinary room conditions.

In the above case about 20.2 per cent. of the total phosphorus had been hydrolyzed on drying at 105° for about 20 hours.

A sample of the pure recrystallized barium inosite hexaphosphate was examined for inorganic phosphoric acid in the same way. After standing in the laboratory for five or six weeks the following results were obtained:

After drying at 105° in vacuum over phosphorus pentoxide 0.2108 gram substance gave 0.0104 gram $\text{Mg}_2\text{P}_2\text{O}_7$, equivalent to 1.37 per cent. of phosphorus.

Before drying:

0.2060 gram (dry substance calculated) gave 0.0030 gram $\text{Mg}_2\text{P}_2\text{O}_7$, equivalent to 0.40 per cent. of phosphorus.

By drying at 105° the inorganic phosphorus increased about $2\frac{1}{2}$ times.

A portion of the inorganic phosphoric acid found in the above determinations was probably due to cleavage of the inosite hexaphosphoric acid by the dilute nitric acid. Such cleavage appears to take place slowly and uniformly, as shown by the following experiment:

Another portion (0.1876 gram substance) of the same barium inosite hexaphosphate without previous drying gave 0.0022 gram $\text{Mg}_2\text{P}_2\text{O}_7$ after heating one hour with the ammonium molybdate, or 0.32 per cent. in organic phosphorus; after heating the solution one-half hour more 0.0010 gram $\text{Mg}_2\text{P}_2\text{O}_7$ was obtained; further heating for one hour gave 0.0022 gram $\text{Mg}_2\text{P}_2\text{O}_7$ and a fourth hour's heating gave 0.0040 gram $\text{Mg}_2\text{P}_2\text{O}_7$.

The total inorganic phosphorus obtained after heating $3\frac{1}{2}$ hours as above was 1.39 per cent. The results indicate that the cleavage under these conditions is slow and that it proceeds at a very uniform rate.

EXPERIMENT TO DETERMINE WHETHER INOSITE IS FORMED IN THE SPONTANEOUS DECOMPOSITION OF INOSITE HEXAPHOSPHORIC ACID

The sample of old inosite hexaphosphoric acid previously referred to was used. As shown by the analysis, the preparation contained 3.10 per cent. inorganic phosphorus. Of this acid, 12.8 grams (corresponding to 10 grams of the dry substance) were dissolved in about 500 cc. of water and barium hydroxide (Kahlbaum, alkali free) added to slight alkaline reaction. The precipitate was filtered and washed several times in water. The barium precipitate was reserved for special examination.

The filtrate was examined for inosite as follows: The excess of barium hydroxide was precipitated with carbon dioxide filtered and evaporated on the water-bath nearly to dryness. The residue was taken up in a few cc. of hot water, filtered from a small amount of barium carbonate and the filtrate mixed with alcohol and ether and allowed to stand for several days in the ice chest. A trace of a white, amorphous precipitate had separated, but absolutely no inosite crystals appeared.

In case the organic part of the inosite hexaphosphoric acid molecule, corresponding to the inorganic phosphoric acid present, had separated as inosite, the above quantity, 10 grams, should have contained about 0.3 grams of inosite and such a quantity could not have escaped detection. Since no inosite could be isolated, it seems fair to assume that under the above conditions of spontaneous decomposition inosite hexaphosphoric acid does not decompose into inosite and phosphoric acid, but phosphoric acid plus some unknown substance.

EXAMINATION OF THE ABOVE BARIUM PRECIPITATE

In the hope of throwing some light upon the nature of this unknown substance, the barium precipitate obtained on the addition of

barium hydroxide was examined as follows: It was rubbed up with about 400 cc. of 0.5 per cent. hydrochloric acid and brought into solution by the careful addition of dilute hydrochloric acid. After filtering it was precipitated by adding an equal volume of alcohol. The precipitate was filtered, washed in dilute alcohol, dissolved in 0.5 per cent, hydrochloric acid and reprecipitated by barium hydroxide. The substance was then precipitated twice from 0.5 per cent. hydrochloric acid with alcohol, finally filtered, washed in dilute alcohol, alcohol and ether and dried in vacuum over sulfuric acid. A white, amorphous powder was obtained which weighed 14 grams. It was free from chlorides and inorganic phosphate. After drying at 105° in vacuum over phosphorus pentoxide the following results were obtained:

Found: C = 7.83; H = 1.46; P = 16.72; Ba = 36.96 per cent.

