XVII. THE SYNTHESIS OF IMINAZOLYLGLYCINE, THE LOWER HOMOLOGUE OF HISTIDINE.

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ENGELAND [1908] isolated from human urine a small quantity of a picrolonate, melting at 244° , to which he assigned the formula $C_5H_7O_2N_3$, $C_{10}H_8O_5N_4$. From this, by decomposition with hydrochloric acid, he obtained a solution, which gave a red coloration with sodium diazobenzenesulphonate. He concluded from these observations that the substance was the lower homologue of histidine.

It was believed that the synthesis of this amino-acid would be useful, partly as it might to some extent support Engeland's conclusion as to the identity of his substance, and partly because a knowledge of its properties might facilitate its isolation from other natural sources.

The only method available for the synthesis appeared to be that of Strecker in which, by the joint action of potassium cyanide and ammonium chloride, an aldehyde is converted into an amino-nitrile, from which the amino-acid is obtained by hydrolysis. The iminazoly-4 (or 5)-formaldehyde [Pyman, 1916] was prepared by the oxidation of iminazoly-4 (or 5)-methyl alcohol, and the latter substance was obtained from citric acid by the method used by Pyman [1911] in his fundamental syntheses of histamine and histidine. The additional experimental details supplied by Koessler and Hanke [1918] were useful in securing good yields in the various stages of this synthesis.

The iminazolyglycine was obtained impure in rather small yield and had to be purified through the picrolonate. The highest melting point of this synthetic salt, 243° (uncorr.) is in close agreement with Engeland's value of 244° . So far as this slender evidence goes, it tells in favour of Engeland's view as to the nature of the substance which he isolated from urine. As was to be expected, iminazolyglycine closely resembles histidine; it does not, however, give Knoop's reaction.

EXPERIMENTAL PART.

Preparation of iminazoly-4 (or 5)-glycine.

Equimolecular proportions of iminazoly-formaldehyde (1 g.), potassium cyanide (0.65 g.) and ammonium chloride (0.53 g.) were dissolved in the minimum of water (5 cc.) and allowed to stand at room temperature for 48 hours. The solution gradually darkened in colour and after a few hours became almost opaque. Attempts were made to prevent this by filling the

reaction vessel with nitrogen and hydrogen, but were unsuccessful. It is important that the concentration of the reacting substances be kept as high as possible; on one occasion, when about twice the usual volume of water was used, scarcely a trace of amino-acid was obtained.

The amino-nitrile was not isolated, but was straightway hydrolysed by adding to the reaction mixture an equal volume of concentrated hydrochloric acid and heating on the boiling water-bath for three hours. The liquid was then evaporated to dryness and the residue dissolved in water. Chlorides were precipitated by silver nitrate, in the presence of nitric acid. After filtration, silver nitrate was added in sufficient quantity to give a brown precipitate with barium hydroxide, and the silver compound of the amino-acid, mixed with silver oxide, was filtered off, washed, and decomposed by hydrogen sulphide. The filtrate from the silver sulphide was boiled to expel hydrogen sulphide, freed from barium by sulphuric acid, and evaporated. The resulting crude amino-acid was recrystallised from dilute alcohol. After several recrystallisations it formed sheaves of microscopic colourless plates, which darkened at 220° and melted with decomposition at 254° . It gave Pauly's reaction for glyoxalines, the triketohydrindene reaction for amino-acids, but not Knoop's test for histidine, neither in its original form, nor as modified by Hunter [1922]. It was readily soluble in water, slightly in methyl, almost insoluble in ethyl alcohol.

Nitrogen by micro-Kjeldahl.

4.92 mg. (dried at 120°) gave 1.275 mg. N N = 25.9 %.

Calculated for $C_5H_7N_3O_2$ N = 26.4 %.

The yield was variable and somewhat low: from 1 g. of the aldehyde, 0.25 to 0.50 g. of crude amino-acid was obtained. The *phosphotungstate* crystallises from hot water in glistening rhombic plates.

