

ON THE PHOSPHOTUNGSTATES OF CERTAIN AMINOACIDS.

BY P. A. LEVENE AND W. A. BEATTY.

(From the Rockefeller Institute for Medical Research, New York.)

In a communication to the Chemical Society¹ one of us reported on the property of aminoacids to form insoluble phosphotungstates. In a later publication² the writers showed the degree of precipitability of aminoacids by means of phosphotungstic acid, and stated that a separation of aminoacids may be accomplished by means of this reagent. The attempt was made to apply the method to the study of the acids resulting from the hydrolysis of gelatine by means of mineral acids. Unfortunately, through an accident in the laboratory, the greater part of the material for this study was lost, and hence in this place there will be reported a method of the separation of oxyprolin and of an acid having the composition $C_7H_{14}N_2O_2$.

Oxyprolin.—This communication is prompted partly by a statement made by Emil Fischer in a recent address delivered to the German Chemical Society in Berlin.³ Fischer points out that considerable difficulty is experienced in obtaining oxyprolin from the mixture of the products resulting from hydrolysis of proteids. The process employed by Fischer consisted in removing most of the aminoacids first by esterification, then by crystallization, and finally by means of phosphotungstic acid. From the mother liquid, remaining after all these precipitations, oxyprolin was separated.

We have observed that oxyprolin can be isolated as a phosphotungstate without great difficulty. It precipitates in the form of an oil which crystallizes on standing and is soluble in an excess of the reagent. Its separation was accomplished in the following manner:

¹ Jan. 5, 1906; see *Science*, 1906, p. 347.

² *Zeitschr. f. physiol. Chem.*, 1906, xlvii, 149.

³ *Berichte d. deut. Chem. Gesellschaft*, 1906, xxxix, 597.

The product of hydrolysis (after the mineral acid has been removed in the usual manner) was concentrated to a thick syrup, acidulated with sulphuric acid, and treated with a concentrated solution of phosphotungstic acid (4 to 1) so long as an oily precipitate was formed. The supernatant liquid was decanted and the oil treated with an excess of the phosphotungstic acid solution until the oily character of the precipitate disappeared. A part of the oil passed into solution and another part formed a precipitate with the excess of the reagent. The soluble part contained the oxyprolin. On concentration of the solution (after the phosphotungstic acid was removed) leucin first crystallized. The mother liquid from this acid was further concentrated, after which a second precipitate formed. This substance was removed by filtration, washed with alcohol and ether, dried, and analyzed. The crude substance had the following composition:

0.1271 gram of the substance gave on combustion 0.2242 gram CO_2 and 0.1033 gram of H_2O ; C = 48.09%, H = 9.03%.

It was then redissolved in little water and two volumes of absolute alcohol were added. A precipitate appeared consisting of typical crystals of oxyprolin. The crystals tasted very sweet and formed pyrrol on sublimation. The melting point was at 270°C .; the composition was as follows:

0.1342 gram of the substance gave on combustion 0.2266 gram CO_2 and 0.0843 gram H_2O . Hence for $\text{C}_7\text{H}_9\text{NO}_3$

Calculated.	Found.
C = 45.80%	46.05%
H = 6.87%	6.9%

From this analysis the substance is oxyprolin.

On the Substance $\text{C}_7\text{H}_{14}\text{N}_2\text{O}_5$.—This substance was obtained from the fraction of the products of hydrolysis consisting chiefly of glycocoll. It had been stated in a previous communication that glycocoll and alanin under favorable conditions may be separated nearly quantitatively by means of phosphotungstic acid. These phosphotungstates possess typical crystalline forms and are slightly soluble in an excess of the reagent. A phosphotungstate of this nature was obtained from the products of hydrolysis of gelatine. The glycocoll was removed in the form of

a picrate. From the mother liquid of the glycocoll picrate, the picric acid was removed in the usual manner, and the solution concentrated to a very small volume and then treated with an excess of absolute alcohol. An oily sediment was formed which on standing was beaded with granular crystalline deposits, and similar deposits were formed on the walls of the vessel. The substance possessed a very sweet taste, gave on sublimation pyrrol, had a melting point of 236° C., and yielded the following composition:

0.1854 gram of the substance gave on combustion 0.2772 gram CO_2 and 0.1196 gram H_2O ; C = 40.76%, H = 7.17%.

In another experiment the substance was obtained in an identical manner and the attempt to purify it by recrystallization was made. For that purpose it was redissolved in little water and treated with alcohol. But as in the course of twenty-four hours no crystal formation appeared, a great excess of alcohol was added. An amorphous precipitate was formed, having a melting point of 232° C. and the following composition:

0.2468 gram of the substance gave on combustion 0.3710 gram CO_2 and 0.1880 gram H_2O ; C = 40.99%, H = 7.13%.

In a third experiment the substance obtained in the same manner was not absolutely free from glycocoll and it contained 37.00% of carbon and 8.00% of hydrogen. This substance was then extracted with a great excess of methyl alcohol. A part passed into solution. The insoluble part was pure glycocoll. It was then attempted to purify the bodies of the first and the second experiment by means of methyl alcohol. It was, however, found that they were completely dissolved in an excess of the reagent. The combined methyl alcoholic solutions were concentrated to a small volume and precipitated with acetone. The precipitate thus formed was amorphous and not absolutely white. It was therefore extracted with a very small quantity of boiling methyl alcohol, washed with alcohol and ether, dried in the xylol bath, and analyzed. It had the following composition:

0.1682 gram of the substance gave on combustion 0.2469 gram CO_2 and 0.1077 gram of H_2O ; C = 40.75%; H = 7.12%.

0.149 gram of the substance gave on combustion 17.5 c.c. (over 50% KOH) Nitrogen at $T^{\circ} = 22.5^{\circ}$ C. and $p = 775$ m.m.: N = 13.73%.

The remaining part of the substance was dissolved in a large excess of boiling absolute alcohol. It all went into solution leaving practically no residue. The excess of alcohol was then removed under diminished pressure and the solution further concentrated on a water bath. A crystalline deposit consisting of prismatic needles was formed. In an aqueous solution it reacted acid to litmus, possessed a sweet taste, and had a melting point of 236°–240° C. On sublimation pyrrol was given. An analysis gave the following composition:

0.1214 gr. of the substance gave on combustion 15.00 c.c. nitrogen (over 50% KOH) at $T^{\circ} = 25^{\circ}$ C. and $p = 763$ m.m.; $N = 14.25\%$. For $C_7H_{14}N_2O_5$:

Calculated.	Found.			
	Sample I.	Sample II.	Sample III.	Sample IV.
C = 40.75%	40.76%	40.99%	40.75%	
H = 6.8%	7.17%	7.13%	7.12%	
N = 13.54%	13.33%		13.73%	14.25%

Thus it was impossible by means of the various methods to alter the composition of the substance, and it is, therefore, probable, that the analyzed body was not a mixture but an individual substance of the composition $C_7H_{14}N_2O_5$. The substance may perhaps be a homologue of Fischer's acid $C_{12}H_{24}N_2O_5$.