# 202. *Peptides. Part X.** β-*Hydroxyleucine.*

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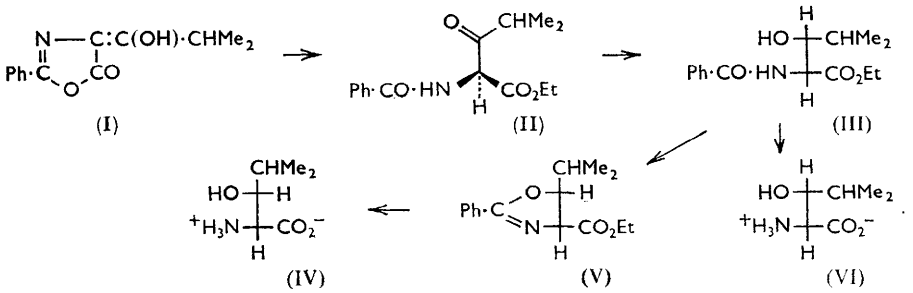
The racemates of both *erythro-* and *threo*-β-hydroxyleucine have been synthesised by stereospecific methods based on ethyl α-benzamido-γ-methyl-β-oxovalerate (II) and ethyl *trans*-5-isopropyl-2-phenyl-Δ²-oxazoline-4-carboxylate (V). The *threo*-racemate has been resolved to L-*threo*-β-hydroxyleucine, identical with a natural product. Condensation of isobutyraldehyde with the potassium salt of glycine gives almost entirely the *threo*-amino-acid, and this is the best preparative procedure.



Recently, we described[1] the isolation, from the acidic hydrolysate of an antibiotic (I.C.I. No. 13,959), of three α-amino-acids not hitherto found in natural peptides. Of these, α-methylalanine (α-amino-α-methylpropionic acid) was rigorously identified, but β-hydroxyleucine and γ-methylproline were identified only tentatively by chromatographic comparisons. We now report stereochemically specific syntheses of DL-*erythro-* and DL-*threo*-β-hydroxyleucine, and resolution of the latter to L-*threo*-β-hydroxyleucine, identical in infrared spectrum and optical rotation with the natural product. Synthesis of the diastereoisomeric γ-methylprolines will be the subject of a future paper.

Synthetic β-hydroxyleucine has been obtained previously from isopropylacrylic acid (4-methylpent-2-enoic acid) by Abderhalden,[2] and by Buston and Bishop,[3] and a convenient one-stage preparation from isobutyraldehyde and glycine has been described by Wieland, Cords, and Keck.[4] However, these syntheses do not allow the rigorous assignment of *threo-* and *erythro*-configurations to the products. To obtain β-hydroxyleucine of defined configuration, we have therefore adapted the procedures developed by Elliott and his co-workers[5,6] for the synthesis of the diastereoisomeric threonines. Thus isobutyryl chloride and 2-phenyloxazolone or, more reproducibly, isobutyric anhydride and




sodium hippurate condensed in the presence of β-picoline, yielding the enolic compound (I), which was converted by boiling ethanol into the keto-ester (II). The latter was smoothly reduced by sodium borohydride to *N*-benzoyl DL-β-hydroxyleucine ethyl ester, estimated to contain 80% of the *erythro*-isomer (III).†

* Part IX, *J.*, 1959, 4100.

† The approximate ratio of the isomers was conveniently shown by acidic hydrolysis and paper chromatography in solvent systems[7,8] capable of separating the β-hydroxyleucine stereoisomers. Control experiments showed that no detectable epimerisation occurred during the hydrolysis.

[1] Kenner and Sheppard, *Nature*, 1958, **181**, 48.
[2] Abderhalden, *Z. physiol. Chem.*, 1938, **251**, 164.
[3] Buston and Bishop, *J. Biol. Chem.*, 1955, **215**, 217.
[4] Wieland, Cords, and Keck, *Chem. Ber.*, 1954, **87**, 1312.
[5] Attenburrow, Elliott, and Penny, *J.*, 1948, 310.
[6] Elliott, *J.*, 1949, 589; 1950, 62.
[7] Hardy and Holland, *Chem. and Ind.*, 1952, 855.
[8] Shaw and Fox, *J. Amer. Chem. Soc.*, 1953, **75**, 3421.

