The transport number of the lithium ion has been calculated and it has been found to increase in each solvent as the concentration of the salt is diminished.

The free energy of dilution and the activity ratios of both the ions and the molecules, on the other hand, decrease with the dilution.

An attempt has been made to explain the decrease in the activity ratios, and hence the free energy, on the assumption of effects due to hydration and the change in dielectric constant. For the higher alcohols, it has been found necessary to assume a polymerization and subsequent complex ionization of the salt molecules.

For a given pair of concentrations the activity ratios of the ions increase with increase in the dielectric constant of the solvent. The ratio $(\xi''/\xi')/\sqrt{K_d}$ increases with increase in concentration and at the same sufficiently high equivalent concentrations attains an approximately equal value for all solvents. For these concentrations the ration (ξ''/ξ') is very nearly directly proportional to the square root of the dielectric constant of the solvent. For similar cells in water and methyl alcohol these ratios are practically identical.

The activity of the ions has been found to increase both with the concentration and with the dielectric constant of the solvent, or of the solution.

IOWA CITY, IOWA.

THE NATURE OF THE INOSITE PHOSPHORIC ACIDS OF SOME IMPORTANT FEEDING MATERIALS.[1]

BY J. B. RATHER.

Received December 6, 1917.

The theory that phytic acid, an inosite phosphoric acid found in seeds of plants as a complex salt known as phytin, corresponds in composition to the formula $C_2H_8P_2O_9$ was first proposed by Posternak.[2] Much of the work of European investigators does not agree with this view. Investigations made in America by Patten and Hart[3] and Hart and Tottingham[4] and the earlier work of Anderson[5] gave results in accord with the theory of Posternak. It was found by the present writer however, that cottonseed meal and wheat bran yielded an inosite phosphoric acid richer in carbon and poorer in phosphorus than is required for the formula proposed by

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[1] That portion of this work relating to corn and kafir corn and a part of that relating to wheat bran was read before the American Chemical Society in Kansas City, April, 1917.

[2] *Rev. Gen. Bot.*, **12**, 5 and 65; *Compt. rend.*, **137**, 35–8.

[3] N. Y. (Geneva) Expt. Sta., *Bull.* **250**.

[4] Wisconsin Expt. Sta., *Tech. Bull.* **9**.

[5] N. Y. (Geneva) Expt. Sta., *Tech. Bulls.* **19** and **21**.

Posternak.[1] Anderson[2] believed that "cottonseed meal contains an organic phosphoric acid very similar to phytic acid," and that wheat bran[3] contains an inosite phosphoric acid of the formula $C_{25}H_{65}P_9O_{54}$ containing pentose in the molecule, and which decomposes into an inosite phosphoric acid of the formula $C_{20}H_{55}P_9O_{49}$ with the loss of the pentose group. The examination of wheat bran made by Patten and Hart, which preceded that of other workers, led them to conclude that the acid corresponded in composition to the phytic acid of Posternak, namely, $C_2H_8P_2O_9$. The present writer concluded[4] that the acid corresponded in composition to the formula $C_{12}H_{41}O_{42}P_9$ and showed that Anderson's pentose theory was based on inadequate evidence. Later Anderson repeated his work and at first concluded[5] that wheat bran contains a number of inosite phosphoric acids, and later[6] that wheat bran contains inosite monophosphate, inosite triphosphoric acid and inosite hexaphosphoric acid, and also that cottonseed meal contains inosite hexaphosphoric acid.[7] He does not appear to repeat his claim that wheat bran contains an inosite pentose phosphoric acid. Boutwell[8] was unable to obtain the inosite monophosphate from wheat by Anderson's method, nor was he able to separate the inosite hexaphosphoric acid claimed to be present by Anderson. Boutwell's products failed to correspond in composition to that of any simple phosphoric acid ester of inosite.

Anderson has criticised the method of preparation used by the present writer and claims that products so prepared are impure, and in the case of wheat bran highly contaminated with orthophosphoric acid. It has recently been shown however[9] that an entirely different method of preparation yields the same results on cottonseed meal, and that cottonseed meal contains an inosite phosphoric acid corresponding in composition equally as well to the formula $C_{12}H_{41}O_{42}P_9$ previously proposed and to the formula $C_6H_6(OH)(H_2PO_4)_5$, inosite pentaphosphoric acid. The first formula divided by 2 gives figures essentially the same as the latter formula, and since the latter is that of a theoretically possible compound it was adopted as the more desirable. These products, as was with the case criticised by Anderson, were shown to be free from inorganic bases, orthophosphoric acid and other impurities.

