SYNTHESES WITH STABLE ISOTOPES: DL-VALINE-$^{13}C_3$

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SUMMARY

The synthesis of DL-2-amino-3-methyl-^{13}C-butanoic-3,4-$^{13}C_3$ acid (DL-valine-$^{13}C_3$) is described. Acetonitrile-2-^{13}C was converted to 2-methyl-^{13}C-2-thiazoline, which was dialkylated with methyl-^{13}C iodide to give 2-(isopropyl-$^{13}C_3$)-2-thiazoline. Subsequent reduction and hydrolysis of the isopropylthiazoline afforded 2-methyl-^{13}C-propanal-2,3-$^{13}C_2$, which was isolated as the bisulfite addition product. A modified Strecker synthesis with the bisulfite compound gave DL-valine-$^{13}C_3$ in good overall yield.

Key Words: Amino Acids, Carbon-13, 2-Methyl-2-thiazoline, Strecker Synthesis, Valine

INTRODUCTION

Tanaka, et al. (1) have described the metabolism of valine in patients with methylmalonic acidemia using DL-valine with carbon-13 at the α and at the α and β positions of the amino acid. We have provided a side chain labeled molecule for additional studies on valine metabolism and wish to report its synthesis. The synthetic method has been applied to the preparation of DL-2-amino-3-methyl-^{13}C-butanoic-3,4-$^{13}C_2$ acid (1, DL-valine-$^{13}C_3$);

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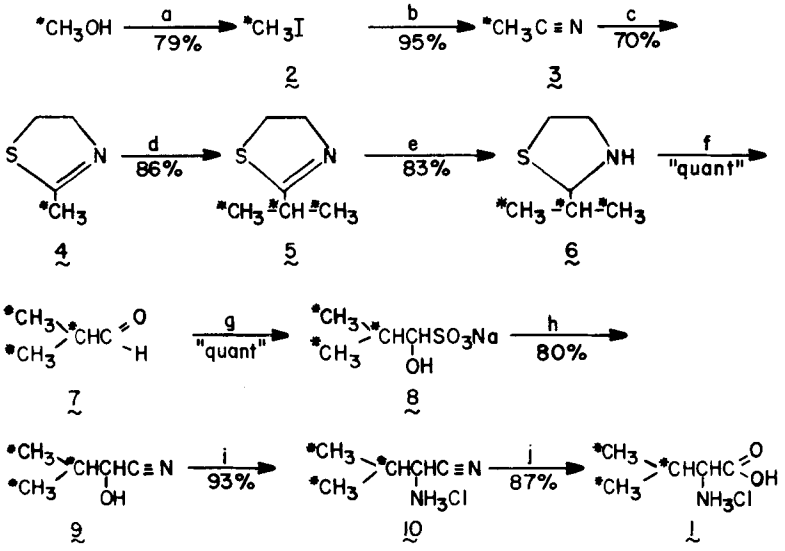
however, it can be used to label other combinations of positions in the amino acid.

DISCUSSION

We chose to investigate the Strecker synthesis of DL-valine as described by Gaudry (2) as a facile route to 1 from the appropriately labeled isobutyraldehyde. In our hands, Gaudry's method, in which isobutyraldehyde is converted to valine without isolation of intermediates, often gave erratic results. Reproducible yields could be obtained by simply isolating the various intermediates and conducting each transformation as a separate reaction. The method of Meyers and Durandetta (3) for the synthesis of dialkylacetaldehydes from 2-methyl-2-thiazoline appeared to be a useful approach to isotopically labeled isobutyraldehyde. Scheme I shows the preparation of DL-valine-$^{13}C_3$ using methanol-^{13}C as the source of the three carbon-13 labels.



(a) 57% HI; (b) NaCN, DMSO; (c) $NH_2CH_2CH_2SH$, EtOH;
(d) BuLi, THF; $^{13}CH_3I$; BuLi, $^{13}CH_3I$; (e) Al(Hg), Et_2O-H_2O;
(f) $HgCl_2$, H_2O, CH_3CN; (g) $NaHSO_3$, CH_3CN; (h) NaCN, H_2O;
(i) conc. NH_3; HCl; and (j) conc. HCl; RT 24 h; reflux 7 h.

