47. ESTERS OF PHOSPHORIC ACID
4. PHOSPHORYL HYDROXYAMINO-ACIDS

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PHOSPHOTYROSINE was made by Levene & Schormüller [1933] by the action of $POCl_3$ in CCl_4 on formyltyrosine in presence of MgO. From the Mg salt of formylphosphotyrosine the formyl group was removed by hydrolysis with HCl and phosphotyrosine was obtained through the Ba and Pb salts.

Levene & Schormüller [1934, 1] tried three methods of preparing phospho-*dl*-serine of which only direct phosphorylation with H_3PO_4 and P_2O_5 was successful. The Ba salt was isolated. Later Levene & Schormüller [1934, 2] prepared larger quantities and separated the stereoisomers by fractional crystallization of the brucine salts. They also isolated the Ba salt of phospho-*l*-hydroxyproline after direct phosphorylation. Phosphohydroxyaspartic acid and phosphohydroxyglutamic acid could not be made. Neither phosphoserine nor phosphohydroxyproline were prepared and very little information was given of the properties of the compounds. Further information was desirable, especially concerning the hydrolysis of these esters by acid, alkali and phosphatase to compare with caseinogen, caseo-phosphopeptone and other esters of phosphoric acid.

EXPERIMENTAL

1. *Phosphotyrosine*

The successful phosphorylation of amino-ethanol and choline by Plimmer & Burch [1937] by heating the substances with H_3PO_4 and P_2O_5 led to a trial with tyrosine. On direct heating, the mixture darkened and effervesced, but it was possible to isolate a small yield of the Ca salt of phosphotyrosine. Subsequent experiments were made by heating the mixture (2–5 g. tyrosine, 14–35 g. H_3PO_4 and 2–5 g. P_2O_5) in a small flask with $CaCl_2$ tube on a water bath for 1, 6, 48, 64 hr. to ascertain the condition for the best yield. The resulting syrup was dissolved in water and made to 500 ml. and an aliquot was taken for N determination to give information of the amount of substance in later solutions. The solution was made alkaline to phenolphthalein with a fine suspension of $Ca(OH)_2$, or with $Ba(OH)_2$ solution, and filtered from $Ca_3(PO_4)_2$ and excess $Ca(OH)_2$, or $Ba_3(PO_4)_2$, which was washed once or more with water until all the N was in solution. In one experiment the Ca salt was isolated by concentrating *in vacuo* and adding an equal volume of absolute alcohol. The air-dried salt was heated at 100° *in vacuo* over P_2O_5 for analysis. (Found: N, 4·48; P, 10·02; Ca, 13·55; H_2O, 14·53 %. $C_9H_{10}O_6NPCa$ requires N, 4·68; P, 10·36; Ca, 13·40; $+3H_2O$ requires H_2O, 15·30 %.) The low water content is probably due to diffculty in removing water from phosphoric esters. Some $CaCO_3$ was present.

In another experiment the Ba salt was isolated by adding an equal volume of absolute alcohol to the concentrated filtrate. It came down as a very fine precipitate; if kept in air it became syrupy and set to a glass which was easily

powdered. It was redissolved in water, reprecipitated and dried in a desiccator. For analysis, it was heated at 100° *in vacuo* over P_2O_5. (Found: N, 3·58; P, 7·48; Ba, 35·22; H_2O, 13·22 %. $C_9H_{10}O_6NPBa$ requires N, 3·53; P, 7·79; Ba, 34·65; $+4H_2O$ requires H_2O, 15·38 %.)

Again, the difficulty in removing H_2O from phosphoric esters will account for the low H_2O value. $BaCO_3$ was also present.

In other experiments lead acetate solution was added to the filtrate until there was no further precipitation. The Pb salt, after washing, was suspended in water and decomposed with H_2S and the filtrate from PbS concentrated and treated with an equal volume of absolute alcohol. The resulting crystals were filtered off and washed with 50 % alcohol. Further crops of phosphotyrosine were obtained on concentrating the solution and adding alcohol. The crops crystallizing out first consisted of nearly pure phosphotyrosine; later crops were admixed with tyrosine. The pure compound was obtained by redissolving, filtering and adding alcohol. The several preparations dried at 100° *in vacuo* over P_2O_5 gave on analysis: N, 5·32, 5·42, 5·41, 5·20; P, 11·92, 12·13, 11·89, 11·68 %. $C_9H_{12}O_6NP$ requires N, 5·34 %, P, 11·83 %.

