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NO. ....

## THE NATURE OF THE PRINCIPAL PHOSPHORUS COMPOUND IN WHEAT BRAN.

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SUMMARY.

(1) Practically all of the soluble phosphorus of wheat bran is of an organic nature.

(2) The organic compound exists in the bran itself as a magnesium-calcium-potassium salt of a phospho-organic acid.

(3) The free acid corresponds to the formula  $C_2H_8P_2O_6$ , and is probably identical with Posternak's anhydrooxymethylene diphosphoric acid.

(4) The alkali salts of this acid are freely soluble in water. The calcium and copper salts are slightly soluble while the barium and strontium salts are but sparingly so.

(5) The acid and its salts seem to be of wide distribution in the vegetable kingdom, having already been isolated from the seeds of red fir, peas, beans, pumpkin, red and yellow lupine, also from the potato and other tubers and bulbs.

This investigation forms part of the research on the metabolism of phosphorous and sulphur in the animal body conducted under the direction of Dr. Jordan. The physiological role of the acid and its salts in animal metabolism will be made the subject of future researches.

## INTRODUCTION.

From the work reported in Bulletin No. 238 of this Station and from other work done preliminary to an investigation of the metabolism of phosphorus in animals, it was found that in many of our ordinary feeding stuffs a varying percentage of the organic phosphorus is directly soluble in water and in dilute hydrochloric acid. Data illustrating this point will be found in the following table :

TABLE I.—SOLUBLE PHOSPHORUS IN OATS, MALT SPROUTS AND WHEAT BRAN.

	Total phosphorus.	Solvent.	Soluble phosphorus.	Percentage of total phosphorus.
	<i>Per ct.</i>		<i>Per ct.</i>	<i>Per ct.</i>
Oats.....	0.355	{ Water } Hydrochloric	0.180	50.0
			0.096	27.0
Malt sprouts....	0.677	{ Water } Hydrochloric	0.548	81.0
			0.477	70.0
Wheat bran ....	1.22	{ Water } Hydrochloric	1.056	86.5
			0.95	77.8

It will be seen that wheat bran carries a much larger percentage of phosphorus than any of the others and that 86.5 per ct. is soluble in water.

It was at first supposed that the soluble phosphorus was in combination as nucleins or salts of nucleic acid, but determinations of the amount of soluble nitrogen showed that only about 33 per ct. of the phosphorus could be accounted for in this way.

We were therefore led to the conclusion that by far the greater part of the soluble phosphorus was linked up in some other organic combination, since Hart and Andrews<sup>1</sup> have shown that the amount of soluble inorganic phosphorus is exceedingly small.

<sup>1</sup> Bulletin 238, N. Y. Agr. Expt. Station.

## INVESTIGATION OF PHOSPHORUS COMPOUND.

### ATTEMPT TO ISOLATE.

Two lbs. of wheat bran was extracted with six liters of 0.2 per ct. hydrochloric acid for several hours with frequent stirring. The extract was strained through cheese cloth and finally filtered through paper.

The filtrate was treated with a large volume of 95 per ct. alcohol which threw down a voluminous, flocculent precipitate. The precipitate was allowed to settle to the bottom, the supernatant liquid syphoned off and the precipitate washed with alcohol several times by decantation. It was then dissolved in a small volume of 0.2 per ct. hydrochloric acid, filtered from an insoluble residue, reprecipitated by alcohol and washed as before by decantation.

This process was again repeated, the precipitate finally brought upon a filter, washed with absolute alcohol and ether and dried at  $110^{\circ}$  C. The resulting product, which weighed 7.2 grams, was a white amorphous powder readily soluble in water.

Its water solution was acid to litmus and it was precipitated from such solution by solutions of salts of the heavy metals, by the alkali metals and the alkaline earths, also by alcohol and ether.

Chemical analysis showed it to be an organic phosphorus compound coupled with calcium, magnesium and potassium.

Of this substance,

0.5004 gms. gave 0.0082 gms. CaO and 0.1327 gms.  $Mg_2 P_2 O_7$  equivalent to 1.13 per ct. Ca and 5.80 per ct. Mg ;

0.512 gm. substance gave 0.0834 gm.  $K_2 Pt Cl_6$  equivalent to 2.60 per ct. K.

0.2174 gm. substance gave 0.1280 gm.  $Mg_2 P_2 O_7$ , equivalent to 16.38 per ct. P.

0.2122 gm. substance gave 0.1438 gm.  $CO_2$  and 0.072 gm.  $H_2 O$ , equal to 18.52 per ct. C and 3.83 per ct. H.

The substance carried also a small amount of nitrogen (0.37 per ct.) ; but this was considered an impurity and, calculated as a proteid, would lower the carbon and hydrogen to 17.30 per ct. and 3.63 per ct. respectively.

## COMPOSITION OF ISOLATED PHOSPHORUS COMPOUND.

	<i>Per ct.</i>		<i>Per ct.</i>
C .....	17.30	Ca .....	1.13
H .....	3.63	Mg .....	5.80
P .....	16.38	K .....	2.60

## SIMILAR COMPOUNDS REPORTED BY OTHER WORKERS.

Palladin<sup>2</sup>, working in Schulze's laboratory, isolated from the seeds of black mustard (*Sinapis nigra*) a highly phosphorized compound in combination with calcium and magnesium.