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

[2] N. Y. (Geneva) Expt. Sta., *Tech. Bull.* 25.

[3] *Ibid.*, 22.

[4] *Loc. cit.*

[5] *J. Biol. Chem.*, **18**, 425 (1914).

[6] *Ibid.*, **18**, 441 (1914); *Ibid.*, **20**, 463 (1915); N. Y. (Geneva), *Tech. Bull.* 40, 25.

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

[8] THIS JOURNAL, **39**, 491 (1917).

[9] Rather, *Ibid.*, **39**, 777 (1917).

The work described below is a continuation of the study of the nature of the inosite phosphoric acids of feeding materials.

Experimental.

The method of separation and purification of the inosite phosphoric acid of feeding materials has considerable effect on the composition. It is not only necessary to separate the acid from inorganic bases, but to avoid hydrolysis by enzymes or other agencies, to separate it from other acids, known and unknown, which occur in the material under examination, and to prepare a compound which can be purified and demonstrated to be pure. These requirements have not always been observed.

The method of preparation adopted in the present work consists of removing the inorganic bases from the crude acid with alcohol, and fractionating the material by means of the strychnine salt. The process has been shown[1] to free the acid from inorganic bases, orthophosphoric acid, oxalic, hydrochloric and acetic acid and from other compounds of an unknown nature. Numerous experiments indicated that the effect of enzymes during the time of extraction has no effect on the composition of the principal product of the procedure. The strychnine salt can be fractionated by recrystallization to any desired extent and the purity of the material demonstrated by uniformity in composition and melting points of the several fractions. The method briefly is as follows:

Digest the finely ground material with 0.2% hydrochloric acid for 3 hours with frequent stirring. Filter through two layers of closely woven cloth, pouring back until the filtrate comes through clear. Add immediately copper acetate solution in excess, filter the copper precipitate and wash with water. Decompose the copper salt with hydrogen sulfide, filter and evaporate to a small volume at 55–60° under a strong current of air. Take up with 6 volumes of 95% alcohol and filter off the insoluble matter. Dissolve the insoluble matter, consisting of acid salts, in acidulated water, precipitate with copper acetate solution and complete as before. This process should be repeated again on any insoluble residue remaining. Evaporate the combined alcohol solutions to a syrup at 50° under a strong current of air, take up again in alcohol, filter if necessary, and evaporate as before. This process removes all inorganic bases. The inosite phosphoric acid is then separated and purified as follows: Weigh the crude acid, dissolve in about three liters of water, filter off the insoluble matter, if any, add to the warm solution an amount of reprecipitated strychnine equal in weight to the syrupy acid. 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.[1] All of the strychnine should go into solution. Filter if necessary, and concentrate at 55–60° under a strong current of air until crystals begin to form. Then allow to

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[1] Rather, THIS JOURNAL, 39, 777 (1917).

cool. Filter and concentrate the mother liquor as above until crystals begin to appear, cool, filter and concentrate the mother liquor again. Purify the fractions by recrystallization in the usual manner. This process separates the inosite phosphoric acid from orthophosphoric acid, oxalic acid and other acids.

The material which is crystallizable consists almost entirely of the inosite phosphoric acid described below.

The Occurrence of Inosite Pentaphosphoric Acid in Wheat Bran.—The material was prepared as described above. 5 kg. of ground wheat bran and 40 liters of 0.2% hydrochloric acid were used. One hundred grams of the crude acid were obtained and from this 65 g. of a strychnine salt melting at 220–22° (uncorr.). Other crystalline and non-crystalline phosphorus-bearing materials were found.

The fraction melting at 220–2° consisted of large scales made up of microscopic needles and plates. It was recrystallized from water without change in melting point. It was then fractionated into two portions without change in melting point. The mother liquor from the second fraction yielded crystals which gave the same melting point.

