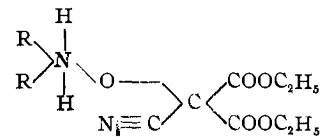
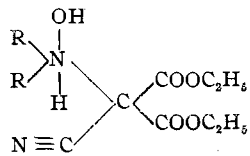
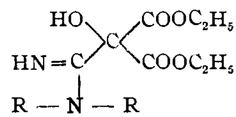


(2) A substituted ammonium hydroxide such as Stadnikoff[1] uses to explain the Strecker reaction:



or

(3) An amidine:



With benzyl amine, however, we have one molecule of the amine adding to two molecules of the cyanotartronate. Evidently this eliminates the possibility of a salt as in (1). In further support of this conclusion, we note that the action of triethyl amine is anomalous and that aromatic amines are evidently without action in spite of their basicity. In (2) a molecule of water must first split off before a second molecule of cyanotartronate could add. Analysis shows that water is not eliminated in this way. We are, therefore, led to conclude that the amidine structure in (3) is the correct one.

URBANA, ILL.

[CONTRIBUTION FROM THE TEXAS AGRICULTURAL EXPERIMENT STATION.]

PHYTIC ACID IN COTTONSEED MEAL AND WHEAT BRAN.

BY J. B. RATHER.[2]

Received April 19, 1913.

It has been shown by the writer[3] that the phosphorus compounds of cottonseed meal are nearly all organic in nature, and especially that cottonseed meal does not contain meta- or pyrophosphoric acid, as has been claimed.[4] The work here presented is a continuation of the study of the phosphorus compounds of cottonseed meal, together with an investigation of the acid-soluble phosphorus compounds of wheat bran.

[1] *Ber.*, 44, 40.

[2] Under the general direction of G. S. Fraps, chemist. Full details of this work may be found in Bulletin 156 of the Texas Experiment Station.

[3] Texas Exp. Sta., *Bull.* 146.

[4] Hardin, S. C. Exp. Sta., *Bull.* 8, new series; Crawford, *J. Pharm. Expt. Therapeutics*, 1, 51.

Historical.—The theory that phytic acid, an inosite-phosphoric acid found in plants as a complex inorganic salt known as phytin, corresponds to the formula $C_2H_8P_2O_9$, was first proposed by Posternak.[1] This theory has been agreed to by a number of investigators, but it seems to rest entirely on the work of Posternak, Patten and Hart,[2] Hart and Tottingham,[3] and Anderson.[4] The method used by these workers for the preparation of the product was a modification of the Posternak method.

Verbrodt[5] and Rising[6] got results which did not agree with the theory of Posternak.

Anderson[7] claims that the organic phosphoric acid of wheat bran, which Patten and Hart[8] considered to be phytic acid, is not phytic acid but an acid corresponding to the formula $C_{25}H_{65}P_9O_{54}$, which decomposes into another, $C_{20}H_{55}P_9O_{49}$, with the loss of one pentose group.

Anderson[9] repeated a number of our tests on the organic phosphoric acid of cottonseed meal and confirms our conclusions in regard to the absence of meta- and pyrophosphoric acid. He states that "cottonseed meal contains an organic phosphoric acid very similar to phytic acid."

Experimental.—In our attempts to purify the organic phosphoric acids of cottonseed meal, we found that the Patten and Hart modification of the Posternak method did not remove all the inorganic matter, and especially iron. The following figures show the results of an analysis of the inosite-phosphoric acid of wheat bran, prepared by the Patten and Hart method:

Iron and aluminum	3.33%
Calcium	0.30%
Magnesium	0.18%
Ortho-phosphoric acid	1.01%

The results show that the product contained almost 5% of inorganic impurities. We have assumed that the iron and aluminum were present in the ignited product as phosphates. Should this not be the case, the total inorganic impurities would be about 10%. It is evident that a formula based on the analysis of such a product would be erroneous. As far as we have been able to find, none of the investigators of the so-called phytic acid have made any attempt to prove their products free from inorganic substances. The method of Patten and Hart is essentially the same as that of Posternak, Patten and Hart using barium chloride instead of

[1] *Rév. Gén. Bot.*, **12**, 5 and 65; *Compt. rend.*, **137**, 35, 8.
[2] N. Y. (Geneva) Exp. Sta., *Bull.* **250**.
[3] Wis. Exp. Sta., *Res. Bull.* **9**.
[4] N. Y. Exp. Sta., *Tech. Bull.* **19** and **21**; *J. Biol. Chem.*, **2**, 471; **12**, 97.
[5] *Anzeiger Akad. Wiss. Krakau*, **1910**, *Series A*, 414.
[6] *Svensk Kemisk Tidskr.*, **22**, 143 (1910).
[7] N. Y. (Geneva) Exp. Sta., *Tech. Bull.* **22**.
[8] *Loc. cit.*
[9] N. Y. (Geneva) Exp. Sta., *Tech. Bull.* **25**.

calcium chloride in the process. The Patten and Hart method was used by Hart and Tottingham and by Anderson. Since the work of these investigators constitutes the proof of the theory that phytic acid corresponds to the formula $C_2H_8P_2O_9$, or a multiple of it, it is plain that this theory is in need of revision.