The seeds freed from fat were extracted with 10 per ct. sodium chloride solution, filtered, and the filtrate heated to about 80° C. A precipitate separated carrying with it all of the proteid. The larger part of the precipitate went into solution again on cooling leaving the coagulated proteid in suspension, which was separated by filtration. The filtrate was again heated and the resulting coagulum collected on a hot water funnel.

Schulze and Winterstein<sup>3</sup> continued the investigation started by Palladin and obtained a compound carrying 9.65 per ct. C, 2.83 per ct. H and 16.13 per ct. P. They thought the substance might be identical with the chief constituent of the globoids found enclosed in the protein kernel of many plant seeds. Pfeffer<sup>4</sup> in conjunction with Brandau found that such globoids consist of a calcium-magnesium salt of an organic body coupled with phosphoric acid. What the nature of this organic combination was they were unable to say.

Winterstein<sup>5</sup> isolated a compound from the seeds of black mustard by extracting with dilute acetic acid. The extract was freed from proteid by boiling and filtering when cold. The filtrate was made slightly alkaline with ammonium hydrate and

<sup>2</sup> Beiträge zur Kenntniss pflanzlicher Eiweissstoffe, *Zeitschrift f. Biologie*, Jahrgang 1894, p. 199

<sup>3</sup> Ueber einen phosphorhaltigen Bestandtheil der Pflanzensamen. *Zeit. f. physiol. Chem.*, 22: 90.

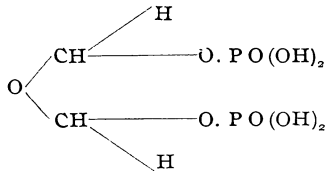
<sup>4</sup> W. Pfeffer: Untersuchung ueber die Proteinkoerner und die Bedeutung des Asparagins beim Keimen der Samen. *Jahrbücher für wissenschaftliche Botanik.*, 8: 147. (1872).

<sup>5</sup> Ueber einen phosphorhaltigen Pflanzenbestandtheil, welcher by der Spaltung Inosit liefert. *Ber. d. deut. chem. Ges.*, 30: 2299.

boiled, the resulting precipitate collected on a hot water funnel and washed with hot water until the wash water reacted neutral. The substance was purified by dissolving in dilute acetic acid and reprecipitating with ammonium hydrate. On removing the calcium with oxalic acid, a product was obtained carrying 18.44 per ct. P and 7.83 per ct. Mg. By heating the magnesium salt in a closed tube with concentrated hydrochloric acid for 30 hours, at a temperature of 130°–140° he obtained inosite as a cleavage product.

The compound obtained by us from bran by the alcohol method, though still impure, is undoubtedly identical with the one described by these investigators.

Posternak<sup>6</sup> isolated the compound from the seeds of red fir, pumpkin, peas, beans, white and yellow lupine and also from the potato. He also isolated and identified the free acid as anhydrooxymethylene diphosphoric acid with the following formula :



The method adopted by Posternak was the following :

The material was extracted with dilute hydrochloric acid and filtered, the filtrate was made alkaline with sodium hydrate and precipitated with calcium chloride. The calcium chloride precipitate was filtered, washed and finally dissolved in dilute hydrochloric acid. Sodium acetate was added to the solution to replace the free mineral acid by acetic acid, and copper acetate added in excess. The copper precipitate was filtered, thoroughly washed with water and decomposed by hydrogen sulphide.

The copper sulphide was removed by filtration and the excess of hydrogen sulphide removed by pumping air through the liquid. It was then evaporated in a vacuum over sulphuric acid.

<sup>6</sup> *Revue Generale de Botanique*, 12 : 5 and 65, 1900.

*Comptes Rendus*, 137 : No. 3. (20 Juillet. 1903).

No. 5. (3 Aout 1903)

No. 8. (24 Aout 1903).

We found it very difficult to remove the last traces of calcium by this method and have modified it by substituting barium chloride for calcium chloride. This admits of a very thorough washing at this stage of the operation as the barium salt is much more insoluble than the calcium salt and also admits of a complete removal of the barium as a sulphate.

#### METHOD OF PREPARATION OF FREE ACID.

The method finally adopted by us is as follows :

The bran was extracted with 0.2 per ct. hydrochloric acid, strained through cheese cloth and filtered through paper. Since the compound is present in bran as a calcium-magnesium-potassium salt, it was precipitated directly with copper acetate, by which means we were able to remove the greater part of these bases. After washing the copper precipitate it was suspended in water and decomposed by hydrogen sulphide. The filtrate from copper sulphide was made alkaline with sodium hydrate and precipitated with barium chloride. The barium salt was washed until free from alkali, suspended in water, and dilute sulphuric acid added in sufficient quantity to decompose the salt and throw down the barium as a sulphate. After removal of the barium sulphate by filtration the filtrate was again precipitated in alkaline solution with barium chloride and treated as before. This process was repeated a third time and after final removal of the barium, copper acetate was added in excess. The copper precipitate was filtered at the pump, thoroughly washed with water and finally suspended in water and decomposed by hydrogen sulphide. The copper sulphide was removed by filtration and the filtrate evaporated on the water bath to a syrupy consistency.

