CONCERNING PHYTIN IN WHEAT BRAN.

FOURTEENTH PAPER ON PHYTIN.

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INTRODUCTION.

It has been shown in earlier reports that the organic phosphorus compounds of wheat bran which have been isolated after digesting the bran in 0.2 per cent hydrochloric acid differ in composition and properties from phytin or inosite hexaphosphate. It has been found that under these conditions several organic phosphoric acids are obtained. Of these we have isolated and identified two; viz., inosite monophosphate and inosite triphosphate.

Patten and Hart, who first investigated the phosphorus compounds of wheat bran, came to the conclusion that the organic phosphoric acid in bran was identical with phytic acid, or the "anhydrooxymethylene diphosphoric acid" of Posternak. These authors isolated the acid preparation which they analyzed after digesting the bran in 0.2 per cent hydrochloric acid. We have shown conclusively, however, that when wheat bran is digested in this strength hydrochloric acid, the organic phosphorus compounds finally isolated are entirely different in composition from phytin or inosite hexaphosphate.

2 Anderson: this Journal, xviii, p. 441, 1914; New York Agricultural Experiment Station Technical Bulletins, No. 36, 1914.
Phytin in Wheat Bran

By the same method as above, i.e., after digesting in 0.2 per cent hydrochloric acid, we have isolated inosite hexaphosphate from corn, cottonseed meal, and oats. All these preparations were found to be identical with the inosite hexaphosphate prepared from commercial phytin.

It would appear, then, as if wheat bran is the only one of all the various plants and seeds examined which does not contain inosite hexaphosphate. Instead, certain lower inosite phosphoric acids appear to be present. It seems difficult to explain why wheat bran should contain different inosite phosphoric acids from other plants.

The work of Suzuki, Yoshimura, and Takaishi, and of Plimmer on the presence of an enzyme in wheat bran which rapidly hydrolyzes phytin with formation of inorganic phosphoric acid, particularly in connection with the two preceding investigations, offered a key to the solution of this problem. The fact that the action of a wheat bran extract on commercial phytin yielded products identical with those which we have previously isolated from wheat bran itself, viz., principally inosite triphosphate and inosite monophosphate, led to the theory that the organic phosphorus compound originally present in bran was probably hydrolyzed during the extraction with 0.2 per cent hydrochloric acid with formation of inorganic phosphoric acid and lower inosite phosphates. This opinion was fully confirmed by the results reported in the preceding paper. It is shown there that inorganic phosphates are liberated rapidly when wheat bran is digested in water, in 0.1 and in 0.2 per cent hydrochloric acid, as well as in 0.2 per cent acetic acid. This rapid formation of inorganic phosphoric acid from the organic phosphorus compound would

naturally preclude the possibility of isolating the original substance from extracts prepared by digesting the material in the above solutions. The only products possible of isolation would be such intermediate substances as had escaped complete hydrolysis by the enzyme. The previous determinations concerning the activity of the enzyme showed, however, that the use of hydrochloric acid stronger than 0.2 per cent materially reduced the amount of inorganic phosphate in the extract, and that the minimum quantity was present when the bran was extracted with 1 per cent hydrochloric acid.

Wheat bran was therefore extracted for five hours with 1 per cent hydrochloric acid and the organic phosphate in the extract isolated as a barium salt (compare experimental part). The substance then obtained had entirely different properties and composition from those obtained when wheat bran is digested in 0.2 per cent hydrochloric acid. After careful purification this salt crystallized in the same form and under the same conditions as the corresponding barium salts of inosite hexaphosphate. So far as crystal form, properties, and composition are concerned, there appears to be no difference between the substances isolated from wheat bran and the barium salts of phytic acid or inosite hexaphosphate obtained from other sources. We conclude, therefore, that all these materials, viz., wheat bran, corn, oats, cottonseed meal, and commercial phytin, contain the same organic phosphates; namely, phytic acid or inosite hexaphosphate, C₆H₁₁O₂₄P₆.

This confirms the conclusions of Patten and Hart¹¹ as to the nature of the organic phosphorus compound of wheat bran. It is somewhat difficult to understand how these authors came to this conclusion, since they state that they had extracted the bran with 0.2 per cent hydrochloric acid, and under these conditions we have shown that phytic acid or inosite hexaphosphate is not obtained, but principally inosite triphosphate and some inosite monophosphate.