The carbon found is much too high for a pure barium inosite hexaphosphate.

PREPARATION OF CRYSTALLIZED BARIUM INOSITE HEXAPHOSPHATE FROM THE ABOVE AMORPHOUS BARIUM SALT

The substance was rubbed up in a mortar with about 150 cc. of cold water and allowed to stand for several hours. The insoluble portion was changed slowly into a semi-crystalline precipitate. It was filtered and washed in water and then recrystallized as follows: It was dissolved in a small quantity of about 3 per cent. hydrochloric acid, the free acid nearly neutralized with barium hydroxide; a concentrated solution of 10 grams of barium chloride was added, the solution filtered and alcohol added gradually with constant shaking until a slight permanent cloudiness was produced. On standing the substance crystallized slowly in the usual crystal form; i. e., in globular masses of microscopic needles. After two days the crystals were filtered off, washed free of chlorides in water and then in alcohol and ether and dried in the air. Yield, 4.5 grams. The substance gave no reaction with ammonium molybdate.

A further quantity of the same-shaped crystals was obtained from the aqueous solution containing the water-soluble portion of the amorphous salt by adding to it 2.5 grams barium chloride and allowing to stand over night. The balance of the water-soluble portion of the substance was recovered by precipitating with an equal volume of alcohol. The resulting precipitate was filtered, washed free of chlorides with dilute alcohol, alcohol and ether and dried in vacuum over sulfuric acid. Yield, 4.1 grams.

These substances were analyzed after drying at 105° in vacuum over phosphorus pentoxide.

The recrystallized salt gave the following results:

Found: C = 6.28; H = 1.28; P = 15.93; Ba = 42.18; H₂O = 11.81 per cent.

The crystalline salt which separated from the aqueous solution gave:

C = 6.47; H = 1.23; P = 15.95; Ba = 42.77; H₂O = 12.62 per cent.

These substances are therefore nearly pure heptabarium inosite hexaphosphate.

Calculated for $(C_6H_{11}O_{24}P_6)_2Ba_7 = 2267$.

C = 6.35; H = 0.97; P = 16.40; Ba = 42.39 per cent.

The water-soluble substance precipitated with alcohol gave the following:

Found: C = 8.58; H = 1.62; P = 15.86; Ba = 38.28 per cent.

This substance was again treated with about 100 cc. of cold water, the insoluble portion filtered off and the filtrate, after adding 1 gram of barium chloride, precipitated with alcohol. After washing in dilute alcohol, alcohol and ether and drying in vacuum over sulfuric acid 1.4 grams of a white, amorphous substance was obtained. For analysis it was dried at 105° in vacuum over phosphorous pentoxide.

Found: C = 8.08; H = 1.68; P = 15.64; Ba = 39.75 per cent.

This water-soluble substance apparently represents a mixture of the barium salts of penta- and tetraphosphoric acid esters of inosite.

CONCERNING THE DECOMPOSITION PRODUCTS OF INOSITE HEXAPHOSPHORIC ACID AFTER DRYING AT 105° UNDER REDUCED PRESSURE

The specimen of old inosite hexaphosphoric acid previously examined was used; 12.8 grams (corresponding to 10 grams dry acid) was dried at 105° for about 48 hours over sulfuric acid under slightly reduced pressure. It was then dissolved in about 200 cc. of cold water. The solution was practically black in color and contained particles of carbonized material. It was decolorized by shaking with animal charcoal. The clear, colorless solution was then precipitated with barium hydroxide to slight alkaline reaction, the precipitated filtered and washed in water and reserved for examination. The filtrate and washings were freed from barium with carbon dioxide and evaporated on the water-bath to dryness. The residue was taken up in a small amount of hot water and filtered. On adding a little alcohol a heavy, voluminous, white, amorphous precipitate was produced. This was

removed from the solution by adding about 3 volumes of alcohol. The precipitate settled, leaving a clear supernatant liquid; adding more alcohol produced no further precipitate. It was then filtered and washed in alcohol and the filtrate reserved.

