LV.—*Phytin and Phytic Acid.*

By George Clarke.

The name phytin has been applied to a white, amorphous substance which has been observed by Palladin (*Zeitsch. Biol.*, 1895, **31**, 199) to occur in the seeds of many plants, and yields inositol and phosphoric acid on hydrolysis under pressure with solutions of mineral acids or alkalis. An examination of the methods used in preparing the material investigated by previous workers left no doubt that different substances have been described as phytin, and that these substances were not always homogeneous. This conclusion was confirmed by the divergent published results representing the composition of phytin (compare Jegorov, *Biochem. Zeitsch.*, 1912, **42**, 433, and Plimmer and Page, *Biochem. J.*, 1913, **7**, 158).

The acid obtained from phytin by the removal of the bases, calcium and magnesium, has been described as phytic acid, but no salt or derivative of it of undoubted purity has been isolated and analysed. Many of the substances described as phytic acid have been mixtures of phosphoric acid and an organic phosphoric acid.

Schulze and Winterstein (*Zeitsch. physiol. Chem.*, 1896, **22**, 90; 1903, **40**, 121) prepared phytin by extracting the fat-free seeds of *Sinapis nigra* with 10 per cent. sodium chloride solution, coagulating the proteins by boiling, and, after cooling and filtering, precipitating phytin from the cold protein-free sodium chloride solution by heating. The substance thus obtained was identical in properties with that prepared by the methods recorded below. These authors mentioned the fact that phytin was less soluble in hot than in cold acetic acid, but did not develop this method of preparation. Winterstein (*Ber.*, 1897, **30**, 2299) prepared phytin by extracting seeds with dilute acetic acid and precipitating it from the solution by ammonia. The free acid was obtained by the removal of the bases by means of oxalic acid, and yielded inositol and phosphoric acid on hydrolysis under pressure with concentrated hydrochloric acid. The substances prepared by the latter methods were undoubtedly mixed with calcium and magnesium phosphates and phosphoric acid.

Posternak (*Compt. rend.*, 1903, **137**, 202, 338, 439) extracted phytin from various fat-free seeds by means of very dilute hydrochloric acid, separating the substance by precipitation of the copper salt, decomposing the latter by hydrogen sulphide, and treating with alcohol the syrupy acid substance obtained by the evaporation of the acid solution. The final product was soluble in water, and thus differed from that obtained by Schulze and Winterstein (*loc.*

cit.) and the present author, which was insoluble. Posternak stated that the substance prepared by him was free from nitrogen and inorganic phosphates.

Treatment with alcohol of the syrupy acid residue obtained by the latter worker would separate a large amount of inorganic phosphoric acid from the final product, but an examination of the free acid liberated from a product obtained by analogous methods, by carefully fractionating the strychnine salt, showed that it contained inorganic phosphoric acid. In this connexion it is of interest to note that Page and Plimmer (*Biochem. J.*, 1913, **7**, 168) have found that samples of commercial phytin always contained inorganic phosphoric acid.

Free phytic acid, obtained by Posternak, was described as a pale yellow, transparent syrup, yielding inositol and phosphoric acid on hydrolysis, and giving no precipitate in the cold, but a characteristic yellow precipitate on warming with ammonium molybdate solution. He ascribed to the acid the formula $C_2H_8O_9P_2$, and the constitution represented by anhydro-oxymethylenediphosphoric acid,

$$[CH_2{\cdot}O{\cdot}PO(OH)_2]_2O.$$

One of the arguments on which this formula was based was the fact that phytin and phytic acid were not hydrolysed by alkalis, a statement that Winterstein (*Zeitsch. physiol. Chem.*, 1908, **58**, 121) has since shown to be incorrect. Neuberg (*Biochem. Zeitsch.*, 1908, **9**, 557) has brought forward additional evidence to show that the inositol ring formation exists in phytin and phytic acid.

The methods used in the preparation of the material, on the analysis of which Posternak based the formula $C_2H_8O_9P_2$, did not preclude the possibility of admixture with phosphoric acid.