In view of the results reported in this as well as in the two preceding papers, it is evident that the compounds which we have previously isolated from wheat bran, viz., inosite triphosphate and inosite monophosphate, do not represent the organic phos-

¹¹ Patten and Hart: loc. cit.
Phytin in Wheat Bran

Phytic acids originally present in the bran, but that they are intermediate products formed from inosite hexaphosphate by the enzyme phytase during the extraction of the bran with the dilute acid.

EXPERIMENTAL PART.

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

The precipitate was still dark colored and it contained some impurities, not completely soluble in the dilute hydrochloric acid, apparently of colloidal nature, which could not be removed by filtration. In order to eliminate these impurities the barium precipitate was suspended in water and the barium removed with a slight excess of dilute sulphuric acid. The barium sulphate was filtered off and the filtrate precipitated with excess of copper acetate. The copper precipitate was filtered and washed free of sulphates with water. It was then suspended in water and decomposed with hydrogen sulphide. After filtering off the copper sulphide a clear and colorless solution of the free acid was obtained. By these various operations the oxalic acid had also been removed, as after nearly neutralizing with barium hydroxide and adding barium chloride, no precipitate or turbidity occurred. The solution was precipitated with barium hydroxide, filtered,
and washed in water. The substance was again twice precipitated with barium hydroxide from 3 per cent hydrochloric acid, and finally twice precipitated with alcohol from the same strength hydrochloric acid. After filtration the substance was washed free of chlorides with dilute alcohol, alcohol, and ether, and dried in vacuum over sulphuric acid. It was a snow-white semicrystalline powder weighing 11 grams. It was free from chlorides and inorganic phosphate and did not contain any bases except barium.

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

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

<table>
<thead>
<tr>
<th>Substance</th>
<th>Weight (gram)</th>
<th>Water Loss (gram)</th>
<th>Water %</th>
<th>Carbon %</th>
<th>Hydrogen %</th>
<th>Phosphorus %</th>
<th>Barium %</th>
</tr>
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<tbody>
<tr>
<td>0.3771</td>
<td></td>
<td>0.0385</td>
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<td>1.20</td>
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<td></td>
<td>0.1218</td>
<td>7.11%</td>
<td>6.53</td>
<td>1.20</td>
<td>10.20</td>
<td>10.22</td>
</tr>
</tbody>
</table>

For heptabarium inosite hexaphosphate, \((C_8H_{11}O_{24}P_6)_{1/2}Ba_7 = 2267\). Calculated: C = 6.33; H = 0.97; P = 16.40; Ba = 42.39 per cent.

Preparation of the crystalline tribarium inosite hexaphosphate.

This was prepared by dissolving 5 grams of the original substance in 2 per cent hydrochloric acid, nearly neutralizing with
dilute barium hydroxide, filtering, and adding alcohol gradually until the solution turned cloudy. It was then allowed to stand for two days at room temperature. The substance separated slowly in the form of globular masses or rosettes of microscopic needles. The crystal form was identical with that previously described for the tribarium inosite hexaphosphate. The substance was filtered and washed in dilute alcohol, alcohol, and ether, and dried in the air. It was recrystallized a second time by dissolving in the minimum quantity of 2 per cent hydrochloric acid, filtering, and adding alcohol until a slight permanent cloudiness remained. After standing for two days the substance had separated in the same form as before. It was filtered, washed free of chlorides with dilute alcohol, and then in alcohol and ether, and dried in the air. It was obtained as a snow-white crystalline powder. It was free from chlorides and inorganic phosphates.

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

0.2922 gram substance lost 0.0334 gram H₂O.
0.2588 gram substance gave 0.0291 gram H₂O and 0.0648 gram CO₂.
0.1421 gram substance gave 0.0044 gram BaSO₄ and 0.0867 gram Mg₃P₂O₇.

Found: C = 6.82; H = 1.25; P = 17.00; Ba = 39.09 per cent.
H₂O = 11.43 per cent.

