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<https://doi.org/10.18297/etd/1021>

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THE ORGANIC PHOSPHORUS COMPOUNDS
OF WHEAT BRAN.

by

J. Howard Mueller.

1914.

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Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Chemistry, in
the Arts Department of the University of Louisville.

The Organic Phosphorus Compounds of Wheat Bran.

A growing interest has manifested itself within the past two or three years in "phytin" and "phytic acid", and in some of the compounds of the latter. Posternak¹ first isolated this substance from the seeds of the red fir, pumpkin, pea, bean, white and yellow lupine and potato. Patten and Hart² obtained the substance from wheat bran, and Hart and Tottingham³ found it in corn meal, oats and barley. The organic phosphorus materials obtained by alcoholic precipitations of aqueous or dilute acid extracts from these various sources are not identical, but ultimate analyses show a fair degree of similarity. Thus the phosphorus varies between 14 and 17 per cent, and they all contain varying amounts of magnesium, potassium and calcium. There has been prepared, also, from the phytin from most of these sources, the free "phytic acid", corresponding to the formula $C_2H_8P_2O_9$, (anhydro-oxy-methylene-phosphoric acid, Posternak⁴), or $C_6H_{24}P_8O_{27}$, (Neuberg⁵, Starkenstein⁶).

In the case of the material extracted from wheat bran, however, there has been some dispute as to its identity with phytin, and its ability to yield phytic acid. Patten and Hart⁷ claim to have obtained an acid containing 10.63% carbon, 3.38% hydrogen, and 25.98% phosphorus, figures agreeing very well with the formula $C_6H_{24}O_{27}P_8$, and therefore called by

¹ Rev. gen. de bot., xii, p. 5 and 65, 1900.

² Compt. rend. de l'acad. des sci., Nos. 3, 5, and 8, exxxvii, 1903.

³ Jour. Biol. Chem., vi, No. 5, p. 564, 1904.

⁴ Loc. cit.

⁵ Biochem. Zeitschr. lx, pp. 551 and 557.

⁶ Biochem. Zeitschr. xxx, p. 56.

⁷ Loc. cit.

them "phytic acid". Anderson¹, on the other hand, is unable to obtain such a compound, and ascribes Patten and Hart's supposed error to contamination with inorganic phosphates and phosphoric acid.

It was with a view to clearing up this matter that the work represented in this paper was undertaken. Wheat bran contains a much larger percentage of organic phosphours extractives than most other materials so far examined, and so should be the best source for phytin and phytic acid for further investigations if they prove to be really present, and at first view, it seems highly improbable that they should not be present, when they are found invariably in all other similar materials examined. Moreover, Anderson obtained his material by a method of procedure different from that used by Patten and Hart, and this suggested itself as a possible explanation of the different results obtained.

We have, therefore, repeated Patten and Hart's work. Their so-called tri-barium phytate has been prepared from wheat bran, taking care to insure the absence of inorganic phosphates by means of the method recommended by Anderson, viz., repeated solution of the salt in dilute hydrochloric acid and reprecipitation with alcohol. We have been able also to prepare by Anderson's² recent method, this barium salt, in crystalline form, identical in properties with that obtained by him from cotton-seed meal, oats and corn, but corresponding more closely in its analysis with the formula $C_6H_{18}O_{24}P_6Ba_3$, than with Anderson's formula, $C_6H_{12}O_{24}P_6Ba_3$. Our data leave

¹ Jour. Biol. Chem., xii, p. 447.

² Jour. Biol. Chem., xvii, No. 2, p. 160. 1914.

no question as to the presence of substances in the bran which yield, by the usual treatment to be described under the experimental part, a substance very similar to phytic acid, but apparently having the composition represented by the formula $C_6H_{24}O_{24}P_6$. From his crystalline tri-barium salt, Anderson obtained an acid to which he ascribed the formula $C_6H_{18}O_{24}P_6$. Hence both in the case of the barium salt, and the free acid, our compounds appear to contain six more hydrogen atoms to the molecule, while in carbon, barium and phosphorus, they agree very well with Anderson's compounds. In explanation of this discrepancy, the method used in combustion must be taken into consideration. It is a well known fact, that in the combustion of organic compounds containing phosphorus, the phosphorus is converted into metaphosphoric acid, HPO_3 , which remains as a glossy coating in the boat, and may occlude more or less carbon. Anderson¹ states that in burning his crystalline barium salts, it was necessary to burn a second time with chromic acid, in order to insure complete combustion of carbon, but that this was unnecessary with the amorphous barium salts. Since he does not mention burning the free acid with chromic acid, we presume that he did not do so. It is inevitable, if this is true, that his hydrogen analyses should give low results with the phytic acid. It is a noteworthy fact that it is just six atoms lower in the molecule than ours, and since the molecule contains six atoms of phosphorus, the formation of the metaphosphoric acid residue would account for it. In the case of the barium salt, the explanation is less evident,