The stereochemical course of such hydride reductions can be predicted by the well-known "rule of asymmetric induction."[9] In the present instance, application of the rule, with the assumption that the ethoxycarbonyl group is *effectively* bulkier than the benzamido-group, leads to the expectation that the hydride ion would be added preferentially from the rear in structure (II) and hence the major product would have the *erythro*-configuration; this assignment is proved in the sequel. Attenburrow, Elliott, and Penny[5] employed catalytic hydrogenation for a similar reduction in the threonine series, but the ratio of isomers was less favourable. Alkaline saponification of the ester (III) yielded *N*-benzoyl-DL-*erythro*-β-hydroxyleucine, which was in turn hydrolysed by acid to DL-*erythro*-β-hydroxyleucine (VI).

For entry into the *threo*-series, the *erythro*-ester (III) was converted by cold thionyl chloride into the oxazoline (V). Elliott[6] has already shown in the threonine series that, under these conditions, cyclisation proceeds with inversion of configuration at the β-carbon atom, and accordingly our oxazoline should have had the *trans*-configuration (V), if our original assignment on the basis of Cram's rule is correct. This *trans*-configuration is in fact proved by the stability of the oxazoline ester to epimerisation by sodium ethoxide: *cis*-oxazoline esters are rapidly converted into the less congested *trans*-forms by this reagent.[6] DL-*threo*-β-Hydroxyleucine (IV) was conveniently obtained by direct acidic hydrolysis of the crude oxazoline ester (V), and it was readily freed from traces of the *erythro*-isomer by recrystallisation.

Resolution of the DL-*threo*-amino-acid was achieved *via* the brucine salt of the phthaloyl derivative.[10] Decomposition of the active brucine salt with alkali yielded *N*-*o*-carboxybenzoyl-L-*threo*-β-hydroxyleucine, which reverted at 100° to the L-phthaloyl derivative. Acidic hydrolysis of the phthalamic acid afforded pure L-*threo*-β-hydroxyleucine monohydrate, $[\alpha]_D^{20}$ −3·5° (*c* 2 in $H_2O$) and $[\alpha]_D^{20}$ +15° (*c* 2 in 5N-HCl). The large positive shift in rotation on passing from neutral to acidic solution is convincing evidence for the L-configuration of the α-carbon atom.[11] Anhydrous L-*threo*-β-hydroxyleucine could be obtained from the monohydrate at 110°, but it was rapidly rehydrated by atmospheric moisture. Both the hydrated and the anhydrous optically active amino-acids gave infrared spectra very different from that of the racemate.[12]

An alternative synthesis provided our first samples of *threo*- and *erythro*-DL-β-hydroxyleucine, but the yields were poor. Ethyl α-chloro-β-hydroxy-γ-methylvalerate was readily obtained from isobutyraldehyde, ethyl dichloroacetate, and magnesium amalgam,[13] but displacement of the chlorine from this ester or the derived acid was unexpectedly difficult. The amination was slow and basic conditions encouraged retrograde aldol reactions. Eventually, small quantities of the pure *threo*-amino-acid were produced by the action of liquid ammonia on the α-chloro-acid, while the *erythro*-isomer was obtained from the α-chloro-ester by treatment with sodium azide and subsequent hydrogenation and hydrolysis. As these displacements would be expected to proceed with retention and inversion of configuration respectively,[14] it is reasonable to conclude, despite the low yields, that condensation between isobutyraldehyde and ethyl dichloroacetate in the presence of magnesium produced mainly *threo*-α-chloro-β-hydroxy-γ-methylvaleric ester. In agreement with this conclusion, acidic hydrolysis of this ester gave, in addition to the α-chloro-acid, an αβ-dihydroxy-γ-methylvaleric acid, presumably formed by inversion at the α-carbon atom.[14] This compound is evidently not identical with that already described,[15] and the latter probably had the *threo*-configuration. These assignments