For tribarium inosite hexaphosphate, C₆H₁₂O₆P₆Ba₃ = 1066.
Calculated: C = 6.75; H = 1.12; P = 17.44; Ba = 38.65 per cent.

For 8 H₂O calculated, 11.90 per cent.

Most authors working with organic phosphorus compounds report much difficulty in obtaining proper values for the carbon. This difficulty is particularly great in burning compounds such as those reported above where the percentage of carbon is so low. Under ordinary conditions it is impossible to obtain a complete combustion—the ash is usually more or less dark colored. Some authors recommend mixing the substance intimately with fine copper oxide. This procedure is very serviceable when burning salts of these organic phosphoric acids with organic bases,—like the strychnine salts which we have previously reported,—but with barium salts we have not found copper oxide to be of much use. In the analyses reported above we have used the following method, for the suggestion of which we are indebted to His Excellency, Prof. E. Fischer, of Berlin.
The substance is first burned in the usual way in a current of oxygen, the combustion lasting about an hour. The calcium chloride tube and the potash bulb are then weighed. The increase in weight of the calcium chloride tube is taken as the correct weight of the water. The residue in the boat, which is dark colored from particles of unburned carbon, is powdered in an agate mortar with some recently fused potassium bichromate and again placed in the boat, the mortar being rinsed out with some more powdered bichromate. The whole is again burned in the usual way. The potassium bichromate fuses and oxidizes all the carbon in the residue. The increase in weight in the potash bulb is added to the first, giving the total carbon dioxide.

Since the barium salts described above agree in crystal form and composition with salts of inosite hexaphosphoric acid or phytic acid, we believe there can be no doubt that wheat bran contains the same phytin as other plants. We would have recognized this relation sooner if we had made a series of inorganic phosphorus determinations in wheat bran extracts prepared with water or dilute acids, such as is reported in a preceding paper. We believed, however, that since phytic acid could be isolated from cottonseed meal, oats, and corn, after digesting in 0.2 per cent hydrochloric acid, that the same procedure should also suffice in the case of wheat bran. Moreover, we were following the method of isolation recommended by previous investigators on this subject. The fact that inosite hexaphosphate is obtained from 0.2 per cent hydrochloric acid extracts of corn, oats, and cottonseed meal only proves that these materials, with the possible exception of oats, do not contain any enzyme of the nature of the phytase contained in wheat bran. In the case of the phytin preparations isolated from oats we found a considerable amount of water-soluble barium salt of practically the same composition as the barium salts prepared from wheat bran after extracting with 0.2 per cent hydrochloric acid. We hope to investigate further this matter concerning the distribution of the enzyme phytase in various plants. We also intend to study more closely certain phases of the action of this enzyme, particularly

12 Anderson: this Journal, xvii, p. 163, 1914; New York Agricultural Experiment Station Technical Bulletins, No. 32, p. 21, 1914.
whether it is capable of inducing synthetic reactions. It appears not improbable that an equilibrium exists in the reaction

\[ C_6H_{12}O_{4}P(OH)_4 + 6H_2O \rightleftharpoons C_6H_{12}(OH)_6 + 6H_3PO_4, \]

particularly at the stage when one-half of the phosphorus has been split off. The fact that the bulk of the substance ordinarily isolated from wheat bran, after digesting in 0.2 per cent hydrochloric acid, is inositol triphosphate, supports this view.

**SUMMARY.**

By digesting wheat bran in 1.0 per cent hydrochloric acid, which is sufficiently strong to destroy the enzyme, phytase, it is possible to isolate from the extract crystalline barium salts of the following composition:

\[ C_6H_{12}O_{4}P_3Ba_3 + 8H_2O \text{ and } (C_6H_{11}O_{24}P_6)Ba_7 + 14H_2O. \]

These salts are identical with the tribarium phytate and heptabarium phytate obtained from oats, corn, cottonseed meal, and commercial phytin. All these materials contain, therefore, the same organic phosphorus compound; \( \text{viz.} \), phytic acid or inositol hexaphosphate, \( C_{6}H_{18}O_{24}P_{6} \).

The author acknowledges with pleasure his indebtedness to Dr. R. H. A. Plimmer for many suggestions which have been of great assistance in carrying out the work reported in this and the two preceding papers.
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