¹ Jour. Biol. Chem. xvii, No. 2, p. 160. 1914.

for there would, of course, be barium phosphate or metaphosphate formed, together with some barium carbonate and metaphosphoric acid, although a reaction between the latter two substances might take place, liberating both the hydrogen and carbon. In our combustions, we burned the material always a second time, in the case of the free acid and the brucine salt to be described, with well dried, powdered lead chromate, and in the case of the barium salts with a mixture of lead chromate and potassium dichromate. There was always an increase in weight in both the potash bulbs and calcium chloride tube following the second burning. It is therefore probable that our compounds from wheat bran are identical with those obtained by Anderson from various other sources.

We believe, however, that in addition to the phytic acid derivative present in the extract of wheat bran, there are at least two other organic phosphorus compounds which we have been prevented from investigating completely by lack of time. It was one of these substances which Anderson¹ investigated, and found to contain an acid to which he ascribed the formula $C_{20}H_{55}O_{49}P_9$, combined with the elements of a pentose. In regard to this substance, we wish to point out that his analytical results show rather wide discrepancies from the calculated formula, and that none of the barium salts were obtained crystalline, and hence may not have been pure. It is also noteworthy that the crystalline brucine salt to which he ascribes the formula $C_{20}H_{55}O_{49}P_9 \cdot (C_{23}H_{26}O_4N_2)_{10}$, gives an analysis according better, except in the case of carbon, which is low, with brucine phosphate, $(C_{23}H_{26}O_4N_2)_3(H_3PO_4)_2$.

¹ Jour. Biol. Chem. xii., p. 450.

than with his calculated formula. Anderson¹ himself shows that phytic acid is broken down into phosphoric acid and other substances by drying at 100°, and even to some extent by drying at ordinary temperatures. The new acid prepared by him from wheat-bran was found to yield inosite and phosphoric acid on hydrolysis with acid, and hence probably also yielded it on drying. At any rate, we have been unable to obtain a crystalline salt of prucine by using a preparation which had not first been heated, while after drying about a gram of the acid at 100° for several hours, we obtained a good yield of crystals, corresponding in physical properties and approximating in analysis, with Anderson's brucine salt, and also with pure brucine phosphate, prepared and analyzed by us.

The material of the experimental part naturally falls into three parts. In the first part is taken up the work done on a precipitate obtained by adding copper acetate solution to an extract of wheat bran; the second part deals with the material resulting from an alcoholic precipitation of the bran extract, while the third part describes a combination of the two methods.

Experimental Part.

I

Preparation of the Impure Barium Salt.

Five kilos of wheat bran were extracted over night in 30 liters of 0.2% hydrochloric acid, the liquid then strained and pressed out of the residue, and 16 more liters of the 0.2% acid added. After stirring at intervals for two hours,

¹ Jour. Biol. Chem., xvii, p. 171. 1914.

this was strained out, and the two extracts united. After standing for sometime to allow suspended matter to settle out, the supernatant liquid was strained through cotton. To the filtrate was added an excess of a concentrated solution of copper acetate, containing some acetic acid. A heavy precipitate was produced. This was allowed to settle over night, and the precipitate was filtered on a Buchner funnel and washed two or three times with water. It was then suspended in water, and hydrogen sulphide run in for several hours, the mixture being stirred constantly by means of a water motor. The mixture was then filtered from the precipitated copper sulphide. To the filtrate was added a solution of 100 grams of barium chloride, and then barium hydroxide solution to strong alkaline reaction. A heavy precipitate was obtained. This was filtered out, dissolved in dilute hydrochloric acid and filtered from a slight insoluble residue. To the filtrate was again added some barium chloride solution and barium hydroxide to alkaline reaction. After filtering and dissolving the precipitate in dilute hydrochloric acid, it was precipitated by three volumes of alcohol, and the precipitate, after being redissolved in weak hydrochloric acid was again precipitated with alcohol, and the process repeated three times more. The material was now free from inorganic phosphates, i.e., it gave no yellow precipitate when warmed with molybdate solution. After washing in alcohol and ether and drying, the product weighed 57. grams.

Dried for analysis at 130°, this material turned slightly gray.

0.2080 g. gave 0.1235 g. $Mg_2P_2O_7$.

0.4736 g. gave 0.2853 g. $BaSO_4$.

1.0058 g. gave 0.00098 g. N by Kjeldahl method.

Found:- P, 16.55%; Ba, 35.54%; N, 0.098%.

Calculated for tri-barium inosite-hexaphosphate, $C_6H_{12}O_{24}P_6Ba_3$:-
P, 17.44%; Ba, 38.65%.