[9] Cram and Greene, *J. Amer. Chem. Soc.*, 1953, **75**, 6005.
[10] Vogler and Lanz, *Helv. Chim. Acta*, 1959, **42**, 209.
[11] Winitz, Birnbaum, and Greenstein, *J. Amer. Chem. Soc.*, 1955, **77**, 716.
[12] Cf. Brockmann and Musso, *Chem. Ber.*, 1956, **89**, 241; Dougal, Newton, and Abraham, *Biochem. J.*, 1957, **66**, 30P.
[13] Darzens, *Compt. rend.*, 1937, **204**, 272.
[14] Cowdrey, Hughes, and Ingold, *J.*, 1937, 1208.
[15] Braun, *Monatsh.*, 1896, **17**, 216.

appear to conflict with a recently suggested mechanism [17] for the Reformatsky and the Ivanov reaction, in which simultaneous co-ordination of the substituted acetate anion and the aldehydic oxygen atom to magnesium is postulated as the factor determining the configuration of the product.

With both *threo-* and *erythro*-isomers of β-hydroxyleucine at hand, it was of interest to examine the distribution of isomers from other preparative procedures. The one-stage preparation [4] from isobutyraldehyde, glycine, and potassium hydroxide is remarkably specific, the *threo* : *erythro* ratio being approximately 10 : 1. Despite the low yield, this procedure remains the method of choice for the synthesis of DL-*threo*-β-hydroxyleucine. When glycine was replaced by its copper salt, a procedure reported to give high yields in the threonine series,[16] the ratio was only 2 : 1. The physical constants of Buston and Bishop's [3] isomer-I show it to be the *erythro*-form, as required by *trans*-addition of hypobromous acid to the *trans*-methylpentenoic acid and subsequent amination with retention of configuration.

## Experimental

4-(1-*Hydroxy*-2-*methylpropylidene*)-2-*phenyloxazol*-5-*one* (I).—(a) *From* 2-*phenyloxazol*-5-*one*. A solution of isobutyryl chloride (21·2 g., 0·2 mole) in dry ether (20 ml.) was added dropwise during 10 min. to a stirred solution of 2-phenyloxazol-5-one (35·5 g., 0·2 mole) in dry β-picoline (160 ml.) while the temperature was maintained below 25°. After a further 10 min. the dark solution was poured into a vigorously stirred mixture of concentrated hydrochloric acid (180 ml.) and ice; the precipitated solid was collected and washed well with water. Crystallisation from chloroform, with careful working up of the mother-liquors, furnished a total of 15·3 g. (33%) of the *oxazolone*. After one further recrystallisation, it had m. p. 188—189°, $\lambda_{max.}$ 323 mμ (ε 24,650) and $\lambda_{min.}$ 254—255 mμ (ε 4770) (Found: C, 67·5; H, 5·8; N, 5·9. $C_{13}H_{13}O_3N$ requires C, 67·5; H, 5·7; N, 6·1%). The compound gave an immediate deep blue colour with ethanolic ferric chloride solution.

(b) *From sodium hippurate*. A mixture of sodium hippurate (5 g., 0·025 mole), isobutyric anhydride (14 ml., 0·075 mole), and β-picoline (7 ml., 0·075 mole) was shaken at room temperature for 4 hr., then warmed (50°) for 1 hr. and set aside overnight. The orange-coloured solution was filtered with the aid of a little warm ether, which was evaporated before decomposition of excess of isobutyric anhydride with ethanol (30 ml.). After 30 min. ice-cold dilute hydrochloric acid was added, and the precipitated solid (3 g.) collected. One recrystallisation furnished the oxazolone (2 g., 33%), m. p. 187—188°.

*Ethyl* α-*Benzamido*-γ-*methyl*-β-*oxovalerate*.—The foregoing oxazolone (7·2 g.) was heated in ethanol under reflux during 2 hr. and the solution was then concentrated to about 20 ml. and kept overnight at 0°. The *ester* (7·9 g., 91%; m. p. 86—88°), recrystallised from ethanol–cyclohexane, had m. p. 89° (Found: C, 65·15; H, 7·0; N, 5·1. $C_{15}H_{19}O_4N$ requires C, 65·0; H, 6·9; N, 5·1%).

