CCCXLII.—*Synthesis of* l-2-*Thiolhistidine.*

By Julius Nicholson Ashley and Charles Robert Harington.

In recent years the 2-thiolglyoxalines have acquired renewed biological interest through the identification of ergothioneine as a constituent of mammalian blood (Newton, Benedict, and Dakin, *J. Biol. Chem.*, 1927, **72**, 367). Although the constitution of ergothioneine, which was first isolated from ergot by Tanret (*J. Pharm. Chim.*, 1909, **30**, 145), must be regarded as established

as the betaine derived from 2-thiolhistidine by the work of Barger and Ewins (J., 1911, **99**, 2336), its synthesis has not yet been accomplished. Further, although the amino-acid, 2-thiolhistidine, has not yet been found among the products of hydrolysis of proteins, the natural occurrence of ergothioneine suggests the strong likelihood that the corresponding amino-acid may represent a part of the hitherto unidentified sulphur-containing constituents of proteins. Indeed, evidence of a physiological character has been adduced (Eagles and Vars, *J. Biol. Chem.*, 1928, **80**, 615) that maize contains a substance which can serve as the physiological precursor of ergothioneine, and, since zein contains an appreciable amount of sulphur which cannot be accounted for as cystine, the identity of this precursor with 2-thiolhistidine is a not unlikely hypothesis.

We have been engaged for some time past in attempts (hitherto abortive) to synthesise ergothioneine, and also in a search for 2-thiolhistidine among the products of hydrolysis of zein and other proteins. In connexion with the latter part of our work a knowledge of the properties of 2-thiolhistidine became very desirable and it was therefore decided to attempt the synthesis of this compound.

The starting point of the synthesis was histidine, which was first converted into the methyl ester dihydrochloride; this was benzoylated as described by Kossel and Edlbacher (*Z. physiol. Chem.*, 1915, **93**, 397) to give methyl $\alpha\gamma\delta$-tribenzamido-Δ^{γ}-pentenoate, which, treated with methyl-alcoholic hydrogen chloride according to the method of Windaus, Dörries, and Jensen (*Ber.*, 1921, **54**, 2754), yielded methyl $\alpha\delta$-dibenzamido-γ-ketovalerate. This ester, when boiled with 20% hydrochloric acid, afforded the *dihydrochloride* of *$\alpha\delta$-diamino-γ-ketovaleric acid*, which was converted, on treatment with one molecular equivalent of sodium thiocyanate, into the monohydrochloride of the desired amino-acid. The free amino-acid was readily obtained on addition of sodium acetate to a concentrated aqueous solution of the hydrochloride.

The proof of the constitution of the synthetic amino-acid was obtained by its almost quantitative conversion, by oxidation with ferric sulphate, into histidine. The histidine employed as the starting point in this synthesis was the natural *l*-amino-acid, and the 2-thiolhistidine obtained was also lævorotatory, having $[\alpha]_{5461}$ $-9{\cdot}5°$. The histidine recovered by oxidation of the 2-thiolhistidine had $[\alpha]_{D}$ $-36{\cdot}0°$ as against the accepted value of $[\alpha]_{D}$ $-39{\cdot}7°$ for pure *l*-histidine. It is evident, therefore, that surprisingly little racemisation had occurred during the series of reactions and that the synthetic 2-thiolhistidine was almost optically pure. Moreover, the isomeride obtained in this synthesis, being configuratively related to naturally occurring *l*-histidine, is the isomeride which

may be expected to occur in nature if this compound be indeed a constituent of proteins.

Experimental.

Methyl αγδ-Tribenzamido-Δ$^{\gamma}$-pentenoate.—The method of preparation of this compound described by Kossel and Edlbacher (*loc. cit.*) gave in our hands minute yields only. More success was attained as follows. A solution of *l*-histidine methyl ester dihydrochloride (4 g.) in ice-cold water (100 c.c.) was treated with powdered crystalline sodium carbonate (40 g.), and benzoyl chloride (20 g.) added in small portions with vigorous shaking, the temperature being maintained at 0°. When, after many hours' shaking, the product, at first oily, had formed hard lumps, it was collected with water, pulverised, extracted with ether, dried in the air, and recrystallised by cautious dilution with water of its solution in boiling alcohol (charcoal). The yield of product (m. p. 214—215° uncorr.) was 25% of the theoretical.

Methyl αδ-Dibenzamido-γ-ketovalerate.—This was prepared according to Windaus, Dörries, and Jensen (*loc. cit.*) by boiling the above ester for 45 minutes with 10% methyl-alcoholic hydrogen chloride (30 parts).

αδ-Diamino-γ-ketovaleric Acid Dihydrochloride.—Methyl αδ-dibenzamido-γ-ketovalerate (1 g.) was boiled under reflux with 20% hydrochloric acid for 1·5 hours. The solution was evaporated to dryness under diminished pressure, the evaporation being several times repeated after addition of small amounts of water. The residue was taken up in boiling water (charcoal), and the solution was filtered and evaporated over potassium hydroxide in a vacuum desiccator. There was thus obtained 0·6 g. of a colourless hygroscopic gum mixed with a few crystals of ammonium chloride. The product could not be obtained crystalline, although analysis indicated that it was approximately pure (Found : N, 11·6; Cl, 33·7. $C_5H_{10}O_3N_2,2HCl$ requires N, 12·8; Cl, 32·4%).