This material approaches the constitution of a phytin derivative more closely than the material prepared by Anderson, but it is too low in both phosphorus and barium. Believing it to be a mixture of tri-barium phytate and some other substance, a means of effecting a separation was sought.

Separation by Dialysis.

One gram of the material, dried at 100°, was dissolved in dilute hydrochloric acid, and placed in an S & S No. 579 dialysing capsule, the latter being placed in a beaker of distilled water. After forty-eight hours the dialysate and the material remaining in the capsule were precipitated by three volumes of alcohol. After filtering both precipitates, and washing in alcohol and ether and drying, it was found that the undialysable material weighed 0.2083 grams, while the material from the dialysate weighed 0.3820 grams. Although the precipitation was evidently incomplete, it was plain that some, at least, of the substance was capable of dialysis. Barium was determined in both fractions.

0.2083 g. gave 0.1213 g. $BaSO_4$. (Undialyzed fraction)

0.3820 g. gave 0.2408 g. $BaSO_4$. (Dialysate)

Found:- in the dialysate, 37.09% Ba; in the non-dialysable fraction, 34.27% Ba.

From this it is evident that there are at least two substances in the crude barium salt obtained from the wheat bran, and that the one having the higher percentage of barium

is dialyzable. Since no attempt was made to effect complete separation, by changing the water in the outer container, the undialyzed material was of course contaminated by some of the dialysate, so that the true barium percentage should be slightly lower.

Separation by Extraction with Water.

This method was suggested and used by Anderson¹ in purifying the phytin derivative from oats.

Ten grams of the crude barium preparation were rubbed up in a mortar with about 50 c.c. of water, and after standing for a time, filtered. The residue was thus extracted twice more with small quantities of water, and finally washed with water, alcohol and ether, and dried. The aqueous extract was slightly yellow. Addition of alcohol produced in the first two portions of extract, a rather abundant precipitate, but in the third portion, only a faint turbidity, showing that the extraction was fairly complete. The precipitate, after filtering out and drying, weighed 1.1 grams. The water insoluble material was pure white, while the water soluble fraction was slightly yellow.

Water-insoluble Fraction.

Dried for analysis at 100°.

0.4972 g. gave 0.3115 g. BaSO_4 .

0.3210 g. gave 0.0650 g. CO_2 and 0.0516 g. H_2O .

0.2946 g. gave 0.1676 g. $\text{Mg}_2\text{P}_2\text{O}_7$.

Found:— Ba, 36.87%; P, 15.86%; C, 5.52%; H, 1.80%.

Calculated for tri-barium inosite-hexaphosphate, $\text{C}_6\text{H}_{12}\text{O}_{24}\text{P}_6\text{Ba}_3$:—
Ba, 38.65%; P, 17.44%; C, 6.75%; H, 1.12%.

Comparison shows that the barium, phosphorus and carbon in our preparation are low, while the hydrogen is high.

¹ Jour. Biol. Chem. xvii. p. 160, 1914.

Water-soluble Fraction.

Dried for analysis at 100° .

0.4448 g. gave 0.2560 g. BaSO_4 .

0.2300 g. gave 0.0630 g. CO_2 and 0.0410 g. H_2O .

Found:- Ba, 33.87%; C, 7.47%; H, 2.00%.

This substance has not been investigated further.

Preparation of the Crystallized Barium Salt.

Five grams of the water insoluble fraction were dissolved in the smallest possible quantity of dilute hydrochloric acid, a solution of pure barium hydroxide was added to nearly neutralize the free acid, together with a strong solution of ten grams of barium chloride. The mixture was filtered, and alcohol added until a slight permanent precipitate resulted. This precipitate was amorphous. After standing over night it had become crystalline, the crystals being in the form of microscopic globules, similar to those described by Anderson from cotton-seed meal, oats and corn. After filtering out, a second, third and fourth crop were obtained by adding more alcohol and allowing to stand. These crystals were united, and recrystallized by the same procedure. About two grams of pure white crystals were thus obtained. (See photograph).

The crystals were dried for analysis at 105° , in vacuum over phosphorus pentoxide.

0.2124 g. gave 0.1274 g. $Mg_2P_2O_7$.

0.2053 g. gave 0.1347 g. $BaSO_4$.

0.3099 g. gave 0.0708 g. CO_2 and 0.392 g. H_2O .

Found:— P, 16.72%; Ba, 38.61%; C, 6.23%; H, 1.42%.

Calculated for tri-barium inosite-hexaphosphate, $C_6H_{12}O_{24}P_6Ba_3$:—

P, 17.44%; Ba, 38.65%; C, 6.75%; H, 1.12%.