N-*Benzoyl*-DL-(erythro *and* threo)-β-*hydroxyleucine Ethyl Ester*.—Sodium borohydride (350 mg.) in methanol was added in four portions to a stirred solution of the foregoing keto-ester (7·86 gm.) in ethanol (200 ml.) and 3N-sodium hydroxide (2 drops). After being stirred for 1 hr., the solution was acidified and evaporated. The residue was extracted with ethyl acetate (200 ml.), and the solution washed successively with portions (50 ml.) of dilute hydrochloric acid, water, sodium hydrogen sulphite solution (twice), and water. Evaporation of the dried ($Na_2SO_4$) ethyl acetate solution furnished the mixed esters as a colourless, viscous oil (7·35 g., 94%). A sample was distilled at 170—180° (bath-temp.)/$10^{-4}$ mm. (Found: C, 64·9; H, 7·7; N, 5·3. Calc. for $C_{15}H_{21}O_4N$: C, 64·5; H, 7·6; N, 5·0%). A sample was hydrolysed with 5N-hydrochloric acid in a sealed tube at 100° for 24 hr. Descending paper chromatography in butan-1-ol saturated with 10% aqueous diethylamine [7] or in the upper phase from butan-1-ol (200 ml.), water (150 ml.), acetone (25 ml.), and aqueous ammonia (*d* 0·880; 25 ml.),[8] and development with ninhydrin, showed the presence of *erythro-* and *threo*-β-hydroxyleucines in a ratio of approximately 4 : 1.

[16] Sato, Okawa, and Akabori, *Bull. Chem. Soc. Japan*, 1957, **30**, 937.
[17] Zimmerman and Traxler, *J. Amer. Chem. Soc.*, 1957, **79**, 1920.

N-*Benzoyl*-DL-erythro-β-*hydroxyleucine*.—The above mixed esters (0·5 g.) were saponified in ethanolic solution with excess of 2N-sodium hydroxide. After 3 hr. at room temperature, the mixture was acidified with dilute hydrochloric acid; the precipitated benzoyl derivative (0·25 g.), when recrystallised from aqueous ethanol, had m. p. 193—195° (Found: C, 62·1; H, 6·7; N, 5·7. Calc. for $C_{13}H_{17}O_4N$: C, 62·1; H, 6·8; N, 5·6%). Buston and Bishop [3] give m. p. 196° for their isomer (I). The *N*-benzoyl derivative was hydrolysed by 6N-hydrochloric acid to the free amino-acid, identical with that described subsequently.

*Ethyl* trans-4-*Isopropyl*-2-*phenyl*-$\Delta^2$-*oxazoline*-3-*carboxylate Hydrochloride*.—A solution of the crude *N*-benzoyl-DL-β-hydroxyleucine ethyl esters (2·2 g.) in dry chloroform (25 ml.) was added during 1 hr. to stirred ice-cold thionyl chloride (40 ml.). The mixture was kept overnight at room temperature, then evaporated at 50° *in vacuo*, and the residual oil crystallised from ethyl acetate–ether (1·3 g., 56%; m. p. 115—117° in a sealed tube). For analysis, the *oxazoline hydrochloride* was recrystallised once more, and dried *in vacuo* at room temperature; it then had m. p. 119—120° (Found: C, 60·3; H, 6·8; N, 4·7. $C_{15}H_{20}O_3NCl$ requires C, 60·5; H, 6·8; N, 4·7%), $\nu_{max.}$ (Nujol mull) 2222, 1745, 1639, 1597, 1580, 1299, 1276, 1250, 1214, 1176, 1155, 1147, 1126, 1078, 1037, 1025, 1012, 973, 967, 940, 890, 868, 850, 803, 767, 756, and 710 cm.$^{-1}$. A sample of the oxazoline hydrochloride (5 mg.) was hydrolysed with 6N-hydrochloric acid during 8 hr. at 100°. A second sample (5 mg.) was treated with excess of sodium ethoxide (10 mg.) in ethanol for 5 min. before similar acid hydrolysis. Both hydrolysates were examined by paper chromatography (after deionisation in the second instance). *threo*-β-Hydroxyleucine predominated over the *erythro*-isomer (ratio greater than 8 : 1) in both cases, but the amount of *erythro*-isomer was apparently rather less in the second experiment; presumably the crystalline oxazoline hydrochloride was not completely free from the *cis*-isomer.