The phytin previously examined had been prepared by one of the methods described, or some slight modification of them.

Starkenstein (*Biochem. Zeitsch.*, 1911, **30**, 59) has stated that air-dried commercial phytin contains a considerable quantity of inorganic phosphoric acid, and that the amount is increased by drying at 100°. He attributed this to the decomposition of phytin into inositol and phosphoric acid at that temperature, but did not record the isolation of inositol. Anderson (*J. Biol. Chem.*, 1912, **11**, 473) failed to confirm his conclusions that phytin was so easily decomposed.

The results of experiments recorded in this communication have shown that the free acid liberated from air-dried phytin of homogeneous composition—which was separated from admixed mineral phosphates by precipitation from cold dilute acetic acid by boiling—consisted of a mixture of approximately equal quantities of an organic phosphoric acid (phytic acid) and phosphoric acid.

A solution of the ammonium salt of the organic phosphoric acid, prepared from a pure strychnine salt, gave no precipitate on warming to 60° with a nitric acid solution of ammonium molybdate, and only a very slight one on remaining at that temperature for several hours.

An explanation of the behaviour of phytin is that it is not simply a salt of an inositolphosphoric acid, but a complex substance, possibly a complex calcium-magnesium salt of an inositolphosphoric acid and phosphoric acid, and, on removing the bases, yields the two acids. The fact that the composition of pure phytin, prepared as described below, corresponded with no calcium-magnesium salt of a simple acid ester of inositol and phosphoric acid, gave support to this view. The strychnine salt of the organic phosphoric acid, isolated from the mixture of acids obtained from phytin, on the other hand, gave results on analysis in agreement with salts of simple inositolphosphoric acids.

Anderson (*J. Biol. Chem.*, 1912, **11**, 471) prepared from commercial phytin obtained from two sources a series of salts, which on analysis gave figures corresponding with salts of inositolhexaphosphoric acid, $C_6H_6O_6[PO(OH)_2]_6$. He described an acid tribarium phytate prepared by precipitation from 0·5 per cent. hydrochloric acid solution by the addition of an equal volume of alcohol. This salt was probably one of the purest derivatives of phytic acid hitherto isolated, but it seemed not impossible from the methods of preparation that the salt as well as the acid prepared from it might contain some phosphoric acid.

Experimental.

Preparation of Phytin.

The seeds of Indian field mustards, a mixture of *Brassica juncea* (H. fil. and T.) and *Brassica campestris* (Linn.), were extracted with petroleum for several days. The petroleum extract was separated by means of a centrifugal machine, and the seeds dried in the sun for a few hours. Twenty-seven kilograms of air-dried, fat-free seeds were extracted for seven days with 100 litres of 4·5 per cent. acetic acid. The extract was separated from the seeds as completely as possible in the centrifuge, boiled for fifteen minutes, and allowed to cool. This procedure coagulated a large portion of the proteins. After remaining overnight, the supernatant extract was easily syphoned off. The dark brown extract thus obtained was again heated to boiling. A small quantity of phytin separated out, but the solution was too dilute and impure for any quantity of material to be obtained in this manner. Aqueous ammonia was