After drying, the above precipitate was obtained as a heavy, white, amorphous powder. It was free from inorganic phosphorus, but contained barium and after combustion the ash gave a heavy yellow precipitate with ammonium molybdate. This substance was purified, as will be described later.

The filtrate from the above precipitate was again evaporated on the water-bath nearly to dryness, taken up in hot water, filtered and mixed with alcohol and ether. On scratching with a glass rod a substance began to crystallize in needles. It was allowed to stand in the ice chest over night. The crystals were then filtered, washed in alcohol and ether and dried in the air. Yield, 0.25 gram. The substance was recrystallized four times in the same manner and was finally obtained in colorless needles free from water of crystallization. It gave the reaction of Scherer and melted at 222° (uncorrected). It was, therefore, no doubt pure inosite. This was further confirmed by the analysis:

0.1215 gram substance gave 0.0737 gram H_2O and 0.1780 gm. CO_2 .

Found: C = 39.95; H = 6.78 per cent.

For $C_6H_{12}O_6 = 180$.

Calculated: C = 40.00; H = 6.66 per cent.

PURIFICATION OF THE BARIUM AND PHOSPHORUS CONTAINING PRECIPITATE REMOVED FROM THE INOSITE SOLUTION WITH ALCOHOL

The substance mentioned above, precipitated with alcohol, was apparently the barium salt of an organic phosphoric acid. It was very soluble in water and was not precipitated from the aqueous solution by barium hydroxide. The dry substance weighed 1.2 grams. It was dissolved in a small quantity of water, a few drops of dilute hydrochloric acid added and 10 cc. of N/1 barium chloride. The solution was heated to boiling and alcohol added until a slight cloudiness was produced. On standing in the cold over night a small amount of a hard crust had separated on the bottom of the flask. This was removed and the solution again heated and more alcohol added when a further quantity separated in the same way. The substance was finally filtered and washed thoroughly in 80 per cent. alcohol, alcohol and ether, and dried in the air. Without further purification the substance was analyzed after drying at 105° in vacuum over phosphorus pentoxide.

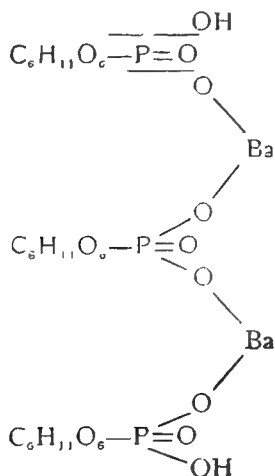
Found: C = 20.03; H = 3.58; P = 8.53; Ba = 25.55 per cent.

The small quantity precluded recrystallization and we are therefore unable to state whether the substance was pure. The analytical result indicates that it was a barium salt of inosite monophosphate and agrees approximately with this formula:



Calculated: C = 20.55; H = 3.33; P = 8.84; Ba = 26.16 per cent.

Such a salt could be represented by the following formula:



EXAMINATION OF THE PRECIPITATE PRODUCED WITH BARIUM HYDROXIDE AFTER DRYING THE ABOVE ACID

A portion of the barium precipitate was dried in vacuum over sulfuric acid and then examined for total and inorganic phosphorus in the same way as before:

Found: Total phosphorus (by Neumann method) 9.98 per cent.

Found: Inorganic phosphorus 7.46 per cent.

As will be noticed from these figures, 74.76 per cent of the phosphorus was present as inorganic phosphoric acid.

The substance was freed from inorganic phosphate by precipitating four times with alcohol from 0.5 per cent. hydrochloric acid. After finally drying in vacuum over sulfuric acid 3.2 grams of a snow-white, amorphous powder were obtained. The substance was free from chlorides and inorganic phosphate.

It was shaken up with about 75 cc. of cold water in which the greater portion dissolved; 10 cc. of N/1 barium chloride was added and allowed to stand for several hours; the insoluble portion was then

filtered off, washed free of chlorides with water and then in alcohol and ether and dried in vacuum over sulfuric acid. It weighed 0.65 gram.