DL-threo-β-*Hydroxyleucine*.—A chloroform solution of the crude *N*-benzoyl-β-hydroxyleucine ethyl esters (6·5 g.) was dehydrated with thionyl chloride as above, and the resulting oxazoline hydrolysed directly by 18 hours' heating with 6N-hydrochloric acid. Benzoic acid was removed, by filtration and ether-extraction, from the cooled solution after it had been diluted with an equal volume of water. The aqueous solution was then evaporated, and the residue transferred in N-acetic acid to a column (9 × 3 cm. diam.) of Dowex-1X2 anion-exchange resin (acetate form; washed with N-acetic acid); ninhydrin-reacting material was eluted between 60 and 160 ml. The pure *threo*-amino-acid (1·6 g., 47%) was obtained from this eluate by evaporation and two crystallisations from aqueous ethanol (Found: C, 49·2; H, 9·1; N, 9·3. Calc. for $C_6H_{13}O_3N$: C, 49·0; H, 8·9; N, 9·5%), $\nu_{max.}$ (KBr disc) 3086, 2941, 2488, 1969, 1629, 1572, 1506, 1471, 1389, 1326, 1258, 1241, 1136, 1111, 1055, 1033, 909, 947, 906, and 866 cm.$^{-1}$. The N-*chloroacetyl derivative* was prepared by acylation with chloroacetyl chloride (3 mol.) in aqueous solution at 0° and pH 9·5 (autotitrator). The yield after recrystallisation from ethyl acetate–cyclohexane was 60%, and the m. p. 146—147° (Found: C, 43·3; H, 6·4; N, 6·5. $C_8H_{14}O_4NCl$ requires C, 43·0; H, 6·3; N, 6·3%). An attempted optical resolution by the action of the enzyme preparation acylase I[18] on this compound was frustrated by very slow hydrolysis. The *phthaloyl derivative* was obtained by heating the amino-acid (10 g., 0·07 mole) and phthalic anhydride (16 g., 0·077 mole) in dioxan (60 ml.) under reflux for 20 hr. The twice crystallised product (17 g., 90%) had m. p. 195—196° (Found: C, 60·9; H, 5·4; N, 5·1. $C_{14}H_{15}O_5N$ requires C, 60·6; H, 5·4; N, 5·05%).

*Brucine Salt of Phthaloyl*-L-threo-β-*hydroxyleucine*.—The foregoing phthaloyl derivative (18 g., 0·067 mole) was added to a solution of brucine dihydrate (28·8 g., 0·067 mole) in 2-methoxyethanol (55 ml.) at 80°, and the mixture shaken and warmed until solution was complete. It was then kept at −5° for 2 days. The crystalline *salt* (14·7 g.) was collected and recrystallised three times from 95% aqueous ethanol to constant optical rotation, $[\alpha]_D^{20}$ −1·3° (*c* 3 in 2-methoxyethanol). A sample, crystallised once further and dried at 80° *in vacuo*, had m. p. 203—204° (Found: C, 64·6; H, 6·6; N, 6·2. $C_{37}H_{41}O_9N_3,H_2O$ requires C, 64·4; H, 6·3; N, 6·1%).

