

hydroxyleucine, from the ornamental shrub *Deutzia gracilis* has also been described,¹¹ but its configuration is not known.

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Experimental¹²

Isolation of β -Hydroxyleucine.—A 2.5-g. sample of Telomycin was heated under reflux for 18 hours with 50 ml. of constant-boiling hydrochloric acid in a nitrogen atmosphere and with magnetic stirring. The mixture was taken to dryness under reduced pressure (Dow Corning Silicone antifoam), and the residue was taken up in water and clarified with activated charcoal. The residue from evaporation of the solution was dissolved in 50 ml. of 10% aqueous isopropyl alcohol and applied to 50 sheets of Whatman No. 31 chromatography paper (1 ml. per sheet) as a streak parallel to the shorter edges. The dried papers were developed (4.5 hours) by descending chromatography with butanol-water-acetic acid (4:5:1).

Narrow guide strips, cut from the paper and developed with ninhydrin (0.25% in acetone), indicated the position of the hydroxyleucine (R_f 0.5 – 0.55) and this region was cut from each sheet. The strips were eluted with water until they were ninhydrin-negative, and the combined eluates were taken to dryness *in vacuo*. The residue was transferred in a few ml. of water to a column (ca. 13 × 1.3 cm.) of 10 g. of Dowex 50-X4 (H^+), the resin washed with water until free from chloride ($AgNO_3$), and the amino acid eluted from the column with N ammonium hydroxide (50 ml.). The eluate was evaporated to dryness under reduced pressure and the residue was dissolved in a few ml. of water, treated with activated charcoal and filtered. The filtrate was taken to dryness and the residue was crystallized from aqueous ethanol, giving 120 mg. of the amino acid as colorless needles, m.p. 218–222° dec., $[\alpha]^{25}_D + 25^\circ$ (c 1.05 in H_2O), $[\alpha]^{25}_D + 35^\circ$ (c 0.41 in N hydrochloric acid).

Anal. Calcd. for $C_6H_{13}NO_3$: C, 48.97; H, 8.91; N, 9.52; C-CH₃, (1) 10.20; CO₂, 29.94. Found: C, 49.31; H, 8.84; N, 9.53; Kuhn-Roth C-methyl, 10.50; ninhydrin CO₂,¹³ 32.25.

Periodate Titration.—One mole of amino acid ($C_6H_{13}NO_3$) consumed 1.01 moles of periodate (standardized against sodium arsenite).

Periodate Degradation.—A mixture of 3 ml. of 0.02 M periodic acid solution and 5 ml. of M sodium bicarbonate solution was added to a solution of 7.4 mg. (0.050 mmole) of the hydroxyleucine (from Telomycin) in 5 ml. of water. After 5 minutes 2.5 ml. of 0.02 M sodium arsenite solution was added, followed by 1.0 ml. of N sulfuric acid. The mixture was then distilled until about 7 ml. of distillate had been collected in a receiver containing 12 mg. (0.061 mmole) of 2,4-dinitrophenylhydrazine in 0.8 ml. of 2 N hydrochloric acid. The product (which separated immediately) was collected and recrystallized from ethanol, giving 10.5 mg. (0.042 mmole) of hydrazone, m.p. 186–186.5°, unchanged on admixture with authentic isobutyraldehyde 2,4-dinitrophenylhydrazone. The infrared spectra were identical.

Phosphorus and Hydriodic Acid Reduction of Hydroxyleucine.—A mixture of 3 mg. of hydroxyleucine (from Telomycin), 18 mg. of red phosphorus and 0.1 ml. of freshly distilled constant-boiling hydriodic acid was heated in a sealed tube at 150–155° for 20 hours. The mixture was diluted with 2 ml. of water and filtered through Celite. The filtrate and washings were taken to dryness *in vacuo*, the residue was dissolved in 3 ml. of water, and the solution was treated with Amberlite resin IR-4B (OH^-) to bring the pH of the solution to 6. The residue was indistinguishable from leucine by paper chromatography in two systems, (a) 1-butanol-water-acetic acid (4:5:1) and

(b) *t*-amyl alcohol saturated with water and containing a little 8-hydroxyquinoline. System b distinguishes among leucine, norleucine and isoleucine.¹⁴

5-Isobutylidene-3-phenyl-2-thiohydantoin.—Reaction of hydroxyleucine (from Telomycin) with phenyl isothiocyanate on a 0.1 mmolar scale by Edman's procedure¹⁵ gave a 65% yield of the dehydrated phenylthiohydantoin of hydroxyleucine, m.p. 219–220° (from aqueous ethanol).

Anal. Calcd. for $C_{13}H_{14}N_2OS$: C, 63.38; H, 5.73. Found: C, 63.54; H, 5.83.