Separation and Purification of the Principal Organic Phosphoric Acid of Cottonseed Meal and Wheat Bran.—The phosphorus compounds of wheat bran and cottonseed meal soluble in 0.2% hydrochloric acid and the phosphorus compounds of cottonseed meal soluble in 0.2% ammonia after extraction with 0.2% hydrochloric acid were studied. The examination of the ammonia extract was made because we found that three times as much phosphorus was soluble in this medium, after extraction with the acid, as was soluble in the acid alone. The method of purification used was a modification of the Patten and Hart method designed to remove inorganic impurities. Briefly it is as follows:

Precipitate the phosphorus compounds in acid solution with copper acetate. Remove the copper with hydrogen sulfide, and evaporate. Dissolve in a small volume of water, add ten times its volume of ammonia, and let set over night. Filter, evaporate to remove excess of ammonia, and take up with water. Precipitate the product a number of times with barium chloride in alkaline solution and finally recover the free acid from its copper salt. Evaporate to less than 100 cc. and add a large excess of purified alcohol. Allow the precipitate to settle, filter and evaporate the filtrate to a syrupy consistency.

Analyses of the products, dried at 110°, gave the following results:

	Wheat bran acid extract.	Cottonseed meal. Acid extract.	Cottonseed meal. Ammonia extract.
Inorganic bases (calculated as Fe)	0.08	0.11	0.14
Ortho-phosphoric acid[1]	0.10	0.12	0.10

These results are low and the error is lessened by more than half in the silver salts subsequently analyzed. The method is satisfactory, because it reduced the inorganic bases in the case of the wheat bran product from 3.81% to 0.08%, and the inorganic phosphoric acid from 1.01% to 0.10%. The other results are correspondingly low. Quantitative tests showed that the products were free from nitrogen and pentosans.

Homogeneity of the Products.—Silver salts of the acid products were prepared under identical conditions, dried, and the silver determined. 0.2000 gram substance yielded 0.1224 and 0.1236 gram from the cottonseed meal products and 0.1206 gram from the wheat bran product. These differences are within the limit of error, and it appears that silver salts containing the same amounts of silver are formed under identical condi-

[1] Calculated from analyses of the silver salts hereinafter described.

tions. This is evidence that the salts are the same. Samples of 0.5000 gram of each of the above silver salts were digested with 200 cc. water for 5 hours at room temperature. The silver dissolved was found to be 0.0178 and 0.0172 gram for the cottonseed meal products, and 0.0162 gram for the wheat bran product. The salts, then, have the same solubility in water, and are evidently identical. This conclusion is confirmed by the qualitative reactions of the cottonseed meal products.

Silver salts of the products were prepared and fractionated, but no attempt was made to keep the conditions the same. As is to be expected in an acid of such high basicity, the silver salts vary in percentage of silver, but they all correspond to salts of the same acid, as the following analyses show:

TABLE I.—PERCENTAGE COMPOSITION OF THE SILVER SALTS OF THE PHOSPHORUS PRODUCTS.

	C.	H.	Ag.	P.
Cottonseed meal:				
Acid extraction:				
Fraction 1, found	4.60	0.91	61.79	9.54
$C_{12}H_{24}Ag_{17}P_9O_{42}$, calculated	4.88	0.81	62.10	9.45
Fraction 2, found	4.87	0.74	59.86	10.06
$C_{12}H_{25}Ag_{16}P_9O_{42}$, calculated	5.06	0.88	60.66	9.81
Ammonia extraction:				
Fraction 1, found	4.78	0.78	62.68	9.40
$C_{12}H_{24}Ag_{17}P_9O_{42}$, calculated	4.88	0.81	62.10	9.45
Fraction 2, found	4.97	1.08	57.15	10.20
$C_{12}H_{27}Ag_{14}P_9O_{42}$, calculated	5.47	1.03	57.41	10.60
Wheat bran:				
Acid extraction:				
Fraction 1, found	5.21	0.82	61.86	9.67
$C_{12}H_{24}Ag_{17}P_9O_{42}$, calculated	4.88	0.81	62.10	9.45
Fraction 2, found	4.75	0.97	59.27	10.12
$C_{12}H_{26}Ag_{15}P_9O_{42}$	5.26	0.95	59.08	10.18

The products correspond to salts of the acid $C_{12}H_{41}P_9O_{42}$. These results are not in accord with those of Patten and Hart, who claim that wheat bran contains an inosite-phosphoric acid corresponding to the formula $C_2H_8P_2O_9$; nor with those of Anderson, who claims that cottonseed meal contains an acid of similar composition; nor with those of Anderson claiming that wheat bran contains an inosite-phosphoric acid corresponding to the formula $C_{20}H_{55}P_9O_{45}$.

Portions of the crude products from cottonseed meal were decomposed into inosite and phosphoric acid, by heating in a closed tube with sulfuric acid, and the inosite separated and crystallized. It was identified by its carbon and hydrogen content, melting point, and the reactions of Gallois and Sherer.