The two fractions were analyzed after drying at 105° in a vacuum over phosphorus pentoxide. They remained unchanged in color upon drying. They were free from bases other than strychnine and from orthophosphoric acid. Nitrogen was determined by the Kjeldahl method, phosphorus by the official volumetric method, after destroying the organic matter by ignition with magnesium nitrate, and carbon and hydrogen by combustion in a current of oxygen, the material being mixed in the boat with fine copper oxide. The results follow:

Calc. for the 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, Fraction 1: C, 56.32, 56.39; H, 5.09, 5.22; N, 5.44; P, 7.89.

Found, Fraction 2: C, 56.18; H, 5.20; N, 5.30; P, 7.45.

The products correspond in composition to the octastrychnine salt of the acid $C_{12}H_{41}O_{42}P_9$, the formula for the acid of wheat bran previously proposed by the writer. They also correspond equally as well in composition to tetrastrychnine inosite pentaphosphate. No other salt of any simple ester of inosite and phosphoric acid has a similar composition. The facts that the melting point of the material remained unchanged upon recrystallization, that different fractions have the same composition, is considered sufficient evidence of the purity of the salt. The writer has shown in a previous publication[1] that under the conditions of the method of preparation there is no formation of a strychnine salt of the inosite

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[1] This Journal, **39**, 777 (1917).

phosphoric acid which has a similar composition to the salts of lower or higher esters of phosphoric acid and inosite.

Earlier work of the writer has shown that the analysis of the acid in a free state gives misleading results. This is also the present opinion of other workers in this field. Boutwell[1] has shown that the inosite phosphoric acid of wheat bran decomposes on mere drying *in vacuo* over phosphorus pentoxide at room temperature. For these reasons the silver salt was prepared and analyzed instead of the free acid.

Three g. of the purified strychnine salt were dissolved in water with the aid of heat, the strychnine precipitated with a slight excess of ammonia, the mixture cooled rapidly and the strychnine filtered off. The filtrate was washed with ether and boiled until the slight excess of ammonia was expelled. Three g. of silver nitrate in solution were added to the hot solution of the ammonium salt and the resulting silver salt was filtered off and washed with water. It was dried *in vacuo* over phosphorus pentoxide at 100°. Whenever protected from light there was only a slight change in color. The analytical 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 nonasilver inosite pentaphosphate, $C_6H_6(OH)H(PO_4)_5Ag_9$: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.

Found, Fraction 1: C, 4.94; H, 0.97; P, 9.77; Ag, 61.77.

Found, Fraction 2: C, 4.89; H, 1.05; P, 9.95; Ag, 62.04.

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

Composition of free acid calc. from composition of Ag salt:

Fraction 1: C, 12.80; P, 26.26.

Fraction 2: C, 12.69; P, 25.82.

The free acid was prepared from the strychnine salt by means of the barium salt, hydrolyzed and the inosite separated in the usual manner. It melted at 215–16° (uncorr.) and gave the reaction of Scherrer.

The silver salt is amorphous and may consist of a mixture of silver salts of the acid in question, and for this reason variation in analyses of different fractions may be expected. Since the strychnine salt from which the silver salt was made has been shown to be pure all of the carbon and phosphorus in the silver salt must be attributed to the acid. The composition of the acid then can be calculated from analyses of the silver salt regardless of the fact that the silver salt may be a mixture of silver salts of the same acid. The error of analysis would of course be multiplied.

The silver salt agrees in composition to nonasilver inosite pentaphosphate and equally as well to a silver salt of the acid of the formula previously proposed by the writer. The percentage of carbon in the free acid as calculated from these analyses agrees with that calculated for inosite pentaphosphoric acid, and the phosphorus agrees likewise, but not so closely.

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[1] *Loc. cit.*

On account of the fact that the large amount of material obtained in the above work on wheat bran added considerably to the time required for purification and fractionation, and that a considerable amount of strychnine orthophosphate was obtained, probably as a product of hydrolysis, the work was repeated using smaller amounts of wheat bran.

1.5 kg. of wheat bran were digested for three hours with 16 liters of 0.2% hydrochloric acid and the process of separation and purification conducted as before. 32 g. of the crude syrupy acid were obtained and from this 35 g. of the purified strychnine salt melting at 220–2°. 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; 7 H_2O, 6.57.

Found, Fraction 1: C, 55.87; H, 5.78; N, 5.48; P, 8.84; H_2O, 6.45.

Found, Fraction 2: C, 55.87; H, 5.70; N, 5.44; P, 8.62; H_2O, 6.25.