Calculated for $C_6H_{18}O_{24}P_6Ba_3$:—

P, 17.35%; Ba, 38.43%; C, 6.72%; H, 1.69%

There is little choice between the two calculated formulae here. While the analysis of this material corresponds to a tri-barium salt, the crystalline substance obtained by Anderson by the same method gave an analysis corresponding to the hepta-barium salt, (R_2Ba_7) . The explanation is that our solution probably contained more free acid than his. When portions of this salt which had been dried in vacuum over sulphuric acid at room temperature, or in a water oven at 100° were further dried at 105° in vacuum over phosphorus pentoxide, a slight loss in weight resulted. This was, however, variable, and the crystal form of the salt was not injured by it, so that it hardly seems probable that there is water of crystallization present, hence the percentages of moisture lost by this drying are not quoted.

Crystallization of the Barium Salt from Dilute Acid Solution.

All of the remaining impure barium salt was extracted with water as described above, and the insoluble portion, weighing about 20 grams, purified as follows. It was dissolved in 0.2% hydrochloric acid, and after filtering from a slight insoluble residue, alcohol was added until a fairly heavy precipitate resulted. This required considerably less than one volume of alcohol. The precipitate was amorphous, but after standing over night, it became crystalline, similar in

appearance to that already described. It was filtered out, washed with alcohol and ether and dried. After securing a second and third crop of crystals, all were united and recrystallized in the same way. After drying in the water-oven for some time at 100° , the product, weighing about seven grams, was a light, powdery material. A part of this was dried at 105° in vacuum over phosphorus pentoxide and analyzed.

0.1400 g. gave 0.0860 g. $Mg_2P_2O_7$.

0.1596 g. gave 0.1001 g. $BaSO_4$.

0.2275 g. gave 0.0575 g. CO_2 and 0.0399 g. H_2O .

Found:— P, 17.12%; Ba, 37.77%; C, 6.89%; H, 1.96%.

Calculated for $C_6H_8O_{24}P_6Ba_3$,
P, 17.35%; Ba, 38.43%; C, 6.72%; H, 1.69%.

Preparation of the Free Acid from the Crystallized Material.

The entire amount of the crystalline material remaining, a little less than seven grams, was decomposed as follows. Somewhat more than the calculated amount of N/1 H_2SO_4 was added to precipitate the barium, and after warming for some time the clear supernatant liquid was filtered out. To this was added an excess of copper acetate solution, and the precipitate filtered out and washed thoroughly with water. It was finally suspended in water, and decomposed by running in hydrogen sulphide. After filtering from the copper sulphide, the liquid containing phytic acid was concentrated to a small bulk by boiling in vacuum, the temperature not rising above 65° , and this was finally dried for ten days in vacuum over sulphuric acid at room temperature. The residue, weighing about three grams, was a very thick, amber colored syrup. For analysis a portion of it was dried at 105° in vacuum over phosphorus pentoxide.

0.1208 g. gave 0.1199 g. $Mg_2P_2O_7$.
 0.2893 g. gave 0.1156 g. CO_2 and 0.0917 g. H_2O .
 Found:- P, 27.67%; C, 10.90%; H, 3.54%.
 Calculated for $C_6H_{18}O_{24}P_8$:-
 P, 27.92%; C, 10.81%; H, 3.63%.
 Calculated for $C_6H_{18}O_{24}P_8$:-
 P, 28.18%; C, 10.90%; H, 2.73%.

The drying at 105° causes a blackening, and presumably a partial decomposition of the material. Anderson¹ shows that it causes the formation of phosphoric acid and we have confirmed his observations.

0.1919 g. unheated acid gave 0.0070 g. $Mg_2P_2O_7$.
 0.2360 g. acid heated to 105° gave 0.0381 g. $Mg_2P_2O_7$.
 0.2570 g. unheated acid gave on heating at 105° , 0.0210 g. H_2O .

The unheated acid therefore contains 8.17% water.

Stating the results on the dry basis, before heating, 4.00% of the total phosphorus was present as phosphoric acid, a part of which may have been produced by the nitric acid of the molybdate solution. After heating for two hours at 105° , 16.26% of the total phosphorus was present as phosphoric acid.

Attempt to Prepare Brucine Phytate.

Hoping that it might be possible to prepare a crystalline brucine salt of phytic acid which could be compared with the brucine salt prepared by Anderson from his more complex acid, we undertook to make it by the same method used by him, except that to begin with, we did not use the dried acid. This work was done before we effected a separation of the crude barium salt by water extraction, and the mixture of the water soluble and insoluble materials was therefore used. To ten grams of this material, the calculated amount of N/1 sulphuric acid was added to precipitate

the barium. After filtering, the filtrate was concentrated by boiling in vacuum to a small bulk. An excess of crystallized brucine was then added, and then 150 c.c. alcohol, 15 c.c. chloroform and ether until a permanent turbidity resulted. After standing for two weeks with occasional addition of ether, at a temperature most of the time below freezing, there was not a trace of crystalline deposit, although there was a small amount of gummy material on the bottom of the flask.