then added to the boiling extract until it was just alkaline, and the boiling continued for a few minutes. A large quantity of dark-coloured precipitate (A) was thus obtained, which was separated by filtration while still hot, and well washed with boiling water. The crude precipitate (A) contained phytin, calcium and magnesium-ammonium phosphates, and a considerable quantity of protein and other organic impurity. It was intimately mixed with 16 litres of 8 per cent. acetic acid, and extracted for two or three hours with constant shaking. The extract was separated from the undissolved organic impurities by filtration through cloth, boiled, allowed to cool overnight, and the cold solution thoroughly stirred. The undissolved and precipitated matter was then easily separated by filtration through paper, and a perfectly clear filtrate obtained. Aqueous ammonia was added to the boiling filtrate until it was just alkaline, the white precipitate (B) being collected on a filter and washed with boiling water until almost free from ammonia. The white precipitate (B) consisted of phytin and calcium and magnesium-ammonium phosphates. It was dissolved in 6 litres of 8 per cent. acetic acid, and a very small amount of insoluble matter separated by filtration. The clear solution thus obtained was boiled for some minutes. Much phytin was precipitated, and it was separated by filtration through a Büchner funnel while the liquid was still hot, then well washed with boiling water, and finally with ethyl alcohol, 82 grams being obtained. The hot filtrate from which the phytin had been separated was again made alkaline with ammonia, and the precipitate (C) separated and washed. It was dissolved in 2 litres of 8 per cent. acetic acid, and phytin separated by boiling, filtering while still hot, and washing with water and alcohol as described above. Fifteen grams were obtained. The filtrate from this was again subjected to similar treatment, a smaller volume of 8 per cent. acetic acid being used (1 litre), and yielded 8 grams of phytin. The residual filtrate on treatment with excess of ammonia yielded a further precipitate, which consisted mainly of calcium and magnesium-ammonium phosphates, and contained only a very small amount of carbon.

The total yield of phytin was 105 grams, or 0·38 per cent. of the air-dried, fat-free seeds.

The following yields of phytin were obtained in other preparations:

27·0	kilograms of air-dried fat-free seeds	gave	125	grams of phytin	=	0·46	per cent.
*28·8	,,	,,	,,	165	,,	,,	= 0·57	,,
31·5	,,	,,	,,	152	,,	,,	= 0·47	,,

* Seeds extracted with 0·2 per cent. hydrochloric acid.

Very dilute hydrochloric acid can be used instead of dilute acetic

acid for the extraction of the seeds, and some of the material used in this investigation was prepared by extraction with 0·2 per cent. hydrochloric acid, the phytin being subsequently purified by separation from 8 per cent. acetic acid. It was found, however, that the extracts obtained by the use of dilute hydrochloric acid were more difficult to handle than those obtained by dilute acetic acid.

Phytin prepared in the manner described above was a snow-white, amorphous powder, resembling in properties the substance described by Schulze and Winterstein (*Zeitsch. physiol. Chem.*, 1896, **22**, 90). It contained carbon, hydrogen, phosphorus, calcium and magnesium, but no trace of nitrogen could be detected. It was insoluble in hot and cold water, readily soluble in very dilute mineral acids, and soluble in cold, but sparingly so in hot dilute acetic acid. It was precipitated from a cold 8 per cent. acetic acid solution on boiling, completely redissolving when allowed to cool.

A solution of phytin in very dilute nitric acid gave an abundant yellow precipitate with acid ammonium molybdate solution on warming to 60°.

The following fractions from different preparations were prepared for analysis:

(A) Fifty grams of phytin dissolved in 2000 c.c. of distilled water and 100 c.c. of glacial acetic acid. The solution obtained was quite clear and free from undissolved material. It was heated, not quite to boiling. The precipitated phytin was separated by filtration, well washed with boiling water until free from acid, finally with alcohol, and dried on a porous plate.

(B) The filtrate from A was boiled for fifteen minutes. Phytin was again precipitated, separated, washed with boiling water, but not with alcohol, and dried on a porous plate.

(C) Seventy grams of phytin from a separate preparation were dissolved in 2500 c.c. of 5 per cent. acetic acid. The clear solution was heated for some time by passing steam into it. The precipitated phytin was separated, washed with water and alcohol, and dried.

(D) Seventy grams of phytin from a preparation made by extracting mustard seeds with 0·2 per cent. hydrochloric acid, and subsequent purification by precipitation from 8 per cent. acetic acid, were dissolved in 2500 c.c. of 5 per cent. acetic acid. Phytin was separated from the perfectly clear solution by heating, washed and dried.