The salt corresponds in composition to that previously prepared.

The silver salt was prepared and analyzed as before. The results follow:

Calc. for nonasilver inosite pentaphosphate: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.

Found, Fraction 1: C, 4.49; H, 1.17; P, 10.68; Ag, 61.67.

Found, Fraction 2: C, 4.88; P, 10.04; Ag, 62.47.

Calc. for inosite pentaphosphoric acid: C, 12.41; H, 26.72.

Composition of free acid as calc. from mean analyses of Ag salt: C, 12.21; P, 26.80.

The results are in accord with those discussed above.

The strength of the acid (1.0%) used in extracting the material from wheat bran by the process of Anderson is greater than that employed in the above work. This might cause a decrease in the enzyme action and affect the composition of the product obtained. While the influence of enzymes has been found to be small under the conditions of the method used by the present writer, it was nevertheless considered desirable to analyze the material prepared with the use of the stronger acid.

1.5 kg. of wheat bran were digested for 3 hours with 16 liters of 1.0% hydrochloric acid and the process of separation and purification conducted as already described. 34 g. of the crude syrupy acid were obtained, and from this 34 g. of the purified strychnine salt melting at 220–2°. Analysis was conducted as before.

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 7 H_2O, 6.57.

Found: C, 56.18; H, 5.73; N, 5.49; P, 8.81; H_2O, 6.50.

The silver salt was prepared and analyzed as before.

Calc. for octasilver inosite pentaphosphate: C, 5.02; H, 0.63; P, 10.80; Ag, 60.13.

Found: C, 4.90; H, 1.11; P, 10.47; Ag, 60.31.

Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.

Composition of free acid calc. from analyses of silver salt: C, 12.17; P, 26.02.

The substances correspond in composition to those prepared with the 0.2% hydrochloric acid.

Separation of the Inosite Phosphoric Acid by the Method of Anderson.—The method by means of which Anderson[1] separated a product from wheat bran which he believed to be a salt of inosite hexaphosphoric acid, $C_6H_6(H_2PO_4)_6$, differs from that employed by the present writer. It is therefore of interest to determine whether this process gives the same results on the sample under examination as that of the present writer. The procedure was carried out as closely as possible as directed by Anderson. The barium salt thus prepared was analyzed after drying to constant weight at 105° in a vacuum over phosphorus pentoxide. Carbon was determined by mixing the sample with a fused and powdered mixture of lead chromate and potassium dichromate and burning in a current of oxygen. The results follow:

Found: C, 7.62; H, 1.96; P, 16.84; Ba, 37.56.

Composition of free acid as calc. from above analyses: C, 11.53; P, 26.73.

Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.

Calc. for inosite hexaphosphoric acid: C, 10.90; P, 28.18.

The phosphorus content corresponds to that of inosite pentaphosphoric acid, and the carbon content lies between that of inosite pentaphosphoric acid and that of inosite hexaphosphoric acid.

Since the barium salt is burned with great difficulty, the silver salt of the acid was prepared from the barium salt by decomposing the latter with sulfuric acid, precipitating the free acid with copper acetate, decomposing the copper salt with hydrogen sulfide, and preparing the silver salt from the ammonium salt as already described. Analysis was made as before. The results follow:

Calc. for octasilver inosite pentaphosphate: C, 5.02; H, 0.63; P, 10.80; Ag, 60.13.
Found: C, 5.03; H, 1.39; P, 10.66; Ag, 60.00.

Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.
Composition free acid calc. from analyses of silver salt: C, 12.37; P, 26.28.

The composition of the silver salt corresponds to that of a salt of inosite pentaphosphoric acid. It does not correspond to that of a salt of inosite hexaphosphoric acid. The composition of the free acid as calculated from analyses of the silver salt corresponds in carbon and phosphorus quite closely to that of inosite pentaphosphoric acid.

On the basis of the analyses reported on the preceding pages, the writer concludes that the inosite phosphoric acid separated from the sample of wheat bran by the several methods corresponds in composition to an inosite phosphoric acid of the formula $C_6H_6(OH)(H_2PO_4)_5$ inosite pentaphosphoric acid. The silver and strychnine salts correspond equally as well in composition to salts of the acid of the formula $C_{12}H_{41}O_{42}P_9$, the formula pre-

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[1] N. Y. (Geneva) Expt. Sta., *Tech. Bull.* 40.

viously proposed by the writer for the acid in wheat bran, but for theoretical reasons the first formula is to be preferred.

