5. The small amount of amino acid found may be due to a small quantity of an amino acid containing phosphatid as impurity.

6. Heart lecithin has about the same amounts of each of the three main constituents as brain lecithin. The two lecithins may be the same compound. It is not to be expected that lecithin from two such different tissues prepared by rather different methods should yield by hydrolysis nitrogen products qualitatively identical and quantitatively so similar, unless each of these constituents was represented in the molecule by about the quantities found.

7. Acetone dehydration left the tissue in the most satisfactory condition for further work on the phosphatids.

BERKELEY, CAL.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE ARKANSAS AGRICULTURAL EXPERIMENT STATION AND JOHNS HOPKINS UNIVERSITY.]

THE INOSITE PHOSPHORIC ACIDS OF COTTONSEED MEAL.[1]

BY J. B. RATHER.

Received January 3, 1917.

In a previous paper on the phosphorus compounds of cottonseed meal,[2] the writer showed that the inorganic phosphorus of that material was only 5% of the total phosphorus, and that cottonseed meal contained an organic phosphoric acid which gave all the reactions relied on by Hardin[3] and by Crawford[4] to prove the presence of meta- and pyrophosphoric acids in that material. The writer concluded that there is no evidence that cottonseed meal contains these acids. Anderson[5] about a year later came to the same conclusion, and further stated that cottonseed meal contains an organic phosphoric acid very similar to phytic acid, $C_2H_8P_2O_9$. In continuation of the study the present writer[6] showed that cottonseed meal contains an inosite phosphoric acid richer in carbon and poorer in phosphorus than phytic acid, and ascribed the empirical formula $C_{12}H_{41}O_{42}P_9$ to this acid on the basis of analyses of silver salts which had been freed from bases and inorganic phosphoric acid. Anderson[7] later repeated his work and concluded that the acid was not the one claimed to be present by the writer, but that it was inosite hexaphosphoric acid,

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[1] Read in part at the Seattle Meeting of the American Chemical Society and the 1916 Meeting of the Association of Official Agricultural Chemists. Full details of this work will be published as a bulletin of the Arkansas Agricultural Experiment Station.

[2] Texas Agr. Expt. Sta., *Bull.* 146.

[3] South Carolina Agr. Expt. Sta., *Bull*, 8, n. s.

[4] *J. Pharm. Expt. Therapeutics*, 1, 51 (1910).

[5] *J. Biol. Chem.*, 13, 311 (1912).

[6] THIS JOURNAL, 35, 890 (1913).

[7] *J. Biol. Chem.*, 17, 41 (1914).

$C_6H_{18}O_{24}P_6$. The work reported in this paper is a further study of the nature of the inosite phosphoric acids of cottonseed meal with different methods and with samples from different sources.

Experimental.

Since there is a possibility of enzymes splitting off inorganic phosphoric acid from organic compounds during the process of extraction of the sample, experiments were made to determine to what extent this took place. The amount of inorganic phosphorus in the extracts was not decreased by the presence of 5% of phenol in the extracting liquid, and it was concluded that there is no appreciable hydrolysis by enzymes under the conditions of the method. This confirms *a priori* reasoning, because the method of preparation of cottonseed meal undoubtedly kills all enzymes.

Clarke[1] was the first worker, so far as the writer is aware, to obtain a crystalline salt of an inosite phosphoric acid on which melting-point determinations could be made, and which could be purified by fractional crystallization. He used strychnine for this purpose and obtained a compound melting at 203–4°. Anderson[2] used this base for the identification of inosite triphosphoric acid in wheat bran. Strychnine is not an ideal base for this purpose on account of its high molecular weight and for other reasons, but attempts by the present writer to obtain crystalline salts of the acid with other bases of an organic nature did not succeed although several weeks were spent in the trials. In the absence of a more desirable base, strychnine was used in the work to be described.