The filtrate from above containing the water-soluble portion of the substance was acidified with a few drops of dilute hydrochloric acid, heated to boiling and alcohol added until a slight, permanent cloudiness remained. On standing over night a portion had separated in the form of a heavy, granular powder. Under the microscope no definite crystal form could be observed, but it appeared to consist of transparent globules. It was filtered off, washed free of chlorides in 30 per cent. alcohol, alcohol and ether, and dried in the air. Yield, 0.67 gram. It was free from inorganic phosphate.

The mother-liquor from above was precipitated with alcohol. After settling, the precipitate was filtered, washed with dilute alcohol, alcohol and ether, and dried in vacuum over sulfuric acid. Yield, 1.55 grams. The substance was a snow-white, amorphous powder. It was free from chlorides and inorganic phosphate.

These three different portions were analyzed after drying at 105° in vacuum over phosphorus pentoxide.

The water-insoluble portion gave:

C = 9.40; H = 1.65; P = 13.76; Ba = 39.56 per cent.

Judging by the analysis this substance consists mainly of barium inosite tetraphosphate.

The granular powder which separated from the hot, dilute hydrochloric acid solution and alcohol on cooling gave the following result:

C = 12.70; H = 2.40; P = 13.84; Ba = 32.29; H_2O = 12.77 per cent.

This substance appears to be mainly barium inosite triphosphate, although not pure. It was mixed probably with some barium inosite diphosphate.

The water-soluble portion precipitated with alcohol gave:

C = 14.07; H = 2.31; P = 12.92; Ba = 33.04 per cent.

Deducting the barium found, allowing for hydrogen and water, and calculating to the free acid these results became:

C = 20.88; H = 4.11; P = 19.16 per cent.

This is approximately the composition of inosite diphosphoric acid, $C_6H_{14}O_{12}P_2 = 340$.

Calculated: C = 21.17; H = 4.11; P = 18.23 per cent.

That these substances, separated from the partially decomposed inosite hexaphosphoric acid, are inosite esters of phosphoric acid and not condensation or other decomposition products, is evident from the fact that on complete cleavage inosite is obtained. Unfortunately, the amount of each of the above substances was too small to permit of

examination in this direction except the last one; viz., the water-soluble product precipitated by alcohol and which analyzed for inosite diphosphate. The remainder (0.94 grams dry substance) was hydrolyzed with dilute sulfuric acid in a sealed tube at 150 to 160° for about 2½ hours and the inosite isolated in the usual way. The amount of inosite obtained was 0.28 gram or about 88 per cent. of the theory. The substance gave the reaction of Scherer and melted at 222° (uncorrected) which leaves no doubt that it was pure inosite.

It is evident that all of the barium precipitates described above are mixtures. It could hardly be expected that a complete separation into pure chemical compounds of the salts of these inosite esters could be effected by the method used. The analytical results, however, show that it is possible to isolate from partially decomposed inosite hexaphosphoric acid certain substances approximating in composition various phosphoric acid esters of inosite which on complete cleavage yielded inosite, just as does inosite hexaphosphoric acid itself. This fact, we believe, supports the view previously expressed that inosite hexaphosphoric acid suffers a gradual and partial decomposition; i. e., molecules of phosphoric acid are eliminated one by one. We believe also that these facts taken in connection with the formation of inosite from the acid on mere drying at 105° must be considered as a strong support of the theory that it is inosite hexaphosphoric acid and not some complex compound, as previously held.

ATTEMPT TO PREPARE A METHYL ESTER OF INOSITE HEXAPHOSPHORIC ACID

The silver salt previously described as hepta-silver phytate (Anderson 1912 (2)) was used. Of this salt, 5.4 grams were suspended in 100 cc. of absolute methyl alcohol and 4 grams of methyl iodide (a little over the required amount) were added and the mixture shaken for several hours, the flask being protected from the light. At the end of this time the white silver phytate had changed into the yellow silver iodide. The precipitate was filtered off and washed several times in absolute methyl alcohol and the filtrate several times evaporated in vacuum to dryness under addition of methyl alcohol for the removal of the excess of methyl iodide. The residue was dissolved in methyl alcohol and evaporated to dryness in vacuum over sulfuric acid. The substance was then obtained as a light-yellow-colored, thick syrup of faint, aromatic odor. It was strongly acid in reaction and of sharp acid taste. For analysis it was dried in vacuum at 105° over phosphorus pentoxide. It then turned very dark in color.