It is somewhat difficult to obtain phytin in an anhydrous condition. After heating for several hours at 110° in a vacuum over phosphoric oxide, it still continued to lose weight. When heated

under similar conditions at 180° for five hours it became constant in weight, and remained so after prolonged heating for many hours. The anhydrous substance, dried at 180° and dissolved in dilute acetic acid, was precipitated again unchanged by boiling.

Plimmer and Page (*Biochem. J.*, 1913, **7**, 162) have referred to the difficulty of analysing phytin, and have critically examined the methods of determining calcium and magnesium in the presence of phosphoric acid. They mention also the difficulty of completely burning carbon in the presence of phosphoric acid.

The following methods were used in the analyses of phytin recorded below:

Carbon and Hydrogen.—The anhydrous substance was intimately mixed with the finest powdered copper oxide.

Calcium and Magnesium.—The organic matter was oxidised by concentrated sulphuric acid. After diluting with water, calcium was separated from the solution as calcium sulphate by the addition of an equal volume of alcohol, and magnesium estimated in the filtrate as $Mg_2P_2O_7$, after the removal of alcohol by evaporation and oxidation with nitric acid as described by Plimmer and Page (*loc. cit.*).

Phosphorus was determined (after the oxidation of the organic matter by concentrated sulphuric acid) by means of ammonium molybdate, and weighed as $Mg_2P_2O_7$.

The following results were obtained on analysis of the fractions described above, after drying in a vacuum over phosphoric oxide at 180°:

Fraction A.

0·4176 gave 0·1482 CO_2 and 0·0620 H_2O. C = 9·67; H = 1·64.
0·4866 „ 0·3044 $CaSO_4$ and 0·041 $Mg_2P_2O_7$. Ca = 18·39; Mg = 1·84.
0·4421 gave 0·3210 $Mg_2P_2O_7$. P = 20·21.
*0·4866 „ 0·359 $Mg_2P_2O_7$. P = 20·53.

Fraction B.

0·4650 gave 0·1694 CO_2 and 0·0698 H_2O. C = 9·93; H = 1·66.
0·4592 „ 0·2812 $CaSO_4$ and 0·0458 $Mg_2P_2O_7$. Ca = 18·01; Mg = 2·18.
0·2820 gave 0·2072 $Mg_2P_2O_7$. P = 20·45.

* Estimated as $Mg_2P_2O_7$, after separation of $CaSO_4$.

Fraction C.

0·4330 gave 0·1530 CO_2 and 0·0756 H_2O. C = 9·63; H = 1·94.
0·4070 „ 0·1440 CO_2 „ 0·0684 H_2O. C = 9·65; H = 1·86.
0·6210 „ 0·3880 $CaSO_4$ and 0·0520 $Mg_2P_2O_7$. Ca = 18·37; Mg = 1·83.
0·4504 gave 0·3370 $Mg_2P_2O_7$. P = 20·82.

Fraction D.

0·3758 gave 0·1334 CO_2 and 0·0530 H_2O. C = 9·68; H = 1·56.
*0·4774 „ 0·1626 CO_2 „ 0·0680 H_2O. C = 9·29; H = 1·58.
0·4950 „ 0·3100 $CaSO_4$ and 0·0404 $Mg_2P_2O_7$. Ca = 18·40; Mg = 1·78.
0·3680 gave 0·2736 $Mg_2P_2O_7$. P = 20·69.
$C_{12}H_{22}O_{44}P_{10}Ca_7Mg$ requires C = 9·70; H = 1·48; Ca = 18·87; Mg = 1·61; P = 20·88.
$C_{12}H_{20}O_{43}P_{10}Ca_7Mg$ requires C = 9·82; H = 1·34; Ca = 19·09; Mg = 1·63; P = 21·14.
$C_{12}H_{16}O_{41}P_{10}Ca_7Mg$ requires C = 10·06; H = 1·12; Ca = 19·58; Mg = 1·67; P = 21·67 per cent.

The composition of separate preparations of phytin, purified by separation from cold dilute acetic acid, was constant.