The Occurrence of Inosite Triphosphoric Acid in Cottonseed Meal.—Two and one-half kilograms of cottonseed meal, purchased in the Baltimore, Maryland, market, were digested for 3 hrs. with 10 liters of 0.2% hydrochloric acid with frequent stirring. The mixture was filtered through 2 layers of closely woven cloth, and copper acetate solution was immediately added to the filtrate until no further precipitation took place. The copper precipitate was filtered off and washed with water, suspended in water, and decomposed with hydrogen sulfide. The copper sulfide was filtered off, the filtrate evaporated to a small bulk at 55–65° under a strong current of air, and taken up with 6 volumes of 95% alcohol. A portion of the substance, consisting principally of acid salts of the crude acid, remained undissolved. This was filtered off, dissolved in acidulated water, and reprecipitated with copper acetate solution. This copper precipitate was treated in the same manner as the preceding. On the addition of alcohol only a small amount remained undissolved. This was discarded. The combined alcohol solutions were evaporated to a syrupy consistency at 50° under a strong current of air, taken up again in alcohol and a small precipitate filtered off. This process removed inorganic

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[1] *J. Chem. Soc.*, **105**, 535 (1914).

[2] *J. Biol. Chem.*, **20**, 463 (1915).

bases. The alcohol solution, when evaporated to a syrupy consistency, weighed 25 g. It was dissolved in about 3 liters of water and 50 g. of powdered strychnine were added. Upon heating all but 10 g. of the strychnine dissolved. The solution was filtered and allowed to cool overnight. Large plates made up of very small needles separated out. These were filtered off, washed with water, alcohol, and finally with ether. They weighed 30 g. and melted at 205–215° (Fraction 1). The mother liquor was evaporated at 55–65°, in a current of air to a volume of about 500 cc. and allowed to cool. Six g. of crystals of the same melting point as the above were obtained (Fraction 2). A further concentration gave 4 g. of needle-shaped crystals melting at 253–256° (Fraction 3). A still further concentration gave 3 g. of a similar substance (Fraction 4). Complete evaporation of the mother liquor gave 20 g. of a semi-solid mass which tended to crystallize only upon long standing. This process separates the inorganic phosphoric acid. Fractions 1 and 2 will be discussed in this paper; the others are reserved for further study.

Fractions 1 and 2 were recrystallized separately from water, and both crops of crystals melted at 203–4° (uncor.). They were recrystallized 4 times from water, the melting point remaining the same. All those lots of crystals, which upon recrystallization had a constant melting point of 203–4°, were combined and dissolved in methyl alcohol with the aid of heat. A few milligrams of a difficultly soluble substance melting at 215–220° were filtered off. The solution was concentrated, an equal volume of water added, and cooled in ice. Crystals melting at 203–4° were obtained. Crystals from the mother liquor had the same melting point. The two fractions were combined, recrystallized from water, washed with water, alcohol, and then with ether, and finally dried in the air. The purified product consisted of microscopic needles and plates and melted at 203–4° (uncor.).

For analysis the crystals were dried at 115°. Phosphorus was determined by the gravimetric method after igniting the salt with magnesium nitrate. Strychnine was determined by extracting the substance in solution in hot water after the base had been set free by ammonia. A mixture of equal parts of chloroform and ether was used as a solvent.

The results follow:

Calc. for tristrychnine inosite triphosphate, $C_6H_6(OH)_3(H_2PO_4)_3(C_{21}H_{22}N_2O_2)_3$: P, 6.54; strychnine, 70.46; 10 H_2O, 11.24,

Found: P, 6.16, 6.28; strychnine, 70.82, 71.48; H_2O, 11.74, 11.13, 11.19.

The composition of the salt agrees with that calculated for tristrychnine inosite triphosphate. Some other salts of inosite phosphoric acids have similar composition, so an analysis of the free acid is necessary to prove the identity of the salt.

Separation of the Free Acid.—Ten g. of the strychnine salt were dissolved in warm water, an excess of ammonia added, the mixture cooled and the strychnine filtered off. The filtrate was acidified with acetic acid, and copper acetate solution was added. The treatment of the copper precipitate was the same as described for the preparation of the crude acid. The product was a colorless, uncrystallizable syrup which darkened on standing and upon heating.

For analysis it was dried in a vacuum over phosphorus pentoxide at 100°. Phosphorus was determined by the volumetric method[1] after decomposition of the organic matter with magnesium nitrate. Carbon and hydrogen were determined in the usual manner, oxygen being used to aid the combustion. The results follow:

Calc. for inosite triphosphoric acid, $C_6H_6(OH)_3(H_2PO_4)_3$: C, 17.14; H, 3.57; P, 22.14.

