CXXXIII. THE ISOLATION OF PURE *l*-PROLINE.

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During the last few years, investigations have been carried out in this laboratory with the object of discovering a method for a more quantitative and simple separation of the hydrolysis products of the proteins than those at present in vogue.

In the course of this work a method was discovered, some 18 months ago, for the separation in almost quantitative yield of pure *l*-proline. The proline thus isolated was found to differ appreciably in its properties from those ascribed to it in the literature. It was found, for example, to have a considerably higher laevorotation ($[\alpha]_D^{18^\circ} = -86{\cdot}7^\circ$) compared with that previously mentioned in the literature ($[\alpha]_D^{18^\circ} = -80^\circ$), and was found furthermore to crystallise from water, and to be only slightly soluble in cold absolute alcohol, although it is more readily soluble in the hot solvent. Recrystallisation from hot alcohol forms, in fact, a very convenient method for its purification.

It was intended to withhold the publication of these results until the general researches had reached a more advanced stage. In the meantime, however, attention has been called to a paper published a few months ago by Kapfhammer and Eck [1927] which has only just come to the notice of the author. As the results obtained by the author confirm, in some measure, those of Kapfhammer and Eck (except that the rotation has been found to be some 2° higher than mentioned by them), and furthermore as the method employed appears to be simple and entails the use only of the most common laboratory reagents, it has been thought advisable to publish it at the present stage, withholding for the present the details of the separation of the other hydrolysis products.

Kapfhammer and Eck employed for the separation a reagent known as Reinecke's acid, $H[(SCN)_4Cr(NH_3)_2]$, the ammonium salt of which is prepared by fusing together ammonium thiocyanate and ammonium dichromate. This reagent has been found by them to be a specific precipitant for proline and hydroxyproline.

The method employed by the author depends, in the first instance, on the separation of the hydrolysis products into three fractions by means of their copper salts, which have differing properties.

I. Copper salts soluble in water and in methyl alcohol.

II. Copper salts soluble in water, but insoluble in methyl alcohol.

III. Copper salts insoluble in both water and methyl alcohol.

The success of the method depends on the employment of a special technique which is described in detail below. The proline is found in the first fraction, together with, in the case of gliadin (which was the protein first employed in the evolution of the method), valine, hydroxyvaline, and the peptide phenylalanylproline, first isolated by Osborne and Clapp [1907]. These are insoluble in absolute alcohol, whereas proline is soluble. From the alcohol-soluble portion, proline can be precipitated almost quantitatively as the picrate. There are also among the copper salts soluble in methyl alcohol (*a*) a product soluble in alcohol but giving no picrate and (*b*) a product giving a picrate soluble in ether; these fractions are at present being investigated.

The fraction giving copper salts soluble in water but not in methyl alcohol contains glutamic acid, the bases, glycine, alanine and serine.

The fraction giving copper salts insoluble in water and in methyl alcohol contains phenylalanine, leucine and aspartic acid. Methods are in use, or are being worked out in this laboratory, for the separation of the constituents of these fractions, and will be published in due course. Pure proline has also been prepared in this laboratory, by the method here described, from glutenin by Mr E. L. Hill, from gelatin by Mr Hand, and by other workers from other sources.

Experimental.

500 g. of dry wheat gliadin were hydrolysed by slowly adding the protein to four times its bulk of hot 25 % sulphuric acid, and boiling for 24 hours under a reflux condenser. The hydrolysate was now cooled, diluted to 4 litres, and the humin material filtered off and washed. To the filtrate and washings was added hot saturated baryta solution roughly equivalent to the sulphuric acid present. The precipitated barium sulphate was filtered off and washed seven times by boiling with water. The filtrate and the washings from the barium sulphate were now concentrated to about 2 litres, and barium and sulphuric acid quantitatively removed.

The solution of amino-acids was next heated in a large porcelain evaporating basin, on a water-bath, and copper carbonate was added, little by little, until there was no more effervescence. A fair quantity of copper carbonate was now added, and the solution concentrated to a thick syrup. The mass was diluted with water, and the excess of copper carbonate, together with any insoluble copper salts, was filtered off and thoroughly washed with water. The filtrate was once again evaporated to a thick syrup, with the further addition of copper carbonate. When quite viscous, the material was treated with dry acetone, and after standing for a few minutes with this liquid the acetone was poured off and a fresh quantity added. After five or six treatments with acetone the copper salts granulated rapidly. The acetone was renewed several times after

this, and finally the copper salts were lightly ground in a mortar, when they fell rapidly to a fine powder. The acetone was removed as far as possible by filtering through a Büchner funnel, and the copper salts further dried by leaving overnight in a vacuum desiccator, over sulphuric acid. The solid was finally dried in an electric oven at 110° for a short time. The whole success of the method depends on the rapid and efficient drying which the acetone effects; no other method has been found for completely drying the copper salts, which is essential to the success of the separation.