Phytin is decomposed by heating under pressure with 30 per cent. sulphuric acid into inositol and phosphoric acid (Posternak, *Compt. rend.*, 1903, **137**, 439; Winterstein, *Ber.*, 1897, **30**, 2299).

Fifteen grams of phytin were heated with 67 c.c. of 30 per cent. sulphuric acid in a sealed tube at 130° for ten hours. The dark-coloured solution was diluted with water, and calcium sulphate, which had separated out, removed by filtration. Excess of sulphuric acid was removed by treatment with finely powdered barium carbonate. The solution, which contained inositol, was evaporated to a small bulk, removing from time to time the slight deposits of mineral matter. The concentrated solution was acidified with two drops of nitric acid, poured into five times its volume of ethyl alcohol, and ether added. Inositol separated out as a viscid mass, which quickly solidified. Yield = 3·5 grams.

Six grams of inositol from the above and other similar preparations were boiled for two hours with 50 grams of recently distilled acetic anhydride and 0·5 grams of zinc chloride, and the reaction mixture poured into cold water. Hexa-acetylinositol separated, and was purified by several recrystallisations from ethyl alcohol. It melted sharply at 211° (uncorr.), and was dried at 110° for analysis.

* Anhydrous substance mixed with powdered lead chromate.

(Found, C = 50·0; H = 5·6. $C_{18}H_{24}O_{12}$ requires C = 50·0; H = 5·5 per cent.)

The decomposition of phytin into inositol and phosphoric acid takes place in accordance with the following equation:

$$C_{12}H_{22}O_{44}P_{10}Ca_7Mg + 8H_2O + 8H_2SO_4 = 2C_6H_{12}O_6 + 10H_3PO_4 + 7CaSO_4 + MgSO_4.$$

Fifteen grams of phytin gave 3·5 grams of inositol.

$C_{12}H_{22}O_{44}P_{10}Ca_7Mg$ requires 3·6 grams of inositol.

Examination of the Acid from Phytin, $C_{12}H_{22}O_{44}P_{10}Ca_7Mg$.

Seventy grams of air-dried phytin were dissolved in 2·5 litres of 5 per cent. acetic acid, and basic lead acetate solution was added until no further precipitate was produced. The lead salt was separated, washed with boiling water until free from acetic acid, and decomposed by hydrogen sulphide. Excess of the latter was removed from the acid solution by boiling under diminished pressure, and sufficient cold saturated copper acetate solution added to precipitate the acid. The copper salt was separated, washed with boiling water, and deprived of copper by means of hydrogen sulphide. The strongly acid solution thus obtained was evaporated to a syrup under diminished pressure, and treated with 500 c.c. of 95 per cent. ethyl alcohol. A large quantity of a white, flocculent substance (I) separated. This was coagulated by boiling for a few minutes, filtered from the alcoholic extract, and washed with a little alcohol.

The white substance (I) contained phosphorus, calcium and carbon, and readily dissolved in cold water. Its aqueous solution was precipitated with cold saturated copper acetate solution. The copper salt was decomposed by hydrogen sulphide, and the acid solution evaporated to a syrup. This syrup was treated with 95 per cent. ethyl alcohol (300 c.c.). A smaller amount of a white substance (II) separated, which was filtered from the alcoholic extract after boiling, and washed with alcohol. Its aqueous solution was again precipitated with copper acetate solution, the copper salt separated, deprived of copper, and the acid solution treated as before. This procedure was repeated until the syrupy acid residue was completely soluble in 95 per cent ethyl alcohol. About five operations were necessary.

The alcoholic solutions of the acid obtained by the above operations were mixed together, evaporated under diminished pressure, and finally dried in a vacuum over sulphuric acid.

The acid thus obtained was a viscid, dark-coloured syrup, free from calcium and magnesium. It was very readily soluble in

water or alcohol, and gave a yellow precipitate with acid ammonium molybdate solution on slightly warming.