Found: C, 17.37, 17.11; H, 4.35; P, 22.02, 22.06.

The composition of the material corresponds with that calculated for inosite triphosphoric acid.

A portion of the acid prepared from the strychnine salt was hydrolyzed with sulfuric acid in a sealed tube at 165° and the inosite separated in the usual manner. It melted at 216° (uncor.) after recrystallization from alcohol and ether. Inosite melts at 217–8° (uncor.). The hexacetate was prepared in the usual manner, and the product melted at 210–212° (uncor.). Mixed with a known sample of inosite hexacetate, the melting point remained unchanged. Inosite hexacetate melts at 212° (uncor.).

Attempts to make hexamethylamine inosite triphosphate using absolute alcohol as a medium did not succeed, due to the highly hydroscopic nature of the methylamine salts of the acid.

The writer believes that the above-described strychnine salt is tristrychnine inosite triphosphate because the acid obtained from it corresponds in composition to inosite triphosphoric acid, and because the composition and melting point of the salt is the same as that of a material of similar composition yielding inosite triphosphoric acid, obtained from wheat bran by another worker.[2]

The Occurrence of Inosite Pentaphosphoric Acid in Cottonseed Meal.—The composition of the inosite phosphoric acid obtained from the sample of cottonseed meal described above differed so widely from that obtained from samples purchased in the Texas market, that it was thought desirable to repeat the work on samples obtained from a different locality. Accordingly, a lot of cottonseed meal was purchased on the Fayetteville, Arkansas, market, and examined as described below.

The preparation of the strychnine salt was essentially the same as described above, except that in this case an *equal* weight of freshly pre-

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[1] *J. Assn. Official Agr. Chemists*, 1, No. 4, 3 (1916).

[2] Anderson, *J. Biol. Chem.*, **20**, 463 (1915).

cipitated strychnine was added to the crude acid after freeing it from bases. Upon recrystallizing from water a series of fractions were obtained melting around 220°, which constituted the principal part of the product, and in addition a semi-solid mass similar to that previously described in this paper. The latter material is reserved for future study. Upon recrystallization of the fractions melting around 220°, products with a constant melting point of 220–222° (uncor.), consisting of microscopic needles and plates, were obtained. The first fraction thus obtained, which will be designated as Product A, was analyzed with the results given below. For analysis the air-dry material was dried to constant weight in a vacuum over phosphorus pentoxide at 105°. Carbon, hydrogen and phosphorus were determined as just described, except that the material was mixed with fine copper oxide previous to burning in the combustion tube. Nitrogen was determined by the Kjeldahl method. The results follow:

Calc. for octastrychnine salt of the acid $C_{12}H_{41}O_{42}P_9$: C, 56.72; H, 5.70; N, 5.88; P, 7.33.

Calc. for tetrastrychnine inosite pentaphosphate, $C_6H_6(OH)(H_2PO_4)_5(C_{21}H_{22}N_2O_2)_4$: C, 56.33; H, 5.48; N, 5.84; P, 8.09.

Found: C, 56.29; H, 6.14; N, 5.66; P, 8.21.

The composition of the strychnine salt agrees fairly well with that calculated for the octastrychnine salt of the acid $C_{12}H_{41}O_{42}P_9$, the formula for inosite phosphoric acid, or phytic acid, previously proposed by the writer. The composition of the salt agrees also with that calculated for tetrastrychnine inosite pentaphosphate, a possible ester of inosite and phosphoric acid not hitherto described.

The statement has been made by other workers[1] that the respective percentages of carbon, hydrogen, nitrogen and phosphorus in the strychnine salts of the possible inosite phosphoric acids are so close together that little indication of the composition of the free acid can be obtained by analysis of the salts. While this is true when the amount of strychnine in the salt is equivalent to or greater than the amount of phosphorus, it is not true when the molecular ratio Strychnine/Phosphorus is less than 1. The present writer has calculated the percentage composition of the 42 possible strychnine salts of the 6 possible inosite phosphoric acids, and has found that, taking the carbon, nitrogen and phosphorus into consideration, when the ratio is as described, the percentage of at least one of the three elements is quite different from that of the same element in other salts.