While this work confirms earlier work of the writer on the subject it is not claimed that the wheat bran examined does not contain other inosite phosphoric acids. The amounts of other acids present in the acid extract must, however, be small. Further evidence to this effect will be given further on.

The Occurrence of Inosite Pentaphosphoric Acid in Corn.—The crude acid was prepared as already described. From 10 kg. of corn meal and 40 liters of 0.2% hydrochloric acid 23 g. of the crude syrupy acid were obtained. Twenty-eight grams of the strychnine salt melting at 220–2° were obtained. This product was similar in crystal form and melting point to those previously prepared.

The melting point of the product remained unchanged on recrystallization and fractionation. The fractions were free from bases other than strychnine and from orthophosphoric acid. Analysis was made as described above. The results follow:

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

Found, Fraction 1: C, 56.37; H, 5.35; N, 5.32; P, 8.63.
Found, Fraction 2: C, 55.95; H, 6.16; N, 5.30; P, 8.74.

The salt corresponds in composition to that calculated for tetrastrychnine inosite pentaphosphate.

The silver salt was prepared and analyzed as before:

Calc. for nonasilver inosite pentaphosphate: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.
Found, Fraction 1: C, 4.91; H, 0.94; P, 10.26; Ag, 62.15.
Found, Fraction 2: C, 4.95; H, 0.90; P, 10.04; Ag, 62.02.
Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.
Calc. for free acid, Fraction 1: C, 12.77; P, 25.69.
Calc. for free acid, Fraction 2: C, 12.83; P, 26.04.

The free acid was prepared from the strychnine salt, hydrolyzed, and the inosite separated in the usual way. It melted at 215–6° (uncorr.), and gave the reaction of Scherer.

The sample of corn examined contained an inosite phosphoric acid similar in composition to that obtained from wheat bran and cottonseed meal by the writer. The melting point of the strychnine salt is the same and it probably is the same acid.

This conclusion is not in accord with the view of Hart and Tottingham and the view of Anderson concerning the composition of the inosite phosphoric acid of corn. It is possible that samples from different localities might yield acids of different composition, and small amounts of other inosite phosphoric acids may be present.

The Occurrence of Inosite Pentaphosphoric Acid in Kafir Corn.—The separation of the crude acid and the preparation of the purified strychnine salt was conducted as described above. The fraction of the salt melting at 220–2° was obtained as for cottonseed meal, wheat bran, and corn. It constituted about 75% of the product. It was purified by recrystallization from water and fractionated as before. The melting point remained unchanged at 220–2°. The fractions melting at this temperature were free from bases other than strychnine and from orthophosphoric acid. Analysis was made as before. The results follow:

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09.
Found, Fraction 1: C, 55.96; H, 6.09; N, 5.56; P, 8.49.
Found, Fraction 2: C, 55.54; H, 5.87; N, 5.44; P, 8.59.

The silver salt was prepared and analyzed as before:

Calc. for nonasilver inosite pentaphosphate: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.
Found, Fraction 1: C, 5.04; H, 1.02; P, 10.42; Ag, 61.93.
Found, Fraction 2: C, 4.72; H, 0.72; P, 10.48; Ag, 62.06.
Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.
Composition of free acid calc. from analyses of silver salt:
Fraction 1: C, 13.04; P, 26.98.
Fraction 2: C, 12.26; P, 27.19.

The free acid was prepared from the strychnine salt by means of the barium salt, hydrolyzed and the inosite separated in the usual way. The inosite melted at 215–16° (uncorr.), and gave the reaction of Scherer. Combustion gave the following results:

Calc. for inosite, $C_6H_{12}O_6$: C, 40.00; H, 6.67. Found: C, 39.74; H, 6.30.

The composition of the strychnine salt, the silver salt, and of the free acid calculated from the composition of the silver salt agree with the formulas of inosite pentaphosphoric acid and its salts.

The principal organic phosphoric acid in kafir corn is an inosite phosphoric acid corresponding in composition to inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$.