Phosphotyrosine crystallizes in shining platelets; on heating it shrinks at 224° and melts at 225°. It dissolves slowly in cold water, more rapidly on warming. 0·2584 g. in 25 ml. 2*N* HCl gave in a 2 dm. tube a rotation of −0·19°, $[\alpha]_D - 9·19°$. These data do not agree with those of Levene & Schormüller [1933] who gave M.P. 253° and $[\alpha]_D - 2·0°$.

A 0·6 % solution of phosphotyrosine in water gave precipitates only with Pb acetate and $HgNO_3$ solutions. The other salts are thus soluble in water. The Pb salt, isolated in one preparation, dried in air and heated at 100° *in vacuo* over P_2O_5, gave on analysis: N, 2·93; P, 6·86; Pb, 43·74; H_2O, 3·88 %. $C_9H_{10}O_6NPPb$ requires N, 3·00; P, 6·65; Pb, 44·42; $+H_2O$ requires H_2O, 3·72 %.

Phosphotyrosine does not give a blue colour with Folin's phenol reagent. With Millon's reagent at room temperature it turns brown on standing; on warming there is slow development of colour, probably due to hydrolysis to tyrosine.

The yields of phosphotyrosine were 28 % on heating for 1 hr.
41 % ,, 6 hr.
48 % ,, 48 hr.
55 % ,, 64 hr.
the extra time thus being advantageous.

2. *Phosphohydroxyproline*

l-Hydroxyproline (Roche) was phosphorylated in the same way as tyrosine with 2 g. at a time. The syrupy residue was dissolved in water, and the solution treated with $Ba(OH)_2$ solution, or $Ca(OH)_2$ suspension. The filtrate and washings from the precipitate were concentrated *in vacuo* and precipitated with alcohol. The yield of Ba salt was 48 % on phosphorylating for 24 hr. and 51 % after 40 hr. At room temperature the phosphorylation was 26 % in 13 days. The Ba salt was dried in air and heated at 100° *in vacuo* over P_2O_5 for analysis. (Found: N, 3·46; P, 8·81; Ba, 39·23; H_2O, 16·24 %. $C_5H_8O_6NPBa$ requires N, 4·05; P, 8·96; Ba, 39·50; $+4H_2O$ requires H_2O, 17·23 %.)

If precipitated from hot solution, H_2O was 3·50 to 3·88 %, and the salt dried at 100° *in vacuo* over P_2O_5 showed N 3·9, P 8·5, Ba 36·6 %, indicating $1H_2O$ and partial conversion into acid salt.

The Pb salt was prepared from the Ba or Ca salt by precipitation with Pb acetate. After filtration and washing it was suspended in water and decomposed with H_2S. The filtrate from PbS was evaporated *in vacuo* to a small volume and alcohol added in small portions. Phosphohydroxyproline came down as an oil, which could be hardened with alcohol and became crystalline, or it could be obtained crystalline directly by very slowly adding alcohol and scratching.

The analyses of the several specimens of phosphohydroxyproline presented difficulty in the interpretation of the results:

On heating the air-dried substance at 100° *in vacuo* over P_2O_5 and analysing the residue:

H_2O varied from 4·96 to 5·75 %;

N varied from 5·81 to 6·11 %; average 6·00 %;

P varied from 13·46 to 14·00 %; average 13·70 %.

The most usual figures were N, 6·10; P, 13·58 %.

$C_5H_{10}O_6NP$ requires N, 6·63; P, 14·69 %. $C_5H_{10}O_6NP$, H_2O requires N, 6·11; P, 13·54; H_2O, 7·86 %.

The air-dried substance showed N, 5·66; P, 13·28 %.

$C_5H_{10}O_6NP$, $1{\cdot}5H_2O$ requires N, 5·88; P, 13·03 %.

The loss of H_2O on heating at 100° is thus between 1·5 and $1H_2O$.

Not until the substance was heated to 130° *in vacuo* over P_2O_5 was the water of crystallization completely driven off. (Found: H_2O, 10·91; N, 6·66; P, 14·96 %. On exposing the dried substance to air it took up H_2O, 7·71 %.)

The air-dried substance would thus be $C_5H_{10}O_6NP$, $1{\cdot}5H_2O$; after heating to 100° $C_5H_{10}O_6NP$, H_2O; after heating to 130° $C_5H_{10}O_6NP$.