Assuming that the salt is one of a simple acid ester of inosite and phosphoric acid, the percentages of nitrogen and of phosphorus limit the possibilities to pentastrychnine inosite hexaphosphate and tetrastrychnine inosite pentaphosphate. The percentage of carbon enables one to decide that the compound is the latter. The percentage of carbon calculated

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[1] Clarke, *J. Chem. Soc.*, 105, 535 (1914); Anderson, *J. Biol. Chem.*, 20, 465 (1915).

for the former salt is 59.44, and the pentastrychnine salt of the same acid would contain 57.14% carbon, 6.01% nitrogen, and 7.98% phosphorus. The results do not exclude the possibility that the material is a mixture of tetra- and pentastrychnine inosite hexaphosphate. Evidence that this is not the case is given below.

The material was isolated again as a strychnine salt from another portion of the same lot of cottonseed meal in the same manner as described above except that all those fractions melting at 220–2° were combined. This material will be designated as Product B. The product was analyzed as before. The results follow:

Calc. for $C_{12}H_{41}O_{42}P_9(C_{21}H_{22}N_2O_2)_8$: C, 56.72; H, 5.70; N, 5.88; P, 7.33.

Calc. for $C_6H_6(OH)(H_2PO_4)_5(C_{21}H_{22}N_2O_2)_4$: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 7 H_2O, 6.57.

Found: C, 55.85; H, 6.21; N, 5.56; P, 8.84; H_2O, 6.75, 5.96.

The composition of the product agrees with that of Product A.

In order to determine whether the composition of different fractions of the same melting point was the same, and to determine whether the compound could be isolated from an entirely different sample of the original material, another lot of cottonseed meal was purchased in the Fayetteville, Arkansas, market. The method of preparation of the strychnine salt was the same as just described. The crystalline salt, melting at 220–2°, was fractionated into 3 crops of crystals which in turn were recrystallized with no change in melting point. Crystals from the mother liquors had the same melting points. These products will be designated as Products C, D and E. Analysis was made as before.

Calc. for $C_{12}H_{41}O_{42}P_9(C_{21}H_{22}N_2O_2)_8$: C, 56.72; H, 5.70; N, 5.88; P, 7.33.

Calc. for $C_6H_6(OH)(H_2PO_4)_5(C_{21}H_{22}N_2O_2)_4$: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 7 H_2O, 6.57.

(Product C) Found: C, 55.72; H, 6.36; N, 5.60; P, 8.58; H_2O, 6.69, 6.66.

(Product D) Found: C, 55.74; H, 5.55; N, 5.61; P, 8.74; H_2O, 6.56, 7.27.

(Product E) Found: C, 56.20; H, 5.73; N, 5.73; P, 8.46; H_2O, 7.08.

The composition of the Products C, D and E are the same and agree with that of Products A and B. This is evidence both that the salt is a pure substance and that the material is not a mixture of two salts of the same acid. This excludes the possibility mentioned above, that the products are mixtures of tetra- and pentastrychnine inosite hexaphosphate. The writer concludes that the various products analyzed are the same, and that they correspond in composition to the octastrychnine salt of the acid $C_{12}H_{41}O_{42}P_9$, and equally as well to the tetrastrychnine salt of inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$.

Preparation of the Strychnine Salt from the Barium Salt of the Acid.—Anderson[1] believes that the acid in cottonseed meal is inosite hexaphosphoric acid, a substance containing less carbon and more phos-

[1] *J. Biol. Chem.*, 17, 141 (1914).

phorus than the acid previously isolated from cottonseed meal by the writer. In order to compare the product as obtained by the process of Anderson with those products obtained as described above, the writer has prepared the material by the barium precipitation method of Anderson. The reader is referred to Anderson's paper for the description of the process. The procedure was followed as closely as possible and the barium salt was dried to constant weight in a vacuum over phosphorus pentoxide and analyzed. Combustion was made after mixing the material with a previously fused and powdered mixture of lead chromate and potassium dichromate. The results follow:

Calc. for $C_6H_6(HPO_4)_6Ba_3$: C, 6.75; H, 1.12; P, 17.44; Ba, 38.65.
Calc. for $(C_6H_6(OH)(HPO_4)_5)_2Ba_5$: C, 7.79; H, 1.84; P, 16.72; Ba, 37.22.
Found: C, 7.02; H, 2.23; P, 15.98, 16.68; Ba, 38.51, 37.72.