The Occurrence of Inosite Pentaphosphoric Acid in Oats.—The separation of the crude acid and the preparation of the purified strychnine salt was conducted as before. Ten grams of the crude acid were obtained from 10 kg. of ground oats and 40 liters of 0.2% hydrochloric acid. The physical condition of the material made complete extraction of the acid difficult. A fraction of the strychnine salt melting at 220–2° (uncorr.) was obtained as with the plant products previously examined. It was purified by recrystallization without change in melting point. The amount of the purified material was too small for satisfactory fractionation. The product was nearly free from inorganic bases and from orthophosphoric acid. Analysis was conducted as before.

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 8 H_2O, 7.51.
Found: C, 56.18; H, 5.98; N, 5.64; P, 7.45; H_2O, 7.45.

The silver salt was prepared and analyzed as before:

Calc. for nonasilver inosite pentaphosphate: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.
Found: C, 4.84; H, 1.02; P, 10.21; Ag, 61.56.
Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.
Composition of free acid calc. from silver salt: C, 12.41; P, 26.15.

The ammonium salt of the acid was prepared from the strychnine salt and hydrolyzed in a sealed tube with sulfuric acid in the usual manner. Inosite was separated as usual and recrystallized from alcohol and ether. The product had the characteristic appearance of inosite, melted at 216–18° (uncorr.) and gave the reaction of Scherer. Since the principal organic phosphoric acid of oats has already been shown to be an inosite phosphoric acid[1] further evidence as to the identity of the inosite was considered unnecessary.

The composition of the strychnine salt, of the silver salt, and of the free acid as calculated from analysis of silver salt, agree with the formula of inosite pentaphosphoric acid and its salts, and the writer therefore concludes that the principal inosite phosphoric acid in the sample of oats examined corresponds in composition to inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$, and not to that of the so-called phytic acid, $C_2H_8P_2O_9$, as obtained by Hart and Tottingham, nor to that of inosite hexaphosphoric acid, $C_6H_6(H_2PO_4)_6$, as obtained by Anderson, from oats.

The Occurrence of Inosite Pentaphosphoric Acid in Wheat Shorts.—The crude acid was separated in the manner already described and the strychnine salt prepared and purified as before. 16 g. of the crude acid were obtained from 1.5 kg. of material. The fraction of the strychnine salt melting at 220–2° (uncorr.) was obtained as before. As usual it constituted the principal fraction of the crude salts. The melting point remained unchanged on recrystallization. The material was free from bases other than strychnine and from orthophosphoric acid. Analysis was conducted as before:

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 7 H_2O, 6.57.
Found, Fraction 1: C, 56.00; H, 5.66; N, 5.64; P, 8.27; H_2O, 7.00.
Found, Fraction 2: C, 56.01; H, 5.83; N, 5.48; P, 8.78; H_2O, 6.00.

The silver salt was prepared and analyzed as before:

Calc. for nonasilver inosite pentaphosphate: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.
Found, Fraction 1: C, 4.73; H, 1.06; P, 10.22; Ag, 61.76.
Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.
Composition of free acid calc. from silver salt: C, 12.19; P, 26.34.

The ammonium salt of the acid was hydrolyzed in the usual manner and inosite separated and recrystallized. It melted at 216–218° (uncorr.) and gave the reaction of Scherer. Analysis gave the following results:

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[1] Hart and Tottingham, Wis. Expt. Sta., *Res. Bull.* 9; Anderson, *J. Biol. Chem.*, 17, 151.

Calc. for inosite, $C_6H_{12}O_6$: C, 40.00; H, 6.67. Found: C, 40.36; H, 6.49.

The composition of the strychnine salt, of the silver salt, and of the free acid as calculated from analysis of silver salt agree with the formula of inosite pentaphosphoric acid and its salts.

The principal organic phosphoric acid in the sample of wheat shorts examined is an inosite phosphoric acid corresponding in composition to inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$.

The Occurrence of Inosite Pentaphosphoric Acid in Rice Bran.—The acid was separated and the strychnine salt prepared and fractionated as already described. A fraction melting at 220–2° (uncorr.) was obtained as before. 36 g. of the crude acid were obtained from 1250 g. of rice bran. The purified strychnine salt melting at 220–2° constituted about 75% of the crude salts. The product was free from bases other than strychnine and from orthophosphoric acid. It was dried and analyzed in the usual manner.

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 7 H_2O, 6.57.

Found, Fraction 1: C, 55.67; H, 5.75; N, 5.33; P, 8.64; H_2O, 6.26.