Phosphohydroxyproline crystallizes in fine needles, M.P. 115°. The anhydrous substance melts at 130–131° with frothing but without decomposition. 0·2738 g. dissolved in 15 ml. H_2O (1·825 %) in a 2 dm. tube gave a rotation of $-1{\cdot}05°$, $[\alpha]_D - 28{\cdot}76°$. The solution gave only an insoluble lead salt.

3. *Phosphoserine*

Poor yields of the Ba salt of phosphoserine were obtained by Levene & Schormüller [1934, 1, 2] by the interaction of serine, H_3PO_4 and P_2O_5 for 40 hr. at room temperature; thus 1·85 g. from 4 g. serine = approx. 15 %, and 106 g. from 200 g. serine = approx. 17 %.

As good yields of phosphotyrosine and phosphohydroxyproline were obtained on heating at 100°, the same procedure of heating 1 g. serine, 7 g. H_3PO_4 and 1 g. P_2O_5 was tried. There was no product either on heating for 24 or 45 hr. On keeping the mixture for long periods of 4, 24 and 46 days yields of Ba salt amounting to 0·5, 16 and 26 % resulted. It was possible therefore that any phosphoserine formed at 100° was hydrolysed.

Heating in an autoclave at 20 lb. pressure for 1–1·5 hr. gave yields of Ba salt varying from 8 to 21 %, no improvement on the yields at room temperature. An advantage is that the preparation takes less time.

The isolation of the Ba or Ca salt was effected in the usual way. The specimens of Ba and Ca salts after drying in air were heated at 100° *in vacuo* over P_2O_5 for analysis. (Found: N, 4·27; P, 9·63; Ba, 42·62; H_2O, 14·69 %. $C_3H_6O_6NPBa$ requires N, 4·37; P, 9·67; Ba, 42·87; $+3H_2O$ requires H_2O, 14·43 %.)

Other preparations contained $BaCO_3$.

The Ba salt of serine could be recovered from the preparations in which there was no phosphorylation and also from the filtrates from Ba phosphoserine. This salt is less soluble in water than expected.

The Pb salt of phosphoserine was precipitated on adding basic Pb acetate to the solution of the Ba salt. The air-dried salt was heated to 100° *in vacuo* over P_2O_5 for analysis. (Found: N, 1·86; P, 4·39; Pb, 61·91; H_2O, 6·09 %. It appears to be a basic salt: $C_3H_6O_6NPPb$. $Pb(OH)_2$ requires N, 2·22; P, 4·91; Pb, 65·61; $+3H_2O$ requires H_2O, 7·88 %.)

Phosphoserine was prepared from the Pb salt in the usual way by precipitating with 3 to 4 vol. alcohol. Only three specimens, amounting to 0·2, 0·14 and 0·3 g., were made. The air-dried substances heated at 100° *in vacuo* over P_2O_5 gave N, 7·94, 7·56, 7·37; P, 16·72, 16·83, 16·81 %. $C_3H_8O_6NP$ requires N, 7·57; P, 16·76 %.

Phosphoserine crystallizes in irregular platelets which darken on heating and melt at 165–166° with decomposition. A 0·5 % solution gave insoluble salts only with Pb acetate and $HgNO_3$.

4. *Phosphoisoserine*

Phosphorylation of *iso*serine, prepared by Burch [1930], did not take place on standing at room temperature for 54 days. Poor yields (13–16 %) resulted on heating 1 g. *iso*serine, 7 g. H_3PO_4 and 1 g. P_2O_5 at 20 lb. pressure for 1–1·5 hr. The isolation was as described under phosphoserine.

The Ba salt could not be obtained pure and with the small amount available conversion into phospho*iso*serine could not be attempted.

5. *Phosphothreonine*

The mixture of stereoisomers of β-hydroxy-α-aminobutyric acid was prepared by the method of West & Carter [1937]. A yield of 89 % of the mercuric acetate compound of β-methoxybutyric acid was obtained, and from this a 92·5 % yield of β-methoxy-α-bromobutyric acid. 10 g. portions were converted into β-methoxy-α-aminobutyric acid by heating with conc. NH_4OH in a pressure bottle in boiling water for 1·5 hr. The yields were 60–63 %. Hydrolysis with HBr gave yields of 60–68 % of β-hydroxy-α-aminobutyric acids. The pure compound, recrystallized from water and alcohol, had N, 11·74 % (calc. 11·76 %) and M.P. 225–226° with decomposition, which corresponds with 228–229° given by Adkins & Reeve [1938] for *dl*-threonine, as compared with 252–253° for *dl-allo*threonine.