To three grams of the impure barium salt was added the calculated amount of N/1 sulphuric acid, and the liquid, after filtering, evaporated on the water bath, and dried in a water oven for twenty-four hours. The black residue was dissolved in a small amount of water, alcohol added and the solution filtered from a small amount of insoluble, carbonaceous matter. Chloroform and ether were then added as before, and after standing for an hour in the laboratory there began to form a deposit of fine needles. After standing in the cold for several days, these were filtered out and recrystallized by the same procedure. About 0.8 gram of crystals were obtained. These were soluble in water and alcohol, but insoluble in ether and chloroform. No sharp melting point could be obtained, the substance gradually softening and turning brown above 187°, but not being entirely melted until the temperature had reached 200°. The remaining material was dried for analysis at 100°.

0.3207 g. gave by Kjeldahl, NH_3 to neutralize 12.84 c.c. N/10 H_2SO_4 .

0.2916 g. gave 0.0684 g. $\text{Mg}_2\text{P}_2\text{O}_7$.

0.1087 g. gave 0.2224 g. CO_2 and 0.0624 g. H_2O .

Found:- C, 55.80%; H, 6.42%; P, 5.55%; N, 5.61%.

Found by Anderson from the other acid, $(\text{C}_{23}\text{H}_{25}\text{O}_4\text{P})_3$:-

C, 56.24%; H, 6.26%; P, 4.69%; N, 5.86%.

Calculated for brucine phosphate, $(\text{C}_{23}\text{H}_{25}\text{N}_2\text{O}_4)_3(\text{H}_3\text{PO}_4)_2$:-

C, 59.53%; H, 6.08%; P, 4.46%; N, 6.04%.

Obtaining thus a crystalline compound agreeing closely with that obtained by Anderson from a different acid, both in properties and analysis, we could account for it only by supposing that the acids were decomposed by heat into some other material, common to both, which could only be phosphoric acid.

Preparation of Brucine Phosphate.

An unweighed amount of syrupy phosphoric acid was diluted somewhat with water, brucine added, then alcohol, chloroform and ether. Almost immediately a deposit of fine needles appeared which increased in amount on standing over night. These were filtered out, recrystallized as before, and dried for analysis at 100° . Their physical properties were identical with the material previously prepared.

0.5658 g. gave 0.0872 g. $\text{Mg}_2\text{P}_2\text{O}_7$.

0.3400 g. gave by Kjeldahl method, NH_3 to neutralize 14.97 c.c. N/10 H_2SO_4 .

0.4198 g. gave by Kjeldahl method, NH_3 to neutralize 18.25 c.c. N/10 H_2SO_4 .

0.1550 g. gave 0.3391 g. CO_2 and 0.0891 g. H_2O .

Found:- C, 59.66%; H, 6.43%; P, 4.53%; N, 6.18% & 5.85%.

Calculated for brucine phosphate, $(\text{C}_{23}\text{H}_{25}\text{N}_2\text{O}_4)_3(\text{H}_3\text{PO}_4)_2$:-

C, 59.53%; H, 6.08%; P, 4.46%; N, 6.04%.

It seems probable therefore, that the substances obtained from the organic phosphorus acids is nothing more than im-

pure brucine phosphate, the phosphoric acid being produced by hydrolysis.

Attempt to Prepare the Ethyl Ester of Phytic Acid.

All attempts previously described to prepare an ester of phytic acid by means of the silver salt and an alkyl halide have failed because the silver salt was prepared by the action of nitric acid on the acid, by which means only an acid ester has been obtained. It would seem reasonable to suppose that by the use of silver oxide, a neutral ester might be prepared, from which molecular weight determinations could be made. Using ten grams of the impure barium salt, (containing both water soluble and insoluble materials, for at this time we had not found that it was separable into two fractions), the calculated amount of N/1 sulphuric acid was added to precipitate barium, the precipitate filtered out, and a large excess of freshly precipitated silver oxide, (about twenty-five equivalents), was added. The solution was shaken and allowed to stand over night. The precipitate was filtered out in a darkened room and washed thoroughly, first by decantation, and then on the filter, and finally suspended in absolute alcohol. This precipitate, was, of course, a mixture of silver phytate and silver oxide. To this was added about 10% more than the required amount of ethyl iodide to combine with all the silver, and the mixture was allowed to stand, protected from the light, in a warm place for about two weeks, being shaken from time to

time. At the end of this time the original gray precipitate was yellow, (silver iodide), and the reaction was probably complete. The liquid was filtered off, concentrated by boiling in vacuum, more alcohol added and reboiled several times to insure as nearly as possible the absence of ethyl iodide. The dark red residue was dried in vacuum over sulphuric acid. It was a very thick syrup, having a peculiar faint odor. On moist blue litmus paper it gave an acid reaction, due probably to hydrolysis, since it had been prepared from neutral solution. It contained, however, a considerable amount of free iodine, from decomposition of the excess ethyl iodide, and since no way of purifying it seemed applicable, and also because it was now evident that the starting material was not pure, no further effort was made to use it.