Paper Chromatography of *erythro*- and *threo*- β -Hydroxyleucines.—Two solvent systems, known⁷ to differentiate readily between the *erythro* and *threo* forms, were used: (a) *n*-butyl alcohol saturated with 10% aqueous diethylamine^{16a} (R_f 's: *erythro*, 0.41; *threo*, 0.48); (b) *n*-butyl alcohol-water-acetone-concentrated ammonium hydroxide (8:6:1:1)^{16b} (R_f 's: *erythro*, 0.30; *threo*, 0.44).

Synthetic *erythro*- β -Hydroxy-DL-leucine.—A solution of 11.15 g. (0.076 mole) of *N*-benzoyl-*erythro* and *threo*- β -hydroxy-DL-leucine ethyl ester⁷ in 100 ml. of 6 N hydrochloric acid was heated under reflux for 20 hours. The cooled solution was filtered, extracted with ether (100 ml. × 2) to remove benzoic acid, and evaporated to dryness under reduced pressure. The residue was dissolved in 0.5 N acetic acid and transferred to a column (20 × 2.5 cm.) of Dowex 1-X4 anion-exchange resin, previously washed with 0.5 N acetic acid. The combined ninhydrin-positive fractions (25 to 135 ml. of 0.5 N acetic acid eluate) were concentrated to a volume of 50 ml., diluted with 60 ml. of ethanol, and stored in a refrigerator. After 16 hours crystals were collected, affording 1.7 g. (29%) of *erythro*-amino acid, shown by paper chromatography to contain only a trace of the *threo* form (solvent system a, previous paragraph).

Anal. Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52. Found: C, 49.23; H, 8.70; N, 9.76.

Concentration of the filtrate to 20 ml. and addition of 20 ml. of ethanol gave a further 2.45 g. (45%) of crystalline product consisting of an approximately 1:1 mixture of the *threo*- and *erythro*-amino acids.

***N*-Acetyl-*erythro*- β -hydroxy-DL-leucine.**—Acetic anhydride (175 mg., 1.72 mmoles) was added during 30 minutes and with vigorous stirring to an ice-cooled solution of 168 mg. (1.14 mmoles) of *erythro*- β -hydroxy-DL-leucine and 183 mg. (1.73 mmoles) of sodium carbonate in 1.5 ml. of water. Sodium carbonate was added to bring the pH to 7.5, and stirring was continued for an additional 30 minutes at room temperature. The solution was stirred for 5 minutes with about 5 ml. of Dowex 50W-X8 cation exchange resin, and the mixture was set aside in a refrigerator for 2 hours, then filtered. Lyophilization of the filtrate afforded 223 mg. of crude product which was crystallized from ethyl acetate, giving 167 mg. (77%) of the acetyl compound, m.p. 174°.

Anal. Calcd. for $C_8H_{15}NO_4$: C, 50.77; H, 7.99. Found: C, 51.20; H, 8.07.

Resolution of *N*-Acetyl-*erythro*- β -hydroxy-DL-leucine. **Isolation of *erythro*- β -Hydroxy-L-leucine.**—The procedure is patterned after that reported for the resolution of *N*-acetyl-O-benzyl-DL-threonine.¹⁷ A suspension of 1 g. of Takadiastase in 10 ml. of water was stirred at 0° for 2 hours and filtered through Celite. The filtrate was stored at 0° until required. A 1.6-ml. portion of the enzyme solution was added to a solution of 155 mg. (0.8 mmole) of *N*-acetyl-*erythro*- β -hydroxy-DL-leucine in 0.26 ml. of 2 N sodium hydroxide solution (pH 7.2). After storage for 24 hours at 37°, the solution was transferred to a column (20 × 1 cm.) of Dowex 50W-X8 (H^+) cation exchange resin and the column was eluted with 2 N ammonium hydroxide. The combined ninhydrin-positive fractions (35–85 ml. of eluate) were evaporated to dryness under reduced pressure at 40°. Solution of the residue (73 mg.) in 1 ml. of water and addi-

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(12) Melting points are corrected. The microanalyses are by Dr. S. M. Nagy and his associates unless otherwise noted.

(13) Schwarzkopf Microanalytical Laboratory, Woodside 77, N. Y.

tion of 5 ml. of ethanol gave a crystalline precipitate which contained *erythro*- β -hydroxyleucine as the sole ninhydrin-positive component. Paper electrophoresis showed the filtrate to contain a number of ninhydrin-positive substances. The *erythro*-amino acid was purified by treating the aqueous solution (10 ml.) with 0.3 g. of activated charcoal, lyophilizing the filtrate, and recrystallizing the residue from water-ethanol; 28 mg. (47%) of *erythro*- β -hydroxy-L-leucine was thus obtained as colorless needles, m.p. 219–222° dec., $[\alpha]_{D}^{20} + 22^{\circ}$ (*c* 1.0 in water). The infrared spectrum (KBr) was identical with that of the hydroxyleucine obtained from the acid hydrolysate of Telomycin.