In comparison with serine and *iso*serine threonine is readily converted into phosphothreonine. 2 g. portions were heated with 10 g. H_3PO_4 and 2 g. P_2O_5 on a water bath for 5–20 hr. The yield of Ba salt averaged 46 %. The air-dried salt heated at 100° *in vacuo* over P_2O_5 gave N, 3·93; P, 8·94; Ba, 42·06; H_2O, 9·56 %. $C_4H_8O_6NPBa$ requires N, 4·19; P, 9·26; Ba, 41·07; $+2H_2O$ requires H_2O, 9·73 %.

The Pb salt was air-dried and heated at 100° *in vacuo* over P_2O_5 for analysis. (Found: N, 2·97; P, 7·22; Pb, 51·74; H_2O, 10·10 %. $C_4H_8O_6NPPb$ requires N, 3·46; P, 7·67; Pb, 51·24; $+3H_2O$ requires H_2O, 11·84 %.)

Phosphothreonine prepared from the Pb salt by adding 2 vol. alcohol and recrystallizing was dried at 100° *in vacuo* over P_2O_5. (Found: N, 6·37; P, 14·24; H_2O, 3·10 %. $C_4H_{10}O_6NP$ requires N, 7·04; P, 15·56 %. $C_4H_{10}O_6NP,H_2O$ requires N, 6·45; P, 14·27; H_2O, 8·34 %.)

More H_2O was driven off at 115°, but the substance turned brown and partially decomposed.

Phosphothreonine crystallizes from water and alcohol in small square plates, massing in cubes on slow crystallization, M.P. 169° with decomposition. A 0·5 % solution gave insoluble salts only with Pb acetate and $HgNO_3$.

6. *Phosphohydroxyaspartic acid*

Hydroxyaspartic acid, prepared by Burch [1930], could not be phosphorylated either at room temperature, at 100° or at 20 lb. pressure.

Hydrolysis of the esters

Phosphotyrosine was used itself. In the other cases solutions of the Na salts were prepared from the Ba salts by decomposition with the calculated quantity of Na_2SO_4 and filtration from $BaSO_4$.

A. HCl. About 100 ml. of solution were prepared. 1 ml. was taken for estimation of total P and 10 ml. portions for inorg. P by precipitation with NH_4Mg citrate. The NH_4MgPO_4 was filtered off, washed with NH_4OH, dissolved in 2*N* HNO_3 and the P estimated by the micro-method of Plimmer [1933]. The results were as follows:

1. *Phosphotyrosine*

By *N* HCl

0·8 g. in 105 ml. *N* HCl

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
48 ,,	0·31	3·2
5 hr. at 100°	4·79	50·0
21 ,,	8·32	86·8
28 ,,	8·55	89·2
48 ,,	8·49	88·6
72 ,,	9·41	98·2
Total P	9·58	

2. *Phosphohydroxyproline*

By *N* HCl

50 ml. Na salt + 50 ml. 2*N* HCl

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 100°	0	0
4 ,,	0·33	12·4
8 ,,	0·55	20·7
24 ,,	1·18	44·3
32 ,,	1·36	51·1
48 ,,	1·66	62·4
72 ,,	1·82	68·4
Total P	2·66	

3. *Phosphoserine*

By *N* HCl

50 ml. Na salt + 50 ml. 2*N* HCl

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
24 ,,	0	0
48 ,,	0	0
24 hr. at 100°	1·23	60·6
48 ,,	1·49	73·4
72 ,,	1·69	83·2
Total P	2·05	

4. *Phosphoisoserine*

By *N*/2 HCl

50 ml. Na salt + 50 ml. *N* HCl

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
42 ,,	0	0
8 hr. at 100°	1·02	44·0
24 ,,	1·95	84·0
48 ,,	1·96	84·5
72 ,,	2·00	86·2
Total P	2·32	

5. *Phosphothreonine*

By *N* HCl

50 ml. Na salt + 50 ml. 2*N* HCl

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
24 ,,	0	0
48 ,,	0	0
6 hr. at 100°	0·89	18·3
12 ,,	1·83	37·6
18 ,,	2·50	51·3
24 ,,	3·04	62·4
48 ,,	4·26	87·5
Total P	4·87	

At 37° there was no hydrolysis by *N* HCl, except of phosphotyrosine which amounted to 3·2 % in 48 hr. At 100° all the esters were hydrolysed fairly rapidly.