We have the following evidence, then, that the compounds from the wheat bran and cottonseed meal are salts of the same acid: (1) The free acids yield inosite on decomposition with sulfuric acid; (2) the silver salts correspond to salts of the same acid, $C_{12}H_{41}P_9O_{42}$; (3) silver salts containing the same amount of silver are formed under the same conditions; (4) silver salts, prepared under the same conditions, have the same solubility in water; (5) the qualitative reactions of the acids from cottonseed meal are the same.

The fact that the various fractions of the silver salts correspond to salts of the same acid, and that the silver salts prepared under the same conditions have the same solubility in water, is evidence that they are pure.

We are therefore justified in concluding that the inosite-phosphoric acids of wheat bran and cottonseed meal are the same, and that the free acid corresponds to the formula $C_{12}H_{41}P_9O_{42}$.

Anderson[1] claims that the inosite-phosphoric acid of wheat bran is not phytic acid but an acid corresponding to the formula $C_{25}H_{65}P_9O_{54}$, and that this acid is easily transformed into one of the formula $C_{20}H_{55}P_9O_{49}$, losing one pentose group, $C_5H_{10}O_5$, in the process. According to his theory, if the product to which he assigns the formula $C_{25}H_{55}P_9O_{54}Ba_5$ contained one pentose group, this salt would contain 6.87% pentose. But his results show that it contained 0.88% pentose. This is 12.8% of 6.87, or *only one-eighth as much as his theory requires.*

From the phosphorus-pentose ratio in his theoretical barium salt, we can get an idea of what Anderson's crude lime-magnesium-potassium salt should contain. It is evident that the ratio would be constant, regardless of the amounts of other substances present. The amount of phosphorus in Anderson's crude salt corresponds to 7.76% pentose. Anderson's analysis shows, however, that it contained 10.94%. *His crude salt, then, contained 41% too much pentose and his barium salt 87% too little.*

It is evident from Anderson's own results that we have no evidence that wheat bran contains an inosite-phosphoric acid with pentose in the molecule.

After an analytical study of Anderson's methods of preparation of these products, we found that the products were contaminated with pentoses associated with very little, if any, phosphorus.

The Composition of Phytic Acid.—In the following table is shown the percentage composition (calculated) of inosite-phosphoric acid, or so-called phytic acid, according to the various investigators who have reported analyses of the acid:

[1] *Loc. cit.*

TABLE II.—PERCENTAGE COMPOSITION OF INOSITE-PHOSPHORIC ACID ACCORDING TO DIFFERENT INVESTIGATORS.

Investigator.	Proposed formula.	C.	H.	P.
Posternak and others	$C_2H_8P_2O_9$	10.08	3.36	26.07
Verbrodt	$C_{12}H_{40}P_{11}O_{46}$	11.42	3.17	27.04
Rising	$C_6H_{20}P_5O_{22}$	12.02	3.34	25.88
This article	$C_{12}H_{41}P_9O_{42}$	12.68	3.61	24.56

Our formula is $C_{12}H_{41}P_9O_{42}$; Rising's, multiplied by 2, would be $C_{12}H_{40}P_{10}O_{44}$; Verbrodt's is $C_{12}H_{40}P_{11}O_{46}$, while the formula proposed by Posternak, and agreed to by other investigators, multiplied by 6, would be $C_{12}H_{48}P_{12}O_{54}$. Both the formula and the percentage composition of our acid agree fairly well with that of Rising. The carbon content of Verbrodt's acid is 1.34% above that of the Posternak acid, and the results of Patten and Hart, Hart and Tottingham, and Anderson were always higher than their theory called for.

We believe our formula, $C_{12}H_{41}P_9O_{42}$, more nearly represents the truth, and propose it as the empirical formula of inosite-phosphoric acid, or the so-called phytic acid of feeding materials.

Summary.

The method for the preparation of inosite-phosphoric acid, or so-called phytic acid, on which rests the theory that this acid corresponds to the formula $C_2H_8P_2O_9$ or a multiple of it, produces an impure product containing notable amounts of inorganic impurities.

The inosite-phosphoric acids of wheat bran and cottonseed meal are identical and correspond to the formula $C_{12}H_{41}P_9O_{42}$.

We have no evidence that wheat bran contains an inosite-phosphoric acid with pentose in the molecule.

The formula $C_{12}H_{41}P_9O_{42}$ is proposed for the inosite-phosphoric acid, or so-called phytic acid, of feeding materials.

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ON SOME CONDITIONS AFFECTING THE ACTIVITY AND STABILITY OF CERTAIN FERMENTS.

BY J. H. LONG AND W. A. JOHNSON.

Received May 6, 1913.

The behavior of soluble ferments, or enzymes, toward other ferments and the action of foreign bodies, especially acids, alkalies and salts, on ferments, have been the subjects of a great number of investigations, some of which have a purely theoretical interest, while others have a more direct practical importance as bearing on questions of digestion and other actions in the animal body.

One of these queries touches the question of the mutual action of pepsin, trypsin and amylopsin and the behavior of each in presence of a great variety of chemical compounds. A special phase of this problem relates