# β-Hydroxyleucine. I. Synthesis by Means of Copper Complex and Separation of the Diastereomeric Racemates

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β-Hydroxyleucine has recently been found by Kenner and Sheppard[1] to be a constituent of an antibiotic (I. C. I. No. 13959), which is unusually active against infection of *Trypanosoma congolense*, and they assigned the structure D- or L-threo isomer to the natural product. This is, as they stated, the first time that β-hydroxyleucine has been obtained from natural sources.

A survey of the literature indicates that the procedures reported hitherto for the synthetic preparation of this amino acid are rather complicated and that yields are poor.

It was synthesized by Abderhalden for the first time from isocaproic acid through isopropyl-acrylic acid in an overall yield of 7%[2], by Wieland and coworkers by adding isobutyraldehyde into a melting mixture of glycine and potassium hydroxide in a 20% yield[3], and by Buston and Bishop by hydroxybromination of isopropylacrylic acid in a 12% yield[4]. The authors have succeeded in preparing β-hydroxyleucine as a diastereomeric mixture in a high yield by the copper complex method[5] by which already threonine[5], serine[6] and β-hydroxy-β-methylaspartic acid[7] were successfully synthesized.

Glycine copper complex was allowed to react in the presence of potassium or sodium hydroxide with isobutyraldehyde. The reaction mixture was treated with aqueous ammonia and the copper was removed by means of an ion exchange resin (Dowex 50). The product thus freed from copper was found to contain no by-product but a trace of unreacted glycine by paperchromatographic analysis. Having two asymmetric carbons in α- and β-positions, β-hydroxyleucine has two diastereomeric racemates. The separation of these diastereomers was effected by utilizing the different solubility of their sodium salts in absolute ethanol. The amino acid (A) derived from the insoluble salt showed a decomposition point of 240~241°C and that of (B) from the soluble salt 255~256°C. Although Buston and Bishop[4] reported that β-hydroxyleucine is separable into two isomers with m. p. 240 and 209°C, the authors could never obtain the latter. Paperchromatography in a solvent system of methyl ethyl ketone—*n*-butanol — concentrated ammonia — water (3 : 5 : 1 : 1) permits the definite separation of these

1) G. W. Kenner and R. C. Sheppard, *Nature*, **181**, 48 (1958).
2) E. Abderhalden, *Z. physiol. Chem.*, **251**, 164 (1938).
3) T. Wieland, H. Cords and E. Keck, *Chem. Ber.*, **87**, 1312 (1954).
4) H. W. Buston and J. Bishop, *J. Biol. Chem.*, **215**, 217 (1955).
5) M. Sato, K. Okawa and S. Akabori, This Bulletin, **30**, 937 (1957).
6) S. Akabori, T. T. Otani, R. Marshall, M. Winiz and J. P. Greenstein, *Arch. Biochem, Biophys.*, **83**, 1 (1959).
7) L. Benoiton, M. Winiz, R. F. Colman, S. M. Birnbaum and J. P. Greenstein, *J. Am. Chem. Soc.*, **81**, 1726 (1959).

isomers. (A-form, $R_f=0.49$; B-form, $R_f=0.32$). The ratio of A- to B-form obtained was 1.8～2.8 as measured by the ninhydrin method[8]. These results are shown in Table I. In comparison with threonine it may be assumed that A- and B-forms correspond to the threo and erythro-forms, respectively. The definite assignment of structure is under way and will be reported later.

Table I. Yields and ratios of the diastereomers obtained with various catalysts

| Catalyst | Yield | Form A | Form B | Form A/Form B |
|---|---|---|---|---|
| KOH | 76% | 63.7% | 36.3% | 1.8 |
| NaOH | 69% | 73.5% | 26.5% | 2.8 |
| $Na_2CO_3$ | Trace | — | — | — |
| $H_2N$-$CH_2$-$CH_2$-$NH_2$ | Trace | — | — | — |