The composition of the barium salt does not correspond with that of any barium salt of either inosite hexaphosphoric acid or inosite pentaphosphoric acid.

The percentage of phosphorus in the free acid, calculated from the above results, would be 25.88, inosite pentaphosphoric acid would contain 26.72 and the hexaphosphoric acid would contain 28.18%.

The strychnine salt of the acid was prepared from the barium salt after the removal of the barium by an equivalent amount of sulfuric acid. An amount of strychnine was used equal in weight to the syrupy acid. The crystals were recrystallized and melted at 220–2°, as in the case of the products prepared as described above. They had also the same crystal form. The analysis was conducted as already described. The results follow:

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09.
Found: C, 55.64; H, 5.65; N, 5.33; P, 8.88.

The material corresponds in composition to the strychnine salts prepared by the method of the present writer. It does not correspond to any strychnine salt of inosite hexaphosphoric acid. Further evidence on this point is given below.

Separation of the Free Acid from the Strychnine Salt.—From Products B, C, D and E, and a strychnine salt from the barium compound described above, the free acid was isolated in the following manner: A portion of the salt was dissolved in water with the aid of heat, and a slight excess of ammonia was added to precipitate the strychnine. The mixture was cooled, filtered, and made slightly acid with acetic acid. Copper acetate solution was added in excess and the copper precipitate filtered off and washed well with water. This process removes nearly all the ammonia and the last trace of strychnine. The copper precipitate was suspended in water and decomposed with hydrogen sulfide. The copper sulfide was filtered off and the filtrate evaporated to a small bulk at 55–65° under a strong current of air and then taken up with alcohol. The small residue of acid ammonium salts was filtered off and the filtrate was evaporated as above, taken up with water and again evaporated to decompose any esters the compound might have formed with the alcohol. The products thus obtained were straw-colored syrups which darkened on standing in a desiccator and assumed a gummy consistency. For analysis the materials were dried to constant weight at 80° in a vacuum over phosphorus pentoxide. They turned quite dark on drying. The analytical results follow:

Calc. for inosite hexaphosphoric acid, $C_6H_6(H_2PO_4)_6$: C, 10.90; H, 2.72; P, 28.18.

Calc. for inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$: C, 12.41; H, 3.23; P, 26.72.

(Acid from Product B) Found: C, 11.48, 11.28, 11.97; H, 3.26, 3.59, 3.75; P, 25.88, 26.18.

(Acid from Product C) Found: C, 11.59; H, 3.46; P, 26.21, 25.90.

(Acid from Product D) Found: C, 10.99, 11.11; H, 3.34, 3.39; P, 26.99, 26.74.

(Acid from Product E) Found: C, 11.24; H, 3.53; P, 26.68, 26.62.

(Acid from barium compound) Found: C, 11.18, 10.65; H, 3.82, 3.43; P, 26.44, 26.88.

The results agree with the calculated composition of inosite pentaphosphoric acid except that the results are too low by about 1% in the case of the carbon. The percentages of carbon in the above materials are too high for inosite hexaphosphoric acid, and the percentages of phosphorus much too low.

A few grams of the free acid were hydrolyzed with sulfuric acid in a sealed tube and the inosite separated in the usual manner. Upon recrystallization from alcohol and ether the product melted at 215° (uncor.), had the sweetish taste of inosite and gave the reactions of Gallois and of Scherer. This was considered sufficient evidence of the presence of inosite, in view of the fact that a chemical analysis has already been reported by the writer on a material obtained from an acid of cottonseed meal of similar composition.

Preparation of a Silver Salt of the Inosite Phosphoric Acid.—The analytical results on the strychnine salts described above probably preclude the possibility of the acid being inosite hexaphosphoric acid, but in order to confirm these results, if possible, silver salts, which are readily obtained and easily burned when mixed with copper oxide, were prepared and analyzed. The fact that the silver salts of this acid are amorphous is no objection, because the strychnine salts from which they are made have been proven to be pure. All of the carbon and phosphorus in a silver salt of this acid must come from the acid itself, and the percentages of these elements in the free acid can readily be calculated from the analysis of the silver salt.