Preparation of the picrolonate.

The *monopicrolonate* was prepared by adding a hot saturated aqueous solution of rather less than the theoretical quantity of picrolonic acid to a concentrated aqueous solution of the amino-acid [Levene and Van Slyke, 1912]. On cooling, the picrolonate separated in deep yellow crystals which, after recrystallisation from water and from ethyl alcohol, melted and decomposed at 243° —a figure in good agreement with that given by Engeland [1908].

Nitrogen by micro-Dumas.

4.14 mg. (dried at 100°) gave 0.854 cc. N_2 at 11° and 762 mm. N = 24.66 %.

Calculated for $C_5H_7N_3O_2 \cdot C_{10}H_8N_4O_5$ N = 24.2 %.

Estimation of picrolonic acid.

Picrolonates are decomposed by nitron, and as in the case of nitrates and picrates, the nitron compound is practically insoluble in water. The reaction can therefore be used for the estimation of picrolonic acid, and gives satisfactory results even on the micro scale.

Thus 21.09 mg. pure picronic acid gave 45.92 mg. nitron picrolonate.

% picrolonic acid = 99.80.

12.16 mg. 4- (or 5)-iminazolyglycine picrolonate gave 17.43 mg. nitron picrolonate.

% picrolonic acid = 65.7.

Calculated for $C_5H_7N_3O_2 \cdot C_{10}H_8N_4O_5$ 65.2 %.

Decomposition of the picrolonate.

The picrolonate was dissolved in dilute hydrochloric acid and the free picrolonic acid thus formed was removed by extraction with benzene and finally with ether. The residual solution of the amino-acid hydrochloride was evaporated on the water-bath and the residue was recrystallised from dilute alcohol. It was, however, hygroscopic, and decomposed when attempts were made to dry it at 100°. Similar results were obtained when the hydrochloride was prepared by dissolving the amino-acid in hydrochloric acid.

C. 4- (or 5)-iminazoly-N. 2. 4-dinitrotolyl-3-glycine.

β - and γ -trinitrotoluene condense with compounds containing a primary amine group, with elimination of nitrous acid [Barger and Tutin, 1918]. In this case, 2. 3. 4-trinitrotoluene was used, the 3-nitro-group disappearing in the process.

20 mg. of the amino-acid and 60 mg. of 2.3.4-trinitrotoluene (a considerable excess) were dissolved in dilute alcohol and heated under a reflux condenser for three hours. The liquid gradually darkened to a deep yellow. It was evaporated to dryness, and the unchanged trinitrotoluene removed by repeated extraction with hot benzene. The deep yellow crystalline condensation product remained behind. It was very soluble in water, but insoluble in ethyl alcohol. Recrystallised from dilute alcohol, it decomposed violently at 270°.

The nitrogen content was estimated by a modified micro-Kjeldahl. Eckert [1913] was able to use the Kjeldahl method in presence of aromatic nitro-groups by adding sulphur as a reducing agent. 0.2-0.5 g. of substance was mixed with 0.4 g. of sulphur and heated on the water-bath for one hour with 20 cc. of 30-40 % fuming sulphuric acid. The further procedure was as usual.

In adapting this method for use on the micro-scale, it was found advisable to increase the amount of sulphur to two or three times the weight of substance, and also to heat on the water-bath for two hours. Shorter heating did not give satisfactory results with the substances tried, which, however, as they contained two nitro-groups, afforded a fairly severe test of the method.

6.95 mg. of *N*. 2.4-dinitrotolyl-3-histidine were found to contain 1.407 mg. nitrogen.

N = 20.3 %.

Calculated for $C_{13}H_{13}N_5O_6$ N = 20.9 %.

3.58 mg. of *C*. 4- (or 5)-iminazoly-*N*. 2.4-dinitrotolyl-3-glycine contained 0.7752 mg. nitrogen.

N = 21.65 %.

Calculated for $C_{12}H_{11}N_5O_6$ N = 21.8 %.

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