B. NaOH. The samples were acidified with HCl before precipitation with NH_4Mg citrate. Silica from glassware caused trouble in filtration and difficulty in washing. It may retain some unhydrolysed ester, which will be subsequently hydrolysed by HNO_3.

1. *Phosphotyrosine*

By *N* NaOH

1 g. in 50 ml. H_2O + 50 ml. 2*N* NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
48 ,,	0·03	0·4
5 hr. at 100°	0·07	1·0
24 ,,	0·25	3·7
48 ,,	0·28	4·2
72 ,,	0·35	5·2
Total P	6·70	

3. *Phosphoserine*

(1) By *N*/2 NaOH

50 ml. Na salt + 50 ml. *N* NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
6 ,,	0	0
25 ,,	0	0
24 hr. at 100°	0·90	50·9
48 ,,	1·24	70·0
Total P	1·77	

(2) By *N*/4 NaOH

50 ml. Na salt + 50 ml. *N*/2 NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 100°	0	0
4 ,,	0	0
8 ,,	0·07	3·7
24 ,,	1·23	65·4
48 ,,	1·24	66·0
72 ,,	1·20	64·9
Total P	1·88	

(3) By *N*/2 NaOH in Cu flask

50 ml. Na salt + 50 ml. *N* NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 100°	0	0
2 ,,	0·05	2·8
4 ,,	1·27	71·7
8 ,,	1·32	74·6
24 ,,	1·26	71·2
Total P	1·77	

2. *Phosphohydroxyproline*

(1) By *N* NaOH

52 ml. Na salt + 52 ml. 2*N* NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 100°	0	0
6 ,,	0	0
24 ,,	0·06	2·3
Total P	2·66	

The inorg. P seemed to be from adsorption on silica from glass vessel. Hydrolysis repeated in Cu flask.

(2) By *N*/2 NaOH

50 ml. phosphohydroxyproline + 50 ml. *N* NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 100°	0	0
3 ,,	0·06	1·8
6 ,,	0·03	0·9
24 ,,	0·34	10·1
48 ,,	0·07	2·1
72 ,,	1·13	33·7
120 ,,	1·27	37·9
Total P	3·35	

Hydrolysis apparently occurred after 24 hr. The low P figure at 48 hr. is probably an error.

4. *Phosphoisoserine*

By *N*/2 NaOH

50 ml. Na salt + 50 ml. *N* NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
40 ,,	0	0
24 hr. at 100°	0	0
48 ,,	0	0
72 ,,	0	0
Total P	2·43	

5. *Phosphothreonine*

By *N* NaOH

50 ml. Na salt + 50 ml. 2*N* NaOH

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
24 ,,	0	0
48 ,,	0	0
6 hr. at 100°	1·08	21·7
12 ,,	1·82	36·5
37 ,,	2·98	59·8
61 ,,	3·77	75·7
85 ,,	4·16	83·5
Total P	4·98	

All the esters are stable to NaOH at 37°. The stability contrasts with the instability of the phosphoric acid groups in caseinogen and caseo-phosphopeptone, which are completely hydrolysed by $N/4$ NaOH in 24 hr. At 100° there is only a small hydrolysis of phosphotyrosine by $2N$ NaOH in 72 hr.; this contrasts with the complete hydrolysis of phenyl phosphate [Plimmer & Burch, 1929]. Phosphohydroxyproline is only appreciably hydrolysed after 48 hr. Phosphoserine is hydrolysed to the extent of 60–70 % in 24 hr., but phospho*iso*serine is not hydrolysed. Phosphothreonine is hydrolysed like phosphoserine.

C. Kidney phosphatase. The enzyme solution was prepared by grinding minced rabbits' kidneys with sand and extracting with water + toluene for 24–48 hr. The extract was strained through muslin. Control experiments with boiled extract were made at the same time. Inorganic P was estimated after acidifying the solutions with HCl and precipitation with NH_4Mg citrate + NH_4OH. The procedure was then as before. The filtration of the solutions was very slow owing to precipitation of protein. The inorganic P of the control was deducted from that of the enzyme solution to give the change in P content due to hydrolysis.