Portions of the strychnine salts of Products A, C and D, and the strychnine salt from the barium compound, were dissolved in warm water, the strychnine precipitated with ammonia and filtered off. The filtrates were extracted with ether to remove the last traces of strychnine, and the slight excess of ammonia boiled off. An excess of silver nitrate solution was added and the precipitate filtered off and washed with water. For analysis the products were dried at 80° to constant weight in a vacuum over a phosphorus pentoxide. They were free from nitrogen. For combustion they were mixed with fine copper oxide and burned in a current of oxygen. The results follow:

Calc. for $C_{12}H_{25}O_{42}P_9Ag_{16}$: C, 5.06; H, 0.88; P, 9.81; Ag, 60.66.

Calc. for octasilver inosite pentaphosphate, $C_6H_6(OH)H_2(PO_4)_5Ag_8$: C, 5.02; H, 0.63; P, 10.80; Ag, 60.13.

(Salt from Product A) Found: C, 4.78; H, 1.02; P, 9.90; Ag, 60.85.

(Salt from Product C) Found: C, 4.84; H, 1.10; P, 9.88; Ag, 60.65.

(Salt from Product D) Found: C, 4.94; 5,18, H, 1.31, 1.10; P, 9.43; Ag, 60.18.

(Salt from barium compound) Found: C, 5.19, 4.94; H, 1.10, 1.12; P, 10.01; Ag, 61.22.

Deducting silver and adding an equivalent amount of H, percentages of carbon calculated for free acid are: Product A, 12.03; Product C, 12.13; Product D, 12.72; Product from barium compound, 12.86.

The above analytical results correspond in every case to the salt of the acid $C_{12}H_{41}O_{42}P_9$, containing 16 atoms of silver. These results, on salts prepared in an entirely different way from those previously reported by the writer,[1] nevertheless agree closely with the latter results. It was on the basis of analyses of silver salts that the writer proposed the formula $C_{12}H_{41}O_{42}P_9$ for the inosite phosphoric acid of feeding materials. If this formula is divided by 2 the quotient would be $C_6H_{20}O_{21}P_{4.5}$, which is quite similar to the formula for inosite pentaphosphoric acid, $C_6H_{17}O_{21}P_5$. The analytical results on the above salts agree with the calculated composition of octasilver inosite pentaphosphate, except that the phosphorus is slightly too low. The strychnine salts described above correspond quite well with the calculated composition of a salt of this acid, and in the analysis of the free acid the percentage of phosphorus agrees with the calculated percentage in the acid in question.

The percentage of carbon in the free acid, calculated from the analyses of the silver salts varies from 12.03 to 12.86 and averages 12.43%. The calculated percentage of carbon in the acid according to the formula previously proposed by the writer is 12.68, that in inosite pentaphosphoric acid is 12.41, and that in inosite hexaphosphoric acid, believed to be present in cottonseed meal by Anderson, is 10.90.

On the basis of evidence offered by the analysis of strychnine salts of the inosite phosphoric acid, which salts have been shown not to be mix-

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[1] THIS JOURNAL, **35**, 890 (1913).

tures, and on the basis of analysis of silver salts prepared from these salts, the writer concludes that the percentage of carbon obtained by the analysis of the free acid is lower than the truth. The principal inosite phosphoric acid obtained from the Arkansas samples of cottonseed meal is one corresponding in composition to an acid of the formula $C_{12}H_{41}O_{42}P_9$, the formula previously proposed by the writer, and equally as well to that of inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$, a compound which, so far as the writer is aware, has not been mentioned in chemical literature. Since the first-mentioned formula is empirical, while the second is that of a theoretically possible compound of the class to which the acid in question is believed by many to belong, the last-mentioned formula is probably to be preferred.

The Nature of the Inosite Phosphoric Acid Obtained from the Aqueous Extract of Cottonseed Meal.—It has been shown by the writer that the water-soluble phosphorus of cottonseed meal is more than twice as great as the 0.2% hydrochloric acid-soluble phosphorus, and that this excess of phosphorus is probably combined in a similar way to the acid-soluble phosphorus.[1] In order to verify this conclusion, if possible, the writer has prepared the strychnine salt as described above, using water as a solvent to extract the material from the cottonseed meal. The product of the extraction should contain both the acid-soluble material and the water-soluble material. Two lots of cottonseed meal were extracted and the principal product of the process in each case was a strychnine salt melting at 220–2° as before. These materials will be designated as Products F and G. Analysis was made as described. The results follow:

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 8 H_2O, 7.51.