It is possible, however, that by working along this line, using perhaps ethyl chloride, instead of iodide, a pure ester of phytic acid could be obtained, by means of which molecular weights could be determined through the depression of freezing point in some organic solvent, such as nitro-benzene, or phenol, in which the ester was found to be soluble.

II

The work represented in this part of the paper is of interest chiefly by comparison with the results of part III. Wheat bran extract was treated by the method used by Ander-

son for the preparation of the compound $C_{25}H_{55}O_{59}P_9Ba_5$, and we obtained a substance giving an analysis fairly close to that obtained by him.

Preparation of the Crude Phosphorus Compound.

Ten pounds of wheat bran were extracted over night in 0.2% hydrochloric acid. To the extract, after straining through cloth, was added tannic acid in substance to precipitate the protein material. A very heavy purplish precipitate was produced. This was filtered out, and to the filtrate was added an equal volume of alcohol. A heavy white precipitate appeared at once. This was allowed to settle, the liquid siphoned off, and the precipitate filtered on a large Buchner funnel, without suction, (the method which was found to be most satisfactory in working with all these compounds). It was redissolved in dilute hydrochloric acid, the solution obtained being milky and filtered only with difficulty. In attempting to overcome this trouble, more tannic acid was added, producing a precipitate which soon became gummy, and from which a perfectly clear filtrate could be obtained. An equal volume of alcohol was added, and the resulting precipitate was purified by dissolving in dilute acid and precipitating with alcohol, repeating five times. The precipitate, instead of being light and flocculent, was rather heavy, and soon settled into a gummy mass, which could be removed by means of a glass rod from the solution. In the last precipitation, it was found that three volumes

of alcohol were necessary to throw down the substance completely, so that considerable of the material was lost.

This statement may have some bearing on the results of the third part of this paper: The gummy substance was dried in a vacuum dessicator, a white, opaque solid being produced, weighing ten grams. This could be readily powdered in a mortar. It gave a strong acid reaction to litmus paper.

The substance was dried for analysis at 100° .

0.3052 g. gave 0.1563 g. $Mg_2P_2O_7$.

0.2163 g. gave 0.1385 g. CO_2 and 0.0828 g. H_2O .

Found:- P, 14.27%; C, 17.46%; H, 4.32%.

Quantitative determinations of nitrogen were not made, but qualitative tests showed its presence in traces only. Qualitative tests for bases showed magnesium and potassium in fairly large quantities, calcium and sodium in traces.

This material reduced Fehling's solution after boiling with hydrochloric acid, but not before. The vapors from the boiling mixture colored a strip of aniline acetate paper pink, indicating the production of furfural from a pentose present. Polariscopic examination of a ten per cent solution showed optical inactivity. This solution was boiled for some time with an equal volume of concentrated hydrochloric acid, the water lost by evaporation replaced, and the solution, which had darkened considerably, was decolorized with animal charcoal. Polariscopic examination now showed what was judged to be a very slight dextro-rotation, but so slight as to be uncertain.

Preparation of the Barium Salt.

All the remaining material, weighing 5.6 grams, was dissolved in 200 c.c. dilute hydrochloric acid, and after heating nearly to boiling, barium hydroxide solution was

added until alkaline, and the precipitate filtered out. The filtrate was reserved for further examination. The precipitate was dissolved in dilute hydrochloric acid and barium hydroxide again added to alkaline reaction. The precipitate was again dissolved in dilute acid, and reprecipitated with three volumes of alcohol. After undergoing three more purifications by precipitation with alcohol, the substance was washed with alcohol and ether and dried. The product, weighing 2.5 grams, was a white amorphous powder, having an acid reaction.

Dried for analysis at 130° , the material turned slightly gray.

0.3017 g. gave 0.1568 g. BaSO_4 and 0.1430 g. $\text{Mg}_2\text{P}_2\text{O}_7$.
Found; Ba, 30.59%; P, 13.16%.
Found by Anderson:- Ba, 31.29%; P, 12.71%.

We did not make a carbon and hydrogen analysis of this material. So far as examined, however, this substance appeared very similar to that prepared by Anderson.

Examination of Filtrate from Barium Precipitation of Phytin Solution.