Found, Fraction 2: C, 55.93; H, 6.01; N, 5.69; P, 8.81; H_2O, 6.40.

The silver salt was prepared and analyzed with the following results:

Calc. for nonasilver inosite pentaphosphate: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.

Found, Fraction 1: C, 4.78; H, 0.63; P, 10.24; Ag, 62.19.

Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.

Composition free acid calc. from silver salt: C, 12.45; P, 26.67.

Inosite was separated from the ammonium salt by hydrolysis and purification in the usual manner. It melted at 216–18° (uncorr.), gave the reaction of Scherer and had the crystal form and sweetish taste of inosite. Since the principal organic phosphoric acid of rice bran has already been shown to be an inosite phosphoric acid[1] analysis of the inosite was considered unnecessary.

The composition of the strychnine salt, of the silver salt, and of the free acid as calculated from analysis of silver salt, agree with the formula of inosite pentaphosphoric acid and its salts. These results differ from those obtained by Alice R. Thompson[2] who used Anderson's method of separation. The carbon content of the acid as calculated from her results by the writer would be 10.54 as against 12.45 reported here and the phosphorus content 25.58 as against 26.67 found by the writer.

The writer concludes that the principal inosite phosphoric acid in the sample of rice bran examined corresponds in composition to inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$.

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[1] Alice R. Thompson, *J. Agr. Res.*, **3**, 425 (1915); Suzuki, *et al.*, *Bull. Coll. Agr. Tokyo*, **7**, 495–502, 503–512.

[2] *Loc. cit.*

The Occurrence of Inosite Pentaphosphoric Acid in Rice Polish.—Alice R. Thompson[1] has shown that unpolished rice contains an inosite phosphoric acid and that polished rice contains practically none of this compound. The presence of inosite phosphoric acid in rice polish is therefore rendered certain.

The acid was separated and the strychnine salt prepared and fractionated as already described. 44 g. of the free acid were obtained from 1250 g. of the rice polish, and the strychnine salt melting at 220–2° which was obtained as before constituted about 75% of the crude salts. The substance was free from bases other than strychnine and from orthophosphoric acid. It was dried and analyzed as before:

Calc. for tetrastrychnine inosite pentaphosphate: C, 56.33; H, 5.48; N, 5.84; P, 8.09; 7 H_2O, 6.57.

Found, Fraction 1: C, 56.29; H, 6.01; N, 5.52; P, 8.54; H_2O, 6.50.

Found, Fraction 2: C, 55.56; H, 6.09; N, 5.44; P, 8.78; H_2O, 5.83.

The silver salt was prepared and analyzed as before:

Calc. for nonasilver inosite pentaphosphate: C, 4.67; H, 0.52; P, 10.05; Ag, 62.96.

Found, Fraction 1: C, 4.86; H, 1.01; P, 10.30; Ag, 61.88.

Calc. for inosite pentaphosphoric acid: C, 12.41; P, 26.72.

Composition free acid calc. from silver salt: C, 12.55; P, 26.62.

The ammonium salt of the acid, prepared from the strychnine salt, was hydrolyzed and the inosite separated and purified in the usual way. It melted at 216–18° (uncorr.) and gave the reaction of Scherer. Analysis gave the following results:

Calc. for inosite, $C_6H_{12}O$: C, 40.00; H, 6.67. Found: C, 40.11; H, 6.47.

The composition of the strychnine salt, of the silver salt, and of the free acid as calculated from analyses of the silver salt, agree with the formula of inosite phosphoric acid and its salts. The writer concludes, therefore, that the principal inosite phosphoric acid in the sample of rice polish examined corresponds in composition to inosite pentaphosphoric acid, $C_6H_6(OH)(H_2PO_4)_5$.

Concerning the Presence of Phosphorus Compounds other than Inosite Pentaphosphoric Acid in the Samples.—While the crystalline product described above constituted the principal fraction of the several products, the fact that there was in every case an uncrystallizable residue rendered a quantitative knowledge of the amount of inosite pentaphosphoric acid desirable. A method has been devised[2] which determines the compound the writer believes to be inosite pentaphosphoric acid without being affected by other compounds in the plant extract. Results on the samples of materials under examination are given below:

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[1] *Loc. cit.*

[2] Rather, THIS JOURNAL, 39, 2506 (1917).