(Product F) Found: C, 55.76; H, 6.04; N, 5.64; P, 8.59; H_2O, 7.52, 6.89.

(Product G) Found: C, 55.82; H, 6.23; N, 5.72; P, 8.48, 8.08.

The materials correspond in composition to those obtained from the acid extract of the cottonseed meal.

The free acid was prepared as already described, and analyzed as before. The results follow:

Calc. for inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$: C, 12.41; H, 3.23; P, 26.72.

(Acid Product F) Found: C, 11.40, 10.98; H, 3.30, 3.23; P, 26.54, 26.52.

(Acid Product G) Found: C, 11.69; H, 3.67; P, 25.24.

The percentage composition of the strychnine salt agrees with that calculated for tetrastrychnine inosite pentaphosphate, while in the free acid the carbon is a little too low. It has been shown above that the percentage of carbon in silver salts, prepared from strychnine salts which are identical in composition, crystal form and melting point with the material from

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[1] THIS JOURNAL, 35, 890 (1913).

which the above free acid was obtained, when calculated to the free acid, gave results averaging practically the same as for inosite pentaphosphoric acid. In the light of this fact it was thought unnecessary to prepare silver salts or to hydrolyze the material and isolate inosite.

The principal inosite phosphoric acid obtained by aqueous extraction of cottonseed meal, in spite of the fact that this solvent removes twice as much phosphorus as does 0.2% hydrochloric acid, appears to be the same as that obtained by the use of the latter solvent. This confirms previous conclusions of the writer on the subject.

Efficiency of the Method of Purification of the Inosite Phosphoric Acids.—The method of purification of the inosite phosphoric acid previously described by the writer[1] has been criticized by Anderson,[2] who thinks that the silver salts thus prepared were very impure, especially with reference to inorganic phosphates. This, however, is not the case, as the materials were shown to be practically free from bases, and to contain only about 0.10% of inorganic phosphates.

The purity of the products described in the present article were ascertained as follows: Inorganic phosphates were determined in 0.2 g. of the strychnine salts by dissolving the salts in water, precipitating the strychnine with ammonia, filtering and determining the inorganic phosphorus in the filtrate by the Forbes[3] method. Results of some of the determinations are given below:

(Product A) Found: P, 0.028. (Product C) Found: P, 0.014%.
(Product D) Found: P, 0.014. (Product F) Found: P, 0.042%.

It can be seen from these results that the strychnine salts were practically free from inorganic phosphorus.

Quantitative determinations of nitrogen in the silver salts and in the free acid by the Kjeldahl method, after allowing for a blank determination, gave 0.0% nitrogen in every case.

No precipitate formed when the free acid was made alkaline with ammonia and sodium phosphate added, showing the absence of iron, aluminun, calcium and magnesium. Tests for inorganic bases were made in the strychnine salts as follows: 0.2 g. of the salt was dissolved in water with the aid of heat, made alkaline with ammonia as in the gravimetric method for the determination of magnesium, sodium phosphate added, and the mixture allowed to stand overnight. The precipitate was filtered off, washed with 2.5% ammonia, ignited and weighed. The residue consists of inorganic phosphates of iron, aluminum, calcium, and magnesium. The weight of the residues varied from 0.0001 g. to 0.0003 g. The strychnine salts were thus almost entirely free from inorganic bases.

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[1] THIS JOURNAL, **35**, 890 (1913).

[2] *J. Biol. Chem.*, **18**, 435 (1914).

[3] Ohio Agr. Expt. Sta., *Bull.* 215.

Determination of impurities other than nitrogen in the silver salts was deemed unnecessary in view of the demonstrated purity of the strychnine salts from which they were made.

In addition to the above, the following strychnine salts were made and compared as to physical appearance, melting point, and solubility with the products under investigation: monostrychnine, orthophosphate, distrychnine orthophosphate, monostrychnine oxalate, distrychnine oxalate, strychnine acetate, strychnine hydrochloride. The above materials are possible contaminants, either as occurring in the original material (oxalic and phosphoric acids) or being introduced during the preparation of the product. All of these materials were found to be more soluble in water and to have much higher melting points and entirely different crystal form from the strychnine salt under investigation.