1. *Phosphotyrosine*

25 ml. phosphotyrosine soln. + 40 ml. extract. *p*H 4

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0·32	6·0
24 ,,	2·59	49·1
48 ,,	3·42	64·9
96 ,,	3·62	68·7
Total P	5·27	

2. *Phosphohydroxyproline*

50 ml. Na salt + 50 ml. extract. *p*H 5

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
4 ,,	0	0
7 ,,	0·08	2·9
23 ,,	0·82	29·7
30 ,,	1·00	36·2
48 ,,	1·57	56·9
72 ,,	2·10	76·1
96 ,,	2·07	75·0
Total P	2·76	

3. *Phosphoserine*

50 ml. Na salt + 50 ml. extract. *p*H 6·5

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
6 ,,	0·06	3·3
24 ,,	1·22	67·7
54 ,,	1·41	78·3
74 ,,	1·35	75·0
120 ,,	1·35	75·0
Total P	1·80	

4. *Phosphoisoserine*

50 ml. Na salt + 50 ml. extract. *p*H 7

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
8 ,,	8·5	45·2
24 ,,	10·2	54·2
48 ,,	11·6	61·1
72 ,,	10·8	57·4
96 ,,	11·1	59·0
Total P	18·8	

5. *Phosphothreonine*

50 ml. Na salt + 50 ml. extract. *p*H 7

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
5 ,,	0·40	10·8
24 ,,	1·80	48·6
29 ,,	2·11	57·0
48 ,,	2·60	70·3
72 ,,	3·09	83·5
Total P	3·70	

D. Intestinal phosphatase. The solution of enzyme was prepared from the mucous membranes of rabbits' intestines which were ground up with sand and extracted with water + toluene. Before use the extract was strained through

muslin. The experiments were made as with kidney extracts. Filtration of the solutions was very slow owing to precipitation of proteins.

1. *Phosphotyrosine*

25 ml. phosphotyrosine soln. +40 ml. extract. *p*H 4

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	1·64	29·8
24 ,,	3·56	64·7
48 ,,	3·69	67·1
96 ,,	3·69	67·1
Total P	5·50	

2. *Phosphohydroxyproline*

50 ml. Na salt +50 ml. extract. *p*H 6·5

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
3 ,,	1·05	59·0
7 ,,	1·14	64·0
22 ,,	1·23	69·1
46 ,,	1·18	66·3
72 ,,	1·37	77·0
96 ,,	1·30	73·0
Total P	1·78	

3. *Phosphoserine*

50 ml. Na salt +50 ml. extract. *p*H 6·5

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
24 ,,	+	+
48 ,,	+	+
72 ,,	0·68	30·2
120 ,,	1·37	60·9
168 ,,	1·51	67·1
Total P	2·25	

+ error in determinations.

4. *Phosphoisoserine*

50 ml. Na salt +50 ml. extract. *p*H 6·5

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
8 ,,	1·24	60·2
24 ,,	1·29	62·6
48 ,,	1·27	61·3
72 ,,	1·27	61·3
96 ,,	1·33	65·5
Total P	2·06	

5. *Phosphothreonine*

50 ml. Na salt +50 ml. extract. *p*H 8

	Inorg. P mg.	Hydrolysis %
After 0 hr. at 37°	0	0
5 ,,	0·11	3·0
24 ,,	0·51	13·6
29 ,,	0·60	16·0
48 ,,	0·93	24·7
72 ,,	1·09	29·0
Total P	3·76	

Slow hydrolysis probably due to alkaline reaction.

All the esters are hydrolysed by kidney and intestinal phosphatases. The hydrolyses were never complete even after long periods of action. Comparison of the rates was not made as extracts made at different times were used.

Summary

Phosphotyrosine, phosphohydroxyproline, phosphothreonine, phosphoserine and phospho*iso*serine have been prepared by phosphorylation of the amino-acids with $H_3PO_4 + P_2O_5$; in the case of phospho*iso*serine only the Ba salt was isolated. Phosphohydroxyaspartic acid could not be made in this way. The lead salts of all are insoluble in water, as also the mercurous salts except that of phosphohydroxyproline.

All the esters are hydrolysed by *N* HCl at 100°. All are stable to hydrolysis by *N*/2 or even *N* NaOH at 37°, but all except phospho*iso*serine are hydrolysed at 100°, phosphoserine and phosphothreonine fairly rapidly. All are hydrolysed by phosphatases of kidney and intestine.

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