Anal. Calcd. for $C_6H_{13}NO_2$: C, 48.96; H, 8.90; N, 9.52. Found: C, 49.46; H, 8.62; N, 9.55.

2,4-Dinitrophenyl-*erythro*- β -hydroxy-L-leucine.—Dinitrophenylation¹⁷ of both the isolated and synthetic amino acids gave DNP derivatives which were crystallized from aqueous ethanol and had identical m.p.'s (173–174°) and infrared spectra (KBr).

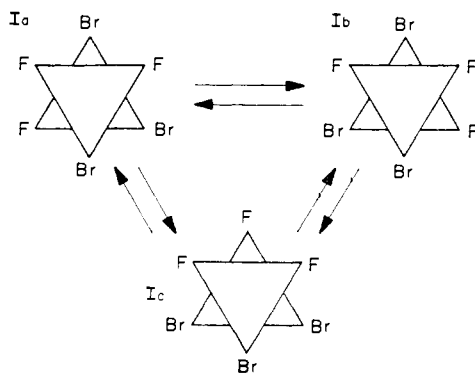
Anal. Calcd. for $C_{12}H_{15}N_3O_7$: C, 46.01; H, 4.82; N, 13.41. Found (for Telomycin product): C, 46.55; H, 5.28; N, 13.70; (for synthetic product): C, 45.83; H, 4.60.

COMMUNICATIONS TO THE EDITOR

OPPOSITE RELATIVE SIGNS OF GEMINAL AND VICINAL FLUORINE-FLUORINE N.M.R. COUPLING CONSTANTS IN A SATURATED FLUOROCARBON BY DOUBLE RESONANCE

Sir:

We wish to report the determination of the relative signs of the geminal and vicinal F^{19} - F^{19} n.m.r. coupling constants in a saturated fluorocarbon by use of the audio side band phase detection decoupling technique.^{1,2} The utility of this decoupling technique for F^{19} - F^{19} decoupling has been demonstrated recently.³ In this communication, we describe the results of double irradiation experiments on 1,1,2-trifluoro-1,2,2-tribromoethane (I). The fluorine n.m.r. spectrum of I was studied previously by Nair and Roberts,⁴ who have also discussed the temperature dependence of the spectrum of I. They demonstrated that molecules of this type are undergoing rapid rotation about the C-C bond at room temperature but that at low temperature it is possible to "freeze" molecules into a mixture of their various possible rotational configurations. The three possible rotational configurations of I are Ia, Ib and Ic. Of these three



configurations Ic would be expected to be the least stable on steric grounds because the 1-bromine atom is flanked by both of the 2-bromine atoms. Ia and Ib are mirror images and obviously have the same energy.

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- (3) D. D. Elleman and S. L. Manatt, *J. Chem. Phys.*, in press.
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We have studied the low temperature spectrum of I (50% in CS_2) at 56.4 Mc. and found that at -110° the spectrum is that of an ABX type with $J_{AB} = |165|$ cps., J_{AX} (or $J_{A'X}$) = 16.2 cps. and J_{BX} (or $J_{B'X}$) = 18.6 cps. (Fig. 1a). The chemical shift between the center of gravity of the AB region and the X region is about 705 cps.

It would appear that at this temperature I has been "frozen" primarily into a mixture of the magnetically equivalent rotational configurations Ia and Ib. However, there are additional lines in the spectrum indicating the presence of Ic. There is a sharp doublet about 388 cps. upfield from the center of gravity of the AB region and a triplet about 250 cps. upfield from the center of the X region of Ia and Ib. The intensity perturbations and spacings of these sets of lines are consistent with those of an A_2X system with a coupling of 18.8 cps. and a chemical shift of 570 cps. Relative area measurements suggest that Ic is only present to the extent of 5–7%.

At -110° we carried out double irradiation experiments at the audio frequencies of 670 and 756 cps. with a $\gamma H_1/2\pi$ of 25 cps. The results are shown in Fig. 1 where each transition has been numbered. By assigning the neighboring nuclear spin states to each transition in Fig. 1a, it is possible to predict what changes in the spectrum will take place on double irradiation between certain sets of the lines for the various possible relative sign assignments for the three coupling constants. This procedure is described in detail elsewhere.^{5,6,7}

When the AB region is observed with a decoupling frequency of 670 cps. lines 10 and 12 are receiving the strong irradiation while lines 5 and 6 are being observed. It is predicted that if J_{AX} and J_{AB} have the same relative signs then no change in lines 5 and 6 should be observed, but if J_{AX} and J_{AB} have different signs then lines 5 and 6 should collapse (as observed in Fig. 1b). When the X region is observed at the same decoupling frequency lines 5 and 6 are being strongly irradiated. If J_{AX} and J_{AB} have the same relative signs then lines 9 and 11 should collapse, but if J_{AX} and J_{AB}

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