Twenty-two grams of acid produced from phytin in the manner described above were dissolved in 3 litres of water, and 50 grams of recently precipitated strychnine, in as fine a state of division as possible, added to the solution. On heating, nearly all the strychnine dissolved. A small amount of resinous impurity separated, and was removed. The solution of strychnine salts was evaporated under diminished pressure to 1 litre, and allowed to remain overnight. Strychnine phytate separated out in small, colourless, needle-shaped crystals, melting at 203—204° (uncorr.). This salt was crystallised many times from water, in which it was only sparingly soluble. The melting point remained unchanged. Strychnine phytate contains water of crystallisation, which it loses very slowly on exposure to air, rapidly when dried at 115°. Its aqueous solution gave an acid reaction with blue litmus.

Separate fractions of strychnine phytate dried at 115° gave, on analysis, the following results (the anhydrous salt was intimately mixed with the finest powdered copper oxide for the estimation of carbon and hydrogen):

0·2208	gave	0·4776 CO_2	and	0·1188 H_2O.	C = 58·99; H = 5·98.
0·2407	„	0·5198 CO_2	„	0·1284 H_2O.	C = 58·89; H = 5·92.
0·3510	„	0·7560 CO_2	„	0·1832 H_2O.	C = 58·74; H = 5·79.
0·1762	„	0·3816 CO_2	„	0·0950 H_2O.	C = 59·05; H = 5·98.
0·1888	„	0·4092 CO_2	„	0·0970 H_2O.	C = 59·11; H = 5·70.
0·2750	„	0·5930 CO_2	„	0·1440 H_2O.	C = 58·80; H = 5·81.
					Mean = 58·92; = 5·86.

0·3936	gave	0·0934 $Mg_2P_2O_7$.	P = 6·59.
0·4896	„	0·1158 $Mg_2P_2O_7$.	P = 6·58.
0·6090	„	0·1440 $Mg_2P_2O_7$.	P = 6·58.
0·5354	„	0·1300 $Mg_2P_2O_7$.	P = 6·75.
0·4574	„	0·1138 $Mg_2P_2O_7$.	P = 6·92.
0·3874	„	0·0960 $Mg_2P_2O_7$.	P = 6·89.
			Mean = 6·72.

0·4176,	air-dried salt,	lost	0·0302 H_2O.	H_2O = 7·23.
0·6916	„	„	0·0480 H_2O.	H_2O = 6·94.
1·2360	„	„	0·0846 H_2O.	H_2O = 6·84.
				Mean = 7·00.

The composition of the strychnine salt agreed with the strychnine salts of several inositolphosphoric acids in which strychnine and phosphorus are in the ratio of 1 molecule of strychnine to 1 atom of phosphorus; for example:

I. $C_6H_8O_2(HPO_4)_2,2C_{21}H_{22}O_2N_2$ requires C = 59·25; H = 5·55; P = 6·37 per cent.

$C_6H_8O_2(HPO_4)_2,2C_{21}H_{22}O_2N_2,4H_2O$ requires H_2O = 6·90 per cent.

II. $C_6H_8O_2(H_2PO_4)_4,4C_{21}H_{22}O_2N_2$ requires C = 58·87; H = 5·66; P = 6·75 per cent.

$C_6H_8O_2(H_2PO_4)_4,4C_{21}H_{22}O_2N_2,8H_2O$ requires H_2O = 7·27 per cent.

III. $C_6H_6(H_2PO_4)_6,6C_{21}H_{22}O_2N_2$ requires C = 59·45; H = 5·63; P = 6·98 per cent.

$C_6H_6(H_2PO_4)_6,6C_{21}H_{22}O_2N_2,12H_2O$ requires H_2O = 7·50 per cent.

II and III are acid salts. The acids in formulæ I and II are capable of yielding a complex calcium magnesium salt with phosphoric acid of the composition $C_{12}H_{22}O_{44}P_{10}Ca_7Mg$, which would decompose on removal of the bases and liberation of the free acid into inositolphosphoric acids and phosphoric acid.