Analysis of the acid dried at  $110^{\circ}$  gave the following results :

0.7207 gm. substance gave 0.2811 gm.  $\text{CO}_2$  and 0.2202 gm.  $\text{H}_2\text{O}$  equivalent to 10.63 per ct. C and 3.38 per ct. H.

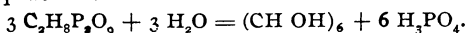
0.1039 gm. substance gave 0.097 gm.  $\text{Mg}_2\text{P}_2\text{O}_7$  equivalent to 25.98 per ct. P.

	Found.	Calculated for $\text{C}_2\text{H}_8\text{P}_2\text{O}_9$
C .....	10.63	10.08
H .....	3.38	3.36
P .....	25.98	26.07



DECOMPOSITION OF COMPOUND INTO INOSITE AND PHOSPHORIC ACID.

Heated with concentrated mineral acids it is broken up quantitatively into inosite and phosphoric acid, after the following equation :



Posternak obtained 97.8 per ct. of the total carbon of the acid decomposed as inosite. In our investigation an unweighed portion of the acid was heated in a closed tube with 50 c. c. of 30 per ct. sulphuric acid at a temperature of  $155^\circ$ – $160^\circ$  for 5 hours. After cooling, the tube was opened and the contents washed into a beaker. The sulphuric and phosphoric acids were removed by barium hydrate and the excess of barium by carbon dioxide. The filtrate was evaporated nearly to dryness, taken up with hot water and filtered from remaining barium carbonate. The filtrate was then treated with absolute alcohol and ether until a cloudiness was produced, when it was allowed to stand in the cold. A crystalline precipitate soon separated which was obtained pure after once recrystallizing. It gave the reactions of Scherer and Gallois and melted at  $218^\circ$ – $219^\circ$  (uncor.). Inosite melts at  $218^\circ$  (uncor.).

Carbon and hydrogen determinations were made on the substance dried at  $110^\circ$ .

	Found	Calculated for Inosite $\text{C}_6\text{H}_{12}\text{O}_6$
C.....	39.59	40.00
H.....	6.78	6.66

PROPERTIES OF ANHYDRO-OXYMETHYLENE DIPHOSPHORIC ACID

The free acid dried slowly over sulphuric acid is a very thick, transparent liquid, of a yellowish brown color. It is soluble in all proportions in water and alcohol, insoluble in ether, benzene, chloroform and glacial acetic acid. It has a very sharp acid taste. Heated to  $110^\circ$  it becomes decidedly brown without decomposition. The alkali salts of the acid are freely soluble in water. Water solutions of the free acid are precipitated by ferric chloride, the precipitate being soluble in an excess of the reagent. Silver nitrate precipitates solutions of the free acid only after a liberal excess of the reagent has been added. The precipitate is insoluble in excess of the reagent. The free acid is not pre-

precipitated by the chlorides of magnesium, calcium, barium or strontium, due to the liberation of free hydrochloric acid. However, when solutions of the alkaline salts of the free acid are treated with the above reagents, a precipitate is formed.

The magnesium and calcium salts are somewhat soluble in water, the barium and strontium salts, very sparingly soluble. The magnesium salt is easily soluble in acetic acid, the calcium salt less so, while the barium and strontium salts are very insoluble. All are readily soluble in mineral acids.

The free acid is tetra-basic and forms two salts, the one a normal salt, the other an acid salt. The normal salt reacts neutral to phenolphthalein while the acid salt reacts neutral to methyl-orange, which may be shown by the following determinations: 10 c. c. of a solution containing 0.0648 gm. of the acid titrated to phenolphthalein with deci-normal barium hydrate required 10.9 c. c. or 0.0748 gm. barium. 0.0648 gm. of the acid should require 0.0749 gm. barium to produce the normal salt ( $C_2H_4P_2O_9Ba_2$ ). 10 c. c. of the same solution titrated to methyl-orange with deci-normal barium hydrate required 5.1 c. c. or 0.035 gm. barium. 0.0648 gm. of the acid should require 0.0343 gm. barium to produce the acid salt ( $C_2H_6P_2O_9Ba$ ).

#### QUANTITATIVE ESTIMATION OF THE ACID IN WHEAT BRAN.

We extracted 10 gms. of bran to 500 c. c. with 0.2 per ct. hydrochloric acid, made 100 c. c. alkaline with sodium hydrate, precipitated with barium chloride, filtered and washed free from alkali.

The phosphorus was determined directly in this precipitate.

Total phosphorus.	Phosphorus in barium precipitate.	Per ct. of phosphorus in barium precipitate to total phosphorus.
<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
1.35	0.92	68.1

Practically all of the phosphorus soluble in 0.2 per ct. hydrochloric acid is in this form, as is shown below:

Total soluble phosphorus.	Phosphorus in barium precipitate.	Per ct. of phosphorus in barium precipitate to total soluble phosphorus.
<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
0.94	0.92	97.9