*Decomposition of the Brucine Salt*.—A solution of the foregoing salt (9 g.) in 2N-sodium hydroxide (30 ml.) and chloroform (30 ml.) was set aside at room temperature for 30 min. with occasional shaking. The aqueous layer was then separated and washed twice with chloroform (15 ml.) which was re-extracted with 0·5N-sodium hydroxide. The combined aqueous solutions were concentrated *in vacuo* and acidified (pH 3) before being kept overnight at 5°. The precipitated *o*-carboxybenzoyl-L-threo-β-hydroxyleucine (2·7 g., 68%) was collected and

[18] Greenstein, *Adv. Protein Chem.*, 1954, **9**, 122.

recrystallised from aqueous ethanol. It had m. p. 218—219°, $[\alpha]_D^{20}$ −12·5° (*c* 2 in 2-methoxyethanol), $\nu_{max.}$ 1709 ($CO_2H$), 1639 and 1527 cm.$^{-1}$ (–CO·NH–) (cf. *o*-carboxybenzoyl-DL-phenylalanine, 1709, 1677, and 1527 cm.$^{-1}$). During drying at 80° for 6 hr. a sample cyclised to the *phthaloyl derivative*, m. p. 210—217° (Found: C, 60·3; H, 5·4; N, 5·3%), having infrared maxima at 1742 and 1695 identical with those of the racemic compound.

L-threo-β-*Hydroxyleucine.*—*o*-Carboxybenzoyl-L-*threo*-β-hydroxyleucine (2·3 g.) was heated with 6N-hydrochloric acid for 7 hr. Next day the precipitated phthalic acid was filtered off, and the filtrate evaporated *in vacuo*. The residue was treated with water (20 ml.), and the mixture filtered from further phthalic acid and evaporated; then the process was repeated. The residual amino-acid hydrochloride (0·91 g., 65%) was dissolved in 0·5N-acetic acid and passed through a column (10 × 1·5 cm.) of Dowex-1X4 anion-exchange resin (acetate form). Elution with 0·5N-acetic acid was continued until the ninhydrin reaction became negative. Evaporation of the eluate and recrystallisation from aqueous ethanol of the residue yielded the pure L-*amino-acid monohydrate* (490 mg.), $[\alpha]_D^{20}$ −3·5° (*c* 2 in $H_2O$), $[\alpha]_D^{20}$ +15° (*c* 2 in 5N-HCl) (Found: C, 43·5; H, 9·0; N, 8·3. $C_6H_{13}O_3N,H_2O$ requires C, 43·6; H, 9·1; N, 8·5%), $\nu_{max.}$ (KBr disc) 3333, 3067, 2959, 1667, 1634, 1567, 1515, 1466, 1397, 1355, 1330, 1256, 1130, 1062, 1006, 952, 923, 903, 858, 788, and 689 cm.$^{-1}$. The anhydrous amino-acid, obtained by drying the monohydrate at 110°/0·1 mm. (Found: C, 49·2; H, 9·0; N, 9·15%), had $\nu_{max.}$ (KBr disc) 3226, 3077, 2950, 2000, 1656, 1634, 1592, 1563, 1504, 1471, 1389, 1330, 1250, 1168, 1126, 1101, 1075, 1057, 1004, 984, 967, 947, 922, 906, 892, 854, 791, 714, and 689 cm.$^{-1}$.

*Ethyl* threo-α-*Chloro*-β-*hydroxy*-γ-*methylvalerate.*[13]—A three-necked flask fitted with stirrer, reflux condenser, and dropping funnel, and containing dry mercury (300 g.) was swept out with dry hydrogen. Magnesium turnings (6·25 g.) were then added, and the flask was warmed until formation of the amalgam was complete. An ethereal solution (250 ml.) of isobutyraldehyde (18 g.) and ethyl dichloroacetate (40 g.) was added in one portion to the cooled mixture. The vigorous reaction, which started almost immediately, was moderated by external cooling with water. After 1 hr. the mixture was poured into glacial acetic acid containing ice, and after a further 30 min. the ethereal layer was separated and washed successively with brine, aqueous sodium carbonate solution, and water. The residual liquid from evaporation of the dried ($Na_2SO_4$) ether was distilled *in vacuo*, the fraction of b. p. 93—97°/1 mm. being collected (28·5 g., 59%) (Found: C, 50·7; H, 8·3; Cl, 18·35. Calc. for $C_8H_{15}O_3Cl$: C, 49·4; H, 7·8; Cl, 18·2%).

