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Preparation of Hydroxylysine-ε-^{14}C and Lysine-ε-^{14}C *)

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In the course of a study of the metabolism of hydroxylysine and lysine it was desired to have these amino-acids labelled with ^{14}C in the ε-position Lysine labelled in this position has been prepared by Olynyk, Camp, Griffith, Woislowski and Helmkamp [1] and by Borsook, Deasy, Haagen-Smit, Keighly and Lowy [2], but these syntheses give a rather poor yield of radioactive material. No synthesis of labelled hydroxylysine has as yet been published.

The lysine-synthesis according to Warner and Moe [3] and the corresponding hydroxylysine-synthesis of Touster [4] appear to be well suited to this work, as the radioactive carbon can be introduced relatively late (Formulas I—V). In these procedures the cyanohydrine of γ-acetamido γ-γ'-dicarbethoxybutyraldehyde (III) is prepared from the corresponding aldehyde (I) and liquid hydrogen cyanide.

Owing to the difficulty of handling small amounts of radioactive liquid hydrogen cyanide the preparation of the desired cyanohydrine (III) *via* the bisulfite-addition complex and potassium cyanide was investigated and found to give good yields. The hydroxylysine (V) prepared by reduction and hydrolysis of (III) according to Touster [4] gave too low values in periodic analysis. Paper-chromatography showed the presence of both hydroxylysine and lysine. Touster also reports that only 60 per cent of the theoretical value of ammonia was split off by periodic acid in his preparation. It was then investigated whether by altered conditions of reduction the formation of lysine could be avoided. Reduction with Adams' catalyst in glacial acetic acid : ethanol 9 : 1 at ordinary pressure and room temperature proceeded smoothly and gave rise to only a small amount of lysine. To obtain pure hydroxylysine it was, however, always necessary to separate the mixture on

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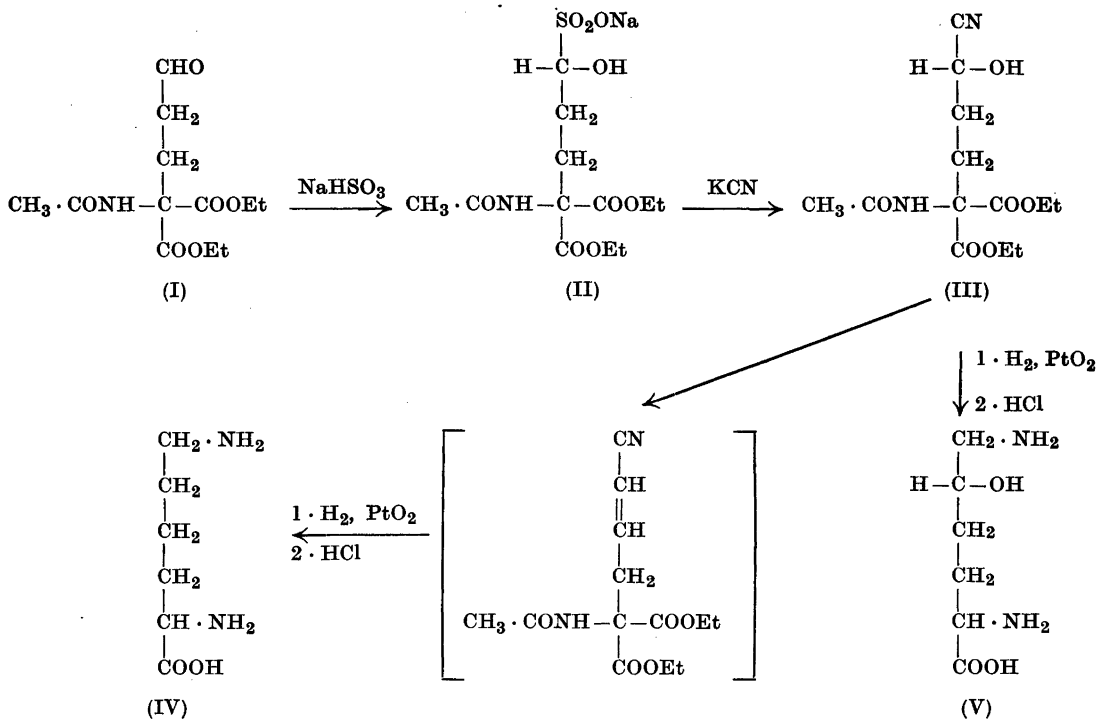

a column of Dowex-50 (NH_4-form) according to Weissiger [5]. The material thus obtained was pure as tested by paper-chromatography and periodic acid analysis.