## Experimental

**Isobutyraldehyde.**—The aldehyde was prepared according to Whitmore's method[9] with slight modification.

**β-Hydroxyleucine.**—In a 500 cc. flask, 30 g. of freshly distilled isobutyraldehyde was added to a suspension of 23 g. of copper complex of glycine in a solution of 8 g. of potassium hydroxide (or 6 g. of sodium hydroxide) in 40 cc. of water. The suspension was swirled for a few minutes, and then placed in a refrigerator overnight. The reaction mixture was transferred to a mechanical shaker and shaken at room temperature for 5 hr. Two hundred cubic centimeters of water and 170 cc. of concentrated aqueous solution of ammonia were added and the resulted solution was filtered. The filtrate was passed through a column of Dowex 50 which had previously been converted into the ammonium form. Then the column was eluted with 5 N aqueous solution of ammonia, and the eluate was collected until no further positive ninhydrin reaction.

The effluent was concentrated under reduced pressure until the crystallization of β-hydroxyleucine began, and then about three volumes of ethanol were added. After being kept in a refrigerator overnight, the crystals of β-hydroxyleucine were collected, washed with ethanol, and dried in vacuo. When potassium hydroxide was used as catalyst, the yield was 22 g. (76%); and in the case of sodium hydroxide the yield was 20 g. (69%).

The reaction product was contaminated with a small amount of unreacted glycine and it was a mixture of two racemic diastereoisomers.

Synthetic β-hydroxyleucine, when recrystallized from water by the addition of ethanol after decolorization with charcoal, showed an m. p. of 240～241°C (decomp.).

Found: C, 48.55; H, 9.07; N, 9.50. Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52%.

Determination of the ratio of A- to B-form was carried out by separating them on paperchromatogram in the solvent system mentioned above and by measuring the optical density of 50% ethanol of each spot treated with ninhydrin.

**Separation of the Diastereomeric Racemates.**—Fourteen and seventenths grams of β-hydroxyleucine was added to a hot solution of sodium ethylate prepared from 2.3 g. of sodium and 50 cc. of absolute ethanol. The suspended mixture was refluxed with agitation for an hour, and placed at room temperature overnight. The mixture was filtered to remove the sodium salt of Form A, and the separeted sodium salt of Form A was added to 50 cc. of ethanol. The mixture was refluxed for an hour and the precipitate was collected and washed with ethanol.

The collected sodium salt of Form A was dissolved in 200 cc. of water and the solution was passed through a column of Amberlite-IRC 50 which had been converted into the H-form. Water was passed through the column until the effluent became ninhydrin negative. The aqueous effluent containing free β-hydroxyleucine (Form A) was concentrated under reduced pressure to a low bulk. Excess ethanol was added, and the resulting precipitate was collected by filtration after storage in a refrigerator overnight and washed with ethanol. The yield was 10 g.

Recrystallization of the product was effected from water by the addition of ethanol after decolorization with a small amount of charcoal. The product, when chromatographed on paper, revealed only a single ninhydrin-positive spot which was identical with Form A, and it showed an m.p. of 240～241°C (decomp.).

Found: C, 48.91; H, 8.72; N, 9.60. Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52%.

On the other hand the filtrate, namely, the ethanol solution of sodium salt of Form B was treated in the same manner as described above and its free amino acid was obtained. The yield was 2.3 g. and m. p. 255～256°C (decomp.).

Found: C, 48.95; H, 8.75; N, 9.60. Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52%.

**Reduction of β-Hydroxyleucine with Hydriodic Acid.** — In a sealed tube a mixture containing 0.05 g. of β-hydroxyleucine, 0.02 g. of red phosphorous and 3 cc. of hydriodic acid ($d=1.60$) was heated for 10 hr. at 150°C. By paperchromatographic analysis of the reaction product it was confirmed that most of the starting material had been reduced to leucine, but a part of β-hydroxyleucine remained unchanged in the reaction mixture. The paperchromatograms also showed three ninhydrin-positive spots of glycine, and unidentified substances with $R_f$-values 0.22 and 0.00.

8) M. Wolfe, *Biochim. et Biophys. Acta*, **23**, 186 (1957).
9) W. L. Evers, H. S. Rothrock, H. M. Woodburn, E. E. Stanly and F. C. Whitmore, *J. Am. Chem. Soc.*, **55**, 1136 (1933).

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