threo-α-*Chloro*-β-*hydroxy*-γ-*methylvaleric and* erythro-αβ-*Dihydroxy*-γ-*methylvaleric Acid.*—A mixture of ethyl α-chloro-β-hydroxy-γ-methylvalerate (5 g.) and 5N-hydrochloric acid (70 ml.) was heated under reflux until a clear solution was obtained (100 min.). Solid sodium carbonate was added, bringing the pH between 9 and 10, and the solution was washed twice with ethyl acetate. The aqueous phase was acidified and re-extracted with ethyl acetate (4 times). Evaporation of the combined extracts and recrystallisation of the residue from benzene afforded the *chloro-acid* (1·27 g., 30%), m. p. 116—121° (Found: C, 43·2; H, 6·6%; equiv., 172. $C_6H_{11}O_3Cl$ requires C, 43·2; H, 6·6%; equiv., 166·5). The mother-liquors from the above crystallisation were then evaporated, and the residual gum set aside for six months. Crystallisation had then occurred, and recrystallisation from ethyl acetate–cyclohexane furnished the *dihydroxy-acid* (0·55 g., 15%), m. p. 131—132° (Found: C, 48·5; H, 8·2%; equiv., 143. $C_6H_{12}O_4$ requires C, 48·6; H, 8·2%; equiv., 148).

DL-threo-β-*Hydroxyleucine.*—α-Chloro-β-hydroxy-γ-methylvaleric acid (312 mg.) was added to liquid ammonia (30 ml.) contained in a thick-walled Carius tube. The tube was sealed and left at room temperature during 2 months. After evaporation of the ammonia, the residue was dissolved in water (20 ml.), acidified, and extracted with ethyl acetate. The aqueous solution was then evaporated, and the residue freed from chloride ions as previously described. Evaporation of the resulting solution in acetic acid, finally at 100°/0·5 mm., and recrystallisation from aqueous ethanol furnished the pure *threo*-amino-acid (15 mg., 5%) (Found: C, 48·7; H, 9·0; N, 9·7%). The infrared spectrum was identical with that of the product previously described.

DL-erythro-β-*Hydroxyleucine Ethyl Ester Hydrochloride.*—A solution of ethyl α-chloro-β-hydroxy-γ-methylvalerate (9 g.) in ethanol (250 ml.) was heated under reflux with finely powdered sodium azide (10 g.) during 25 hr. The solvent was then evaporated and the residue was extracted with ether, which was in turn washed with water and evaporated. The residue was dissolved in 95% ethanol (100 ml.) and hydrogenated over palladium black at room

temperature and pressure during 24 hr., with addition of fresh catalyst at intervals. After removal of catalyst and solvent, the residue was dissolved in ether, and dry hydrogen chloride was passed into the solution. The precipitated hydrochloride was collected, and a second crop obtained by concentration of the solution (0·616 g. in all, 5·5%). One recrystallisation from ethanolic ether furnished the pure *ethyl ester hydrochloride*, m. p. 135° (Found: C, 45·4; H, 8·6; N, 6·4. $C_8H_{18}O_3NCl$ requires C, 45·4; H, 8·6; N, 6·6%).

DL-erythro-β-*Hydroxyleucine*.—The foregoing ethyl ester hydrochloride (64 mg.) was hydrolysed in 5N-hydrochloric acid (2 ml.) at 100° during 2 hr. The solution was then evaporated, and chloride ions were removed as previously described. Evaporation of the resulting solution in acetic acid and recrystallisation of the residue from aqueous ethanol yielded the *erythro*-amino-acid (35 mg., 80%) (Found: C, 48·9; H, 8·8; N, 9·5%), $\nu_{max.}$ (Nujol and hexachlorobutadiene mulls) 3150, 2960, 1635, 1571, 1467, 1457, 1420, 1386, 1369, 1314, 1270, 1170, 1054, 1014, 997, 925, 870, 834, and 670 $cm.^{-1}$.

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