Lysine (IV) was prepared from (III) according to Warner and Moe [3]. The final product contained a small amount of hydroxylysine and was purified by chromatography as described above. The isotopic yield in both syntheses was 30—40 per cent.

EXPERIMENTAL

Bisulfite-addition complex of γ-acetamido-γ'γ-dicarbethoxybutyraldehyde (I). To 21.7 g diethylacetamidomalonate and 2 ml sodium ethoxide (0.5 *N*) in 35 ml benzene was added 7 ml acrolein in 10 ml benzene with stirring. After two hours a solution of 12.6 g $Na_2SO_3 \cdot 7H_2O$ in 35 ml water and 5.8 ml glacial acetic acid was added. The reaction mixture was stirred overnight and filtered from inorganic material and some crystalline bisulfite complex. The resulting clear solution of (II) was diluted to 100 ml with water and used in the following.

Cyanohydrine of (I). To 5 ml of the solution of bisulfite complex was added 0.210 g K ^{14}CN in 2 ml of water. The mixture was shaken for half an hour and then extracted with ethyl acetate. The ethyl acetate solution was dried over sodium sulfate and eva-

porated. Addition of a little ether gave 0.840 g crystallin cyanohydrine. The crystals gave a total of 85×10^7 c.p.m. while the mother liquor gave a total of 10×10^7 c.p.m. After one recrystallization from benzene the cyanohydrine melted at 82°.

Hydroxylysine. 0.650 g cyanohydrine (total 65×10^7 c.p.m.) was hydrogenated in 20 ml glacial acetic acid: ethanol 1 : 9 with 0.1 g Adams' catalyst at ordinary pressure and room temperature. After six hours the reduction was complete. The oil resulting after evaporation of the solvent *in vacuo* was hydrolyzed with 25 ml 6 *N* hydrochloric acid for 15 hours. The hydrochloric acid was removed by repeated evaporation *in vacuo*. The material was dissolved in 100 ml of water and passed through a column of Dowex-50 (35 $mm^2 \times 350$ mm) 100—200 mesh which had been converted to the ammonium salt with *N* ammonia and subsequently washed with water. The column was washed with water until the washings were chloride-free and then eluted with 0.15 *N* ammonia in 4 ml fractions which were tested by paper-chromatography. The fractions containing pure hydroxylysine were combined and evaporated to dryness. The oil was treated with 10 ml *N* hydrochloric acid and the excess removed *in vacuo*. After dissolving in the minimum amount of 50 per cent ethanol, 0.5 ml pyridine was added. The monohydrochloride of hydroxylysine crystallized on scratching and addition of a little acetone. Yield 0.207 g. (total $29 \cdot 10^7$ c. p. m.) Calc. for $C_6H_{15}N_2O_3Cl$ (198.66): N 14.10; Cl 17.85. Found: N 14.01; Cl 17.55; Periodic acid equiv. w. found 198.

Lysine. 0.500 g cyanohydrine was boiled with 5 ml acetic anhydride for 90 minutes and reduced in the same medium with Adams catalyst at 100 atm. pressure and room temperature for 24 hours. The resulting oil was hydrolyzed with 25 ml 6 *N* hydrochloric acid for 15 hours. The material was chromatographed as described for hydroxylysine. The fractions containing only lysine were combined and the dihydrochloride crystallized. Yield 0.130 g. Calc. for $C_6H_{15}O_2N_2Cl_2$: N 13.05; Cl 31.5. Found: N 13.09; Cl 30.5;

SUMMARY

Racemic lysine-ε-^{14}C and hydroxylysine-ε-^{14}C have been synthesized.

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REFERENCES

1. Olynyk, Camp, Griffith, Woislowski and Helmkamp *J. Org. Chem.* **13** (1948) 465.
2. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighly, G., and Lowy, P. H. *J. Biol. Chem.* **176** (1949) 1383.
3. Warner, D. T., and Moe, O. A. *J. Am. Chem. Soc.* **70** (1948) 3918.
4. Touster, O. *J. Am. Chem. Soc.* **73** (1951) 491.
5. Weissiger, J. R. *J. Biol. Chem.* **186** (1950) 591.

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