This filtrate was freed from barium by carbon dioxide, filtered and concentrated on the water bath to a small volume, and again filtered from traces of barium carbonate. The residue, after further concentration, was a yellowish, somewhat viscous liquid, with a very peculiar odor, somewhat like old, but not putrid, egg yolk. The taste was not marked.. It reduced Fehling's solution on boiling, but did not give the aniline acetate test for furfural on

boiling with hydrochloric acid. It gave heavy precipitates with phosphotungstic acid, picric acid and tannic acid. Phosphorus is present, as is also nitrogen. Dried in vacuum, the material seemed somewhat crystalline, but was sticky and hygroscopic.

III

From the fact that the two different methods used in the first and second parts of this paper yielded different products, it appeared possible that neither method alone was sufficient to secure a complete removal of all the organic phosphorus compounds in the wheat bran extract. Supposing this to be true, it should be possible by combining the two methods, to obtain two fractions of precipitate, thus not only securing a more nearly complete precipitation, but also establishing the presence of two different substances in the extract. By precipitating the acid extract first with three volumes of alcohol, removing this precipitate, and treating the filtrate with copper acetate solution, we hoped to effect this separation. The results obtained are somewhat surprising in the light of the first two parts of this paper, and we are unable at this time to offer an adequate explanation for them. The precipitate produced by alcohol, upon purification and formation of the barium salt, yielded, instead of the 30% barium salt of part two, prepared by the same method, the 36% barium salt of part one, prepared by

the copper acetate method, and like it, separable into a water-soluble and water-insoluble material, the latter obtainable only in an impure form, but semi-crystallizable. The copper acetate precipitate from the alcoholic filtrate gave a heavy precipitate, consisting almost entirely of inorganic phosphate, since, after being converted to the barium salt, it failed to precipitate with alcohol.

Preparation of the Alcoholic Precipitate.

Five pounds of wheat bran were extracted as before with 0.2% hydrochloric acid, over night, and the extract, after filtering, precipitated directly with three volumes of alcohol, without previous purification with tannic acid, which would have interfered with the copper precipitation of the filtrate. The precipitate, after settling, was filtered out, dissolved in 0.2% hydrochloric acid, and tannic acid added in excess. The precipitate formed was filtered out, and the filtrate precipitated with alcohol. The precipitate was then purified by four more alcoholic precipitations, and was finally washed with alcohol, and ether and dried. Yield, 40 grams, which was free from inorganic phosphates, and was slowly, but perfectly soluble in water.

Copper Acetate Precipitation of Filtrate.

To the alcoholic filtrate obtained above was added a concentrated solution of 100 grams copper acetate. A very heavy precipitate was produced. This was filtered and washed, suspended in water, and decomposed with hydrogen

sulphide. The filtrate from the copper sulphide was made alkaline, after the addition of a solution of 100 grams of barium chloride, with barium hydroxide solution, a heavy white precipitate resulting. This was filtered out, dissolved in 0.2% hydrochloric acid, and three volumes of alcohol added. Only a faint turbidity was produced, and after long standing a slight precipitate formed, which after filtering and drying, without further purification, weighed only about 0.2 gram. Hence it was discarded. It is possible that the copper precipitate at first contained more organic material, but it stood in the laboratory at 20° - 25° for two or three days, and may have undergone decomposition, although this does not seem probable.

Preparation of the Barium Salt by Direct Precipitation
with Barium Hydroxide.

Twelve grams of the crude material were dissolved in water to which a small amount of hydrochloric acid was added, and the solution boiled. Barium hydroxide solution was now added to strong alkaline reaction. The precipitate was filtered out, dissolved in dilute hydrochloric acid, and reprecipitated with barium hydroxide solution. It was then purified by three alcoholic precipitations in the usual manner. The resulting precipitate weighed, after drying, 12.8 grams.

It was dried for analysis at 105° , in vacuum, over phosphorus pentoxide.

0.2050 g. gave 0.1261 g. BaSO_4 .
0.2019 g. gave 0.1186 g. $\text{Mg}_2\text{P}_2\text{O}_7$
0.3348 g. gave 0.0834 g. CO_2 and 0.0555 g. H_2O .

Found:- Ba, 36.20%; P, 16.37%; C, 6.79%; H, 1.85%.

This method of preparation does not seem to replace entirely the bases originally present, for on fusing the salt with potassium hydroxide and potassium nitrate for phosphorus determination, a faint trace of green was produced, showing the presence of a trace of manganese, which was present in somewhat greater quantity in the original alcoholic precipitate.

Preparation of the Barium Salt by the Copper Acetate Method.