1-2-*Thiolhistidine.*—αδ-Diamino-γ-ketovaleric acid dihydrochloride was dissolved in a little water, sodium thiocyanate (1·1 mols.) added, and the mixture heated on the steam-bath for 1 hour. The solution was then treated with saturated aqueous sodium acetate until it was no longer acid to Congo-red. Crystallisation of the amino-acid set in almost immediately and was complete at 0° after some hours. The yield of almost pure product was about 40% of the theoretical. A further small amount could be obtained by dilution of the mother-liquor and precipitation with a solution of 10% mercuric sulphate in 5% sulphuric acid. The first sticky portion of the precipitate was rejected and the remainder was

collected, washed with water, and decomposed with hydrogen sulphide. The filtrate from the mercuric sulphide was freed from sulphuric acid with barium hydroxide and concentrated to a small volume under diminished pressure; the amino-acid then crystallised.

For analysis the 2-thiolhistidine was recrystallised from water (charcoal), from which it formed colourless plates of somewhat irregular shape (Found: C, 38·45, 38·3; H, 4·8, 4·4; N, 22·4; amino-N, 8·1; S, 16·65, 16·7. Calc. for $C_6H_9O_2N_3S$: C, 38·5; H, 4·8; N, 22·45; S, 17·1%).

When heated, the amino-acid darkened at about 290° but was still not melted at 310°. It had $[\alpha]_{5461}$ — 9·5° (c = 2·01 in *N*-hydrochloric acid; l = 0·5).

l-2-Thiolhistidine is readily soluble in hot water but sparingly so at the ordinary temperature; it has, however, a marked tendency to form supersaturated solutions. It is insoluble in alcohol and other organic solvents. It gives the ninhydrin reaction and, with sodium *p*-diazobenzenesulphonate, an orange-red colour which deepens to a reddish-brown with a purple fluorescence on addition of excess of sodium hydroxide. Aqueous solutions of the amino-acid instantly decolorise alkaline permanganate in the cold. On warming with ferric chloride, sulphuric acid is liberated, and the solution, after removal of iron, gives the cherry-red colour with sodium *p*-diazobenzenesulphonate which is characteristic for glyoxalines.

Although the glyoxaline nucleus in *l*-2-thiolhistidine, as in other 2-thiolglyoxalines, is of a very feebly basic character, the amino-acid forms a well-defined *dihydrochloride* when it is evaporated in a desiccator with excess of concentrated hydrochloric acid. This salt, which forms large colourless prisms, m. p. 197—199° (decomp.), is very soluble in water and sparingly so in alcohol (Found: Cl, 26·6. $C_6H_9O_2N_3$,2HCl requires Cl, 27·3%). No other well-characterised derivative was obtained. The *monopicrate*, prepared by dissolving equimolecular proportions of *l*-2-thiolhistidine and picric acid in water and allowing the solution to evaporate spontaneously at the ordinary temperature, forms orange-red prisms, m. p. 156° after sintering. This salt could not, however, be obtained analytically pure, the picric acid content of recrystallised samples, even when the solution contained excess of picric acid, being 1—2% below the theoretical (*e.g.*, found: picric acid, 52·8. $C_6H_9O_2N_3S$,$C_6H_3O_7N_3$ requires picric acid, 55·0%). *l*-2-Thiolhistidine is precipitated from its aqueous solution by silver salts at about p_H 5·0; it is also precipitated by mercuric sulphate in presence of 5% sulphuric acid, and by mercuric chloride at a faintly acid

reaction. Phosphotungstic acid precipitates it from concentrated solutions only.

Oxidation of l-2-*Thiolhistidine.*—The amino-acid (100 mg.) was heated for one hour on the steam-bath with a solution of ferric sulphate (1·9 g.) in water (10 c.c.). The solution was then diluted, heated to boiling, and treated with barium hydroxide in slight excess. The precipitate was collected, boiled with water, and again filtered off. The combined filtrates were treated at the boiling point with dilute sulphuric acid until faintly acid to Congo-red. After removal of the barium sulphate the solution was concentrated under diminished pressure to about 5 c.c. and treated with 0·4 g. of flavianic acid. Separation of a crystalline precipitate soon set in. The solution was kept over-night at the ordinary temperature and then for a few hours at 0°, and the precipitate was collected. After drying, it weighed 0·35 g., corresponding with an 83% yield of histidine diflavianate calculated on the thiolhistidine taken. A portion was recrystallised from a 3% aqueous solution of flavianic acid and formed bunches of small yellow needles, m. p. 241—242° (decomp.; uncorr.); an authentic sample of *l*-histidine diflavianate had m. p. 242—243° (decomp.; uncorr.) and a mixture of the two melted at 241—242° (decomp.; uncorr.).

The remainder of the flavianate was dissolved in warm dilute sulphuric acid and the flavianic acid was removed by extraction with butyl alcohol. The aqueous layer was freed exactly from sulphuric acid with barium hydroxide, and the filtered solution was evaporated to dryness under diminished pressure. The crystalline residue was taken up with a little hot water and the solution was treated with warm alcohol until a permanent turbidity was produced. On being kept over-night at 0°, it deposited colourless plates, which had m. p. 275° (decomp.; uncorr.) and corresponded in all qualitative reactions with histidine. The oxidation product had $[\alpha]_D$ — 36·0°, $[\alpha]_{5461}$ — 41·4° in water ($c = 3$; $l = 0·5$) and was therefore almost optically pure *l*-histidine, the rotation of which is recorded in the literature as $[\alpha]_D$ — 39·7°. The histidine remaining in the mother-liquor from the crystallisation was converted into the monopicrolonate, which, after recrystallisation from water, formed bunches of microscopic needles, m. p. 227—229° (decomp.; uncorr.); this m. p. was not depressed by admixture with authentic *l*-histidine monopicrolonate.

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL,
LONDON.

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