The solution from which strychnine phytate (m. p. 203—204°) had separated was further evaporated under diminished pressure. After the separation of a small additional quantity of salt, melting at 203—204°, the mother liquors, on concentrating to small bulk, deposited a large quantity of a readily soluble salt (m. p. 252—253°). The amount of this salt was approximately equal to the weight of strychnine phytate obtained. It was easily separated in a state of purity from the sparingly soluble strychnine phytate, and proved, on examination, to be strychnine dihydrogen phosphate.

0·3130 gave 0·0824 $Mg_2P_2O_7$. P = 7·32.

0·2962 „ 0·0754 $Mg_2P_2O_7$. P = 7·08.

$C_{21}H_{22}O_2N_2,H_3PO_4$ requires P = 7·17 per cent.

Phosphoric acid was prepared from the strychnine dihydrogen phosphate described above by decomposing an aqueous solution with excess of sodium carbonate solution, separating the strychnine, precipitating the phosphate as lead phosphate in the presence of dilute acetic acid, and decomposing the lead salt by means of hydrogen sulphide. An aqueous solution of the phosphoric acid was treated with sufficient benzylamine to form benzylamine dihydrogen phosphate, and the latter salt purified by recrystallisation from water. When dried at 110° it gave the following results on analysis:

0·3030 gave 0·1642 $Mg_2P_2O_7$. P = 15·08.

C_7H_9N,H_3PO_4 requires P = 15·12 per cent.

Additional proof that the acid liberated from phytin contains much phosphoric acid, in addition to the organic phosphoric acid already described, was obtained during attempts to prepare the *l*-menthylamine salt.

Nineteen grams of *l*-menthylamine were neutralised by a solution of the mixed acids prepared from phytin, and an equal volume of the same solution was added. This strongly acid solution on slow

evaporation in a vacuum over sulphuric acid deposited, in the form of rhombic prisms, 15 grams of *l*-menthylamine dihydrogen phosphate.

After purification and drying at 100° it gave, on analysis, the following result:

0·2268 gave 0·0984 $Mg_2P_2O_7$. P = 12·07.
0·3334 „ 0·1442 $Mg_2P_2O_7$. P = 12·04.

$C_{10}H_{21}N,H_3PO_4$ requires P = 12·21 per cent.

The solution from which *l*-menthylamine dihydrogen phosphate had been separated, on further evaporation in a vacuum over sulphuric acid, deposited *l*-menthylamine phytate in the form of an uncrystallisable oil.

Preparation of Inositol from Strychnine Phytate.

Nineteen grams of pure air-dried strychnine phytate (m. p. 203—204°) were dissolved in the least possible quantity of boiling water, and decomposed by a slight excess of aqueous ammonia. Strychnine was separated after remaining overnight, and the solution of ammonium phytate evaporated to dryness and dried in a desiccator over sulphuric acid. Ammonium phytate is a non-crystallisable gum, very readily soluble in water. In the presence of a few drops of dilute nitric acid it gives no precipitate with acid ammonium molybdate solution on warming to 60°, but if allowed to remain for several hours at that temperature a very slight yellow precipitate is formed, owing to the slow hydrolysis of phytic acid.

Five grams of dry ammonium phytate were dissolved in 25 c.c. of 30 per cent. sulphuric acid and heated in a sealed tube at 120—130° for eight hours. The dark-coloured solution was diluted with water and filtered from a small deposit of carbon. Sulphuric acid was removed by treatment with barium carbonate, and the solution containing inositol evaporated to small bulk, acidified with a few drops of nitric acid, and poured into alcohol. Inositol separated as a solid, crystalline mass (1·5 grams). The hexa-acetyl derivative, prepared by boiling with acetic anhydride and a trace of zinc chloride, was purified by crystallisation from ethyl alcohol, and melted sharply at 211° (uncorr.). It was dried at 110° for analysis. (Found, C = 50·27; H = 5·47. $C_{18}H_{24}O_{12}$ requires C = 50·00; H = 5·55 per cent.).

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