Twelve grams of the alcoholic precipitate were dissolved in water, a few drops of hydrochloric acid added, and then an excess of a concentrated solution of copper acetate. The conversion of the resulting copper salt to the barium salt was accomplished by the same procedure described previously for this method, and the product, free from phosphates, weighed 12.2 grams.

It was dried for analysis at 105° in vacuum over phosphorus pentoxide.

0.2333 g. gave 0.1418 g. BaSO_4 .
0.2042 g. gave 0.1160 g. $\text{Mg}_2\text{P}_2\text{O}_7$.
0.3342 g. gave 0.0862 g. CO_2 and 0.0497 g. H_2O .

Found:- Ba, 36.90%; P, 16.32%; C, 7.03%; H, 1.66%.

The material was free from manganese, and probably this

method insures a more thorough separation of the bases than the former method.

Purification of the Barium Salt by Water Extraction.

Ten grams of this material were extracted with five successive twenty-five c.c. portions of water, being rubbed up thoroughly in a mortar with each portion. The final portion gave only a cloudiness with alcohol. The united extracts were treated with three volumes of alcohol, the precipitate filtered, washed with alcohol and ether, and dried. Weight, 4 grams.

It was dried for analysis at 105° in vacuum over phosphorus pentoxide.

0.1974 g. gave 0.4211 g. BaSO_4 .

0.2068 g. gave 0.1116 g. $\text{Mg}_2\text{P}_2\text{O}_7$.

0.3655 g. gave 0.0794 g. CO_2 and 0.0629 g. H_2O .

Found:- Ba, 36.10%; P, 15.53%; C, 5.93%; H, 1.93%:

Attempt to Crystallize the Water-insoluble Fraction.

All the water insoluble material was dissolved in 0.2% hydrochloric acid, and alcohol added until a fairly heavy amorphous precipitate was obtained. After standing several days, this precipitate had in part crystallized, but there was considerable amorphous matter mixed with the crystals. The form of the crystals was identical with those obtained before in pure form. Upon standing for several days, complete crystallization failed to take place, and the mixture of crystals and amorphous matter was filtered and dried for analysis at 105° in vacuum over phosphorus pentoxide.

0.1569 g. gave 0.0942 g. BaSO_4 .
0.1525 g. gave 0.0889 g. $\text{Mg}_3\text{P}_2\text{O}_7$.
0.2455 g. gave 0.0570 g. CO_2 and 0.0391 g. H_2O .

Found:- Ba, 35.35%; P, 16.87%; C, 6.33%; H, 1.78%.

None of these substances bear any resemblance to the material obtained by Anderson by a similar method, nor to that obtained by us as described in the second part of this paper. No satisfactory reason suggests itself for this fact, although there were two differences in the preparation:- first, a larger amount of alcohol was used, securing a more complete precipitation, and second, whereas in making the first preparation, about two weeks were consumed, the second preparation was completed in three days. The latter fact could only make a difference if the material tends to decompose. Believing that neither of these reasons is adequate to explain the facts, we leave the question open for further investigation.

Conclusions.

1. A large part of the inorganic phosphorus of wheat bran exists as phytin, similar to that from many other sources, and a crystalline tri-barium salt may be readily prepared from it. This material is most readily obtained by the copper acetate method.

2. There is, in addition to this, a considerable amount of another substance, very similar in analysis, the barium salt of which contains only 34% barium, instead of the 37%

of barium phytate. The fact that this substance does not dialyze would seem to indicate that its molecule is larger than that of barium phytate.

3. There is, finally, a compound differing widely from phytin in having more carbon and less phosphorus in the molecule, which splits off a reducing sugar (pentose), on hydrolysis, and whose barium salt contains only about 31% barium. The composition of this substance we do not believe to be definitely fixed yet. It has not been obtained in crystalline form, the crystalline brucine salt prepared by Anderson probably being simply brucine phosphate.

4. The formulae $C_6H_{18}O_{24}P_6Ba_3$ for the tri-barium salt, and $C_6H_{24}O_{24}P_6$ for the acid, accord more closely with our analytical results than any other formulae, although the agreement is far from satisfactory. A table of analytical results is appended.

Table I..

	From wheat bran (crystallized) Found	Calculated for tri-barium inosite- hexaphosphate $C_6H_{18}O_{24}P_6Ba_3$	Calculated for $C_6H_{18}O_{24}P_6Ba_3$
P	17.12%	17.44%	17.35%
Ba	37.77%	38.65%	38.43%
C	6.89%	6.75%	6.72%
H	1.96%	1.12%	1.69%

Table II.

	From wheat bran Found	Calculated for inosite- hexaphosphate $C_6H_{18}O_{24}P_6$	Calculated for $C_6H_{24}O_{24}P_6$
P	27.67%	28.18%	27.92%
C	10.90%	10.90%	10.81%
H	3.54%	2.72%	3.63%