The dried copper salts were now extracted by shaking mechanically in a stoppered bottle with twice the bulk of dry methyl alcohol. After shaking for 2 hours, the alcohol was filtered off and the residue shaken once again with methyl alcohol for 1 hour. This extraction was repeated six times, by which time only negligible quantities of material were being dissolved out.

This methyl alcohol-soluble portion of the copper salts was freed from the solvent by distillation, again granulated with acetone and finally dried for an hour at 110° as before. It was then re-extracted with methyl alcohol, when the whole material dissolved. On dilution, and standing overnight, however, a quantity of copper salt gradually settled out. This, when dry, weighed only 10 g.

The alcohol was again distilled off and the syrupy copper salts were taken up with water and freed from copper by means of hydrogen sulphide. The free amino-acids, after careful washing of the copper sulphide precipitate, were concentrated to a syrup and treated with absolute alcohol. A large quantity of material was precipitated; this was filtered off and washed, and the filtrate again evaporated down and taken up with absolute alcohol. When a fraction was obtained which was quite soluble in absolute alcohol, the watery solution of the same was treated with picric acid in sufficient quantity to combine with the nonamino-nitrogen present, and the solution boiled. On cooling, a somewhat soft and oily precipitate was obtained, which on filtration and extraction once with ether yielded a crystalline mass of proline picrate. This was recrystallised once from water and decomposed by acidifying with sulphuric acid, extracting the picric acid with ether and evaporating the solution to dryness after removal of the sulphuric acid, when pure proline was obtained. Large quantities of proline have been obtained by the author using this method, several hundred grams of the picrate having been prepared from gliadin alone.

Properties. Pure proline is a white, non-deliquescent solid. It crystallises quite easily from strong aqueous solutions in the form of long needles. It is not very soluble in cold absolute alcohol, but dissolves readily in the hot solvent, crystallising out on cooling, also in needle-shaped crystals. It may also be recrystallised from *iso*-propyl alcohol. It melts with decomposition at 215°. It gives absolutely no amino-nitrogen in the van Slyke apparatus.

Analysis. N (Kjeldahl)—Found: 12·20 %. Calculated: 12·17 %. C and H—2·370 mg. gave 4·540 mg. CO_2, 1·682 mg. H_2O. Found: C, 52·25 %;

H, 7·90 %. Calculated: C, 52·14 %; H, 7·88 %. Rotation: (i) 0·6206 g. in 50 cc. water gave, in a 2 dcm. tube, a rotation of − 2·15°; whence $[\alpha]_D^{18°} = -86·6°$. (ii) 1·734 g. in 50 cc. water gave, in a 2 dcm. tube, a rotation of − 6·02°; whence $[\alpha]_D^{18°} = -86·8°$.

Derivatives. The picrate may be prepared by adding the requisite quantity of picric acid to the hot aqueous solution of proline and cooling; the picrate is thus readily obtained pure. It is practically insoluble in cold water, although it has a large temperature-coefficient of solubility. Attempts to prepare the picrate by the method of Alexandroff [1905] were not so successful. The picrate thus prepared had M.P. 148°. The M.P. quoted in the literature is 152–4°; only once has a picrate of this M.P. been obtained. A mixed M.P. of this sample with that of M.P. 148° gave 153–4°. The picrate melting at 148° crystallised in long golden-yellow needles, while that melting at 154° was in the form of short, dull brown needles. Both picrates on decomposition gave a proline with the same optical rotation. Picrates of proline prepared by other workers in this laboratory have been found invariably to melt at 148°.

The phenylhydantoin is formed quantitatively by shaking proline with phenyl isocyanate in molecular proportions, keeping alkaline with caustic soda; the solution is then acidified with hydrochloric acid, and the strongly acid solution boiled for 5 minutes. On cooling, the phenylhydantoin crystallises out; M.P. 143–4°. If *l*-proline and phenyl isocyanate are warmed together for a few minutes and the resultant product crystallised from alcohol, pure *dl*-prolinephenylhydantoin is formed in good yield; M.P. 118°.

SUMMARY.

A method is given for the preparation of pure *l*-proline, which has a distinctly higher rotation than that stated in the literature. The method depends on a technique for separating the copper salts of protein hydrolysis products into three fractions. From the fraction which is soluble in methyl alcohol the proline can be prepared by isolation in the form of a picrate. The yield appears to be nearly quantitative. The pure compound is only slightly soluble in cold alcohol, though readily soluble in the hot solvent, from which it may be easily recrystallised.

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