0.1985 gram substance gave 0.0608 gm. H₂O and 0.1016 gm. CO₂.

Found: C = 13.95; H = 3.42 per cent.

This agrees with a dimethyl ester of inosite hexaphosphoric acid.
For $C_6H_{16}O_{24}P_6(CH_3)_2 = 688$.
Calculated: C=13.95; H=3.19 per cent.

THE WATER-INSOLUBLE SUBSTANCE WHICH SEPARATES FROM INOSITE HEXAPHOSPHORIC ACID AFTER DRYING

As has been mentioned earlier, inosite hexaphosphoric acid, which has been dried to constant weight in vacuum over phosphorus pentoxide, is not completely soluble in water. We have observed this insoluble substance in many instances after drying the acid at 60°, at 78° and at 105°. It always separates, on adding water to the dry substance, in thin gelatinous plates. It appears to be practically insoluble in hot or cold water. Continued boiling in acidulated water is necessary to dissolve it. It is also insoluble in alcohol and ether.

In order to obtain some knowledge of the composition of this insoluble substance 2.7 grams of the acid, containing about 12 per cent. of moisture, were dried to constant weight at 105° in vacuum over phosphorus pentoxide. After treating with water the insoluble portion was filtered, washed thoroughly in water and finally in alcohol and ether and dried in vacuum over sulfuric acid. It was then obtained as a dirty, gray powder which weighed 0.23 gram. It was non-hygroscopic. For analysis it was dried at 105° in vacuum over phosphorus pentoxide at which no change in color was noticeable. The substance was burned with copper oxide and the phosphorus determined in the ash.

0.2118 gram dry substance gave 0.0569 gram H_2O and 0.1357 gm. CO_2 and 0.1822 gm. $Mg_2P_2O_7$.

Found: C = 17.47; H = 3.00; P = 23.98 per cent.

The quantity of the substance obtained was so small that it was only sufficient for one analysis. Of course, we are unable to state whether it was homogeneous or not, but the analytical results agree approximately with inosite triphosphoric acid minus one molecule of water. The substance may therefore be a partial pyrophosphoric acid ester of inosite or it may represent some complex decomposition product of inosite hexaphosphoric acid.

SUMMARY

The evidence from experimental work shows that the organic phosphorus compound known as phytin or inosite phosphoric acid corresponds in composition to inosite hexaphosphoric acid, $C_6H_{18}O_{24}P_6$ or $C_6H_6O_6 \cdot (PO(OH)_2)_6$.

Crystalline barium salts have been prepared and analyzed which correspond to the following formulas, $C_6H_{12}O_{24}P_6Ba_3$ and $(C_6H_{11}O_{24}P_6)_2Ba_7$.

Salts of other metals are generally obtained as amorphous precipitates.

The acid yields readily crystalline strychnine salts.

The free acid may be obtained as a colorless, non-crystallizable syrup.

The acid decomposes on complete hydrolysis, yielding inosite and phosphoric acid.

On partial hydrolysis of the substance phosphoric acid and various lower inosite tetra-, tri-, di- or monophosphoric acids are produced.

When inosite and dry phosphoric acid are heated in vacuum to a temperature of 140 to 160° a reaction occurs and inosite tetraphosphoric acid is formed.

Inosite monophosphoric acid is formed from inosite hexaphosphoric acid on partial hydrolysis with mineral acids or by the action of the enzyme phytase in dilute acid solutions.

Wheat bran contains an enzyme phytase which in faintly acid solution rapidly hydrolyzes the inosite phosphoric acid with the production of inorganic phosphoric acid and various lower inosite phosphoric acids and some free inosite.

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