Material.	Phosphorus in % of total acid-soluble phosphorus. (A).	(B).	(C).
Corn	75	18	7
Cottonseed meal	95	9	0
Wheat shorts	84	8	8
Kafir corn	74	13	13
Wheat bran	89	3	8
Rice bran	87	19	0
Rice polish	93	10	0
Oats	81	8	11

(A) Phosphorus in the form of inosite pentaphosphoric acid.
(B) Inorganic phosphorus (Forbes method).
(C) Phosphorus in other forms, by difference.

From 75 to 95% of the acid-soluble phosphorus is shown by the above figures to consist of the compound which has been found to correspond in composition to inosite pentaphosphoric acid. Of the amount remaining, from 3 to 18% consists of inorganic phosphorus, leaving from 0 to 13% to be accounted for in other forms of phosphorus. Repeated attempts have been made to obtain a definite compound from the noncrystalline material obtained in the preparation of the purified strychnine salt of the inosite pentaphosphoric acid. In a few cases small amounts of a substance corresponding in composition to strychnine orthophosphate and melting at the temperature of distrychnine orthophosphate were obtained. Analysis of silver salts prepared from these indicated that there was practically no carbon in the free acid and the silver salts had the characteristic canary yellow color of silver orthophosphate. In another case small amounts of a crystalline strychnine salt free from phosphorus were obtained.

The method of preparation of the crude inosite phosphoric acid was designed to include all of the phosphorus compounds soluble in the extracting medium, the separation and purification being made by means of the strychnine salt. It is evident that the noncrystalline material separated from the strychnine inosite pentaphosphate would consist of a mixture of all of those acids, whether phosphorus containing or not, which were extracted by the solvent and whose copper salts are difficultly soluble in water. On account of the very large bulk of the copper precipitate, quantitative removal of soluble matter is impracticable, the copper salts, even when compressed by suction on a Buchner funnel, frequently having a bulk of 500 cc. The mother liquors from the strychnine fractionation would then be expected to contain small amounts of hydrochloric acid and acetic acid (both introduced in the process), all of the orthophosphoric acid, and various unknown plant acids, all in the form of strychnine salts. This does not interfere with the separation and purification of the principal compound in the mixture, but it renders the identification of any constituent present in very small amount quite difficult. For that reason the writer is not prepared to state that inosite monophosphate, inosite triphosphate, or other inosite phosphoric acids are not present in the samples in small amounts, but it is evident that the figures given above that they cannot be present in amounts much exceeding ten percent of the total acid-soluble phosphorus.

Acknowledgment.—The writer is indebted to Mr. R. H. Ridgell for the preparation of some of the crude acids and for making some nitrogen and phosphorus determinations.

Summary.

1. An inosite phosphoric acid has been separated from wheat bran by means of its crystalline strychnine salt and its composition ascertained by analysis of strychnine and silver salts. An acid corresponding in composition to the formula $C_{12}H_{41}O_{42}P_9$ is indicated by these results, which formula is the one previously proposed by the writer for this material. The formula $C_6H_6(OH)(H_2PO_4)_5$, inosite pentaphosphoric acid, agrees equally as well with the results, and since it is numerically almost one-half of the first formula and is that of a theoretically possible compound, it is adopted as the more desirable.

2. Increasing the strength of the extracting medium from 0.2% to 1.0% hydrochloric acid in order to inhibit all enzyme action on the compound gave results in accord with the above.

3. The use of the method of preparation on the basis of which it has been stated that inosite hexaphosphoric acid was separated from wheat bran gave results concordant with the above.

4. The principal inosite phosphoric acid of samples of corn, rice bran and oats was found to correspond in composition to inosite pentaphosphoric acid, and neither to inosite hexaphosphoric acid nor to phytic acid, $C_2H_8P_2O_9$.

5. The principal phosphoric acid of samples of kafir corn, wheat shorts, and rice polish, was found to be organic and to be an inosite phosphoric acid corresponding in composition to inosite pentaphosphoric acid.

6. The possibility of the presence of other inosite phosphoric acids in the samples examined is not excluded by this work, but it is shown that the compound corresponding in composition to inosite pentaphosphoric acid together with the orthophosphoric acid constitute on, and average 95% of the acid-soluble phosphorus.

Fayetteville, Ark.