There seems to be little doubt that the products described in this paper are free from inorganic bases and phosphates, oxalic, hydrochloric and acetic acids.

Discussion of Results.

The results of the examination of the Arkansas samples of cottonseed meal are summarized below, together with the calculated composition of inosite phosphoric acids and their compounds more nearly like the materials analyzed.

Material.	C.	H.	N.	P.	Ag.
Found: Product A	56.29	6.14	5.56	8.21	..
Found: Strychnine salt, Ba method	55.64	5.55	5.33	8.88	..
Calculated: Tetrastrychnine inosite pentaphosphate	56.33	5.48	5.84	8.09	..
Calculated: Pentastrychnine inosite hexaphosphate	57.14	5.49	6.01	7.98	..
Calculated: Tristrychnine inosite tetraphosphate	55.09	5.45	5.59	8.25	..
Found: Acid from Product B	11.59	3.53	..	26.53	..
Found: Acid, Ba method	10.92	3.64	..	26.66	..
Carbon in free acid, calculated from analyses of silver salts, Product C	12.13	..	..	..	..
Product, Ba Method	12.86	..	..	..	..
Calculated: Inosite pentaphosphoric acid	12.41	3.23	..	26.72	..
Calculated: Inosite hexaphosphoric acid	10.90	2.72	..	28.18	..
Found: Ag salt, Product C	4.84	1.10	..	9.88	60.65
Found: Ag salt, Ba compound	5.07	1.11	..	10.01	61.22
Calculated: Octasilver inosite pentaphosphate	5.02	0.63	..	10.80	60.13
Calculated: Nonasilver inosite hexaphosphate	4.44	0.56	..	11.52	60.12
Calculated: $C_{12}H_{25}O_{42}P_9Ag_{16}$	5.06	0.88	..	9.81	60.66

The strychnine salts prepared by the writer's process and that prepared from the material purified by Anderson's process, correspond in composition to a salt of inosite pentaphosphoric acid, and not to a salt of inosite hexaphosphoric acid. The percentage composition of the free acid, prepared by the two methods, agrees with that calculated for inosite, pentaphosphoric acid with the exception of the carbon. The percentage of carbon in the free acid, calculated from analyses of the silver salts, agrees with that calculated for inosite pentaphosphoric acid.

On the basis of the evidence offered by the analyses of the strychnine salts, the free acid and the silver salt prepared from the free acid, the writer feels justified in concluding that the principal inosite phosphoric acid in the cottonseed meal examined is higher in carbon and lower in phosphorus than the so-called "phytic acid," or than inosite hexaphosphoric acid, and that the composition of the products obtained from Arkansas samples of cottonseed meal indicate equally as well the presence of inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$, or of an acid of the formula $C_{12}H_{41}O_{42}P_9$, the formula previously proposed for this compound by the writer.

Since the process by which it was claimed that inosite hexaphosphoric acid was isolated from cottonseed meal yielded a material corresponding in composition to salts of inosite pentaphosphoric acid, the present writer concludes that the samples examined contained no inosite hexaphosphoric acid.

The writer has been unable to obtain from Arkansas samples of cottonseed meal the strychnine salt melting at 202–3°, which yielded an acid corresponding in composition to inosite triphosphoric acid. The supply of material purchased in the Baltimore market, from which this substance was obtained, has been exhausted, so the work cannot be repeated. It is evident, however, that inosite triphosphoric acid is not of constant occurrence in cottonseed meal.

The writer wishes to thank Dr. E. Emmet Reid of Johns Hopkins University for valuable suggestions in connection with the conduct of the work described above and the preparation of the manuscript.

Summary.

1. An inosite phosphoric acid corresponding in composition to inosite triphosphoric acid, $C_6H_6(OH)_3(H_2PO_4)_3$, has been isolated from cottonseed meal. It does not appear to be of constant occurrence.

2. From other samples of cottonseed meal an inosite phosphoric acid has been isolated corresponding in composition to inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$, and corresponding equally as well to the acid, $C_{12}H_{41}O_{42}P_9$, the formula previously proposed for this material by the writer. In view of the theoretical possibility of the existence of the former compound, it appears best to ascribe the former formula to the compound.

3. The method of preparation by which it was stated that inosite hexaphosphoric acid was isolated from cottonseed meal, gave results concordant with the above.

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