Scheme I

Methyl-^{13}C iodide (2) was prepared in 79% yield by the reaction of methanol-^{13}C with 57% hydriodic acid and converted to acetonitrile-2-^{13}C (3) in 95% yield by treatment with sodium cyanide in DMSO (4). The reaction of acetonitrile-2-^{13}C with aminoethanethiol in refluxing ethanol (5) afforded a 70% yield of 2-methyl-^{13}C-2-thiazoline (4). Dialkylation (3) of the thiazoline 4 with methyl-^{13}C iodide proceeded smoothly to give 2-(isopropyl-$^{13}C_3$)-2-thiazoline (5) in 86% yield. Reduction of the thiazoline 5 with aluminum amalgam in moist ether afforded an 83% yield of 2-(isopropyl-$^{13}C_3$)thiazolidine (6). Cleavage of the thiazolidine ring of 6 with mercuric chloride in aqueous acetonitrile gave an essentially quantitative yield of 2-methyl-^{13}C-propanal-2,3-$^{13}C_2$ (7). However, preliminary experiments showed that workup of the cleavage reaction could not be carried out as described by Meyers and Durandetta (3) because of loss of the volatile isobutyraldehyde. The aldehyde 7 was conveniently isolated as its bisulfite addition product as follows: distillation of the reaction mixture to give an aqueous acetonitrile solution of the aldehyde 7, removal of water from the distillate with anhydrous potassium carbonate, and extraction of 7 from the acetonitrile with one equivalent of saturated aqueous sodium bisulfite to give the bisulfite addition product 8 in essentially quantitative yield. Treatment of 8 with aqueous sodium cyanide afforded DL-2-hydroxy-3-methyl-^{13}C-butanenitrile-3,4-$^{13}C_2$ (9) in 80% yield. From the reaction of the cyanohydrin 9 with conc. aqueous ammonia, DL-2-amino-3-methyl-^{13}C-butanenitrile-3,4-$^{13}C_2$ was isolated as the hydrochloride 10 in 97% yield. Hydrolysis of the aminonitrile 10 to DL-valine-$^{13}C_3$ was accomplished with conc. hydrochloric acid under conditions of 24 h at room temperature followed by 7 h at reflux. These conditions were determined from preliminary cmr studies on the hydrolysis of 2-amino-3-methylbutanenitrile. These experiments showed that the amino-

nitrile was completely hydrolyzed in 24 h at room temperature to 2-amino-3-methylbutanamide, which was then converted to valine by refluxing for 7 h. Direct hydrolysis of the aminonitrile to valine with conc. hydrochloric acid at 90°C gave lower yields of the amino acid.

EXPERIMENTAL

Materials and Methods--Methanol-^{13}C (ca. 90 mol % ^{13}C) was prepared by the method reported previously (6). Infrared spectra were recorded with a Perkin-Elmer Model 710 spectrophotometer using polystyrene calibration peaks. Cmr spectra were obtained in $CDCl_3$ or D_2O solutions using a Varian Model CFT-20 spectrometer. Peaks were referenced to solvent $CDCl_3$ (76.9 ppm) or internal CH_3OH (3% in D_2O, 49.3 ppm), and are reported relative to TMS.

Acetonitrile-2-^{13}C (3)--A solution of methyl-^{13}C iodide (182.8 g, 1.38 mol) in DMSO (200 mL) was added dropwise to a stirred mixture of NaCN (75.3 g, 1.54 mol) and DMSO (250 mL). The rate of addition of the halide was controlled so as to keep the reaction mixture at ambient temperature. After the addition was complete (2.5 h), the reaction mixture was stirred for an additional 30 min. The acetonitrile-2-^{13}C was distilled directly from the reaction mixture to give 51.2 g (95%) of 3, bp 73-75°C.

2-Methyl-^{13}C-2-thiazoline (4)--A 500-mL flask fitted with a reflux condenser, $CaCl_2$ drying tube, and magnetic stirrer was charged with aminoethanethiol (35.9 g, 0.47 mol), acetonitrile-2-^{13}C (19.5 g, 0.47 mol), and absolute ethanol (95 mL). The mixture was refluxed for 22.5 h, after which time it was shown by cmr that the reaction was over 90% complete. The solvent was removed by distillation and the residue partitioned between ether (100 mL) and H_2O (100 mL). The ether layer was separated and the aqueous layer extracted with ether (8 x 50 mL). The combined ether extracts were dried over anhydrous K_2CO_3, and the ether was removed

by distillation. Distillation of the residue at reduced pressure afforded 33.6 g (70%) of 4, bp 43.5-44.5°C at 17 torr. Ir (film): 1635 (C=N), 1430, 1355, 1130, 1030, 930, 890 cm^{-1}; cmr ($CDCl_3$): 166.2 (C=N, $^1J_{CC}$=53 Hz), 64.3 (CH_2-N, $^3J_{CC}$=7 Hz), 34.2 (CH_2-S), 19.8 (CH_3) ppm [in agreement with reported chemical shift assignments for 2-methyl-2-thiazoline (7)].

2-(Isopropyl-$^{13}C_3$)-2-thiazoline (5)--A 2-L, three-neck flask equipped with a magnetic stirrer, two addition funnels, and nitrogen inlet tube was charged with a solution of 2-methyl-^{13}C-2-thiazoline (52 g, 0.5 mol) in anhydrous THF (500 mL). The reaction mixture was cooled to -78°C in a Dry Ice-isopropyl alcohol bath, and butyl lithium (300 mL, 1.68 M in hexane, 0.50 mol) was added over a period of 40 min. The reaction mixture was stirred at -78°C for an additional 1.5 h, and then methyl-^{13}C iodide (71 g, 0.50 mol) was added dropwise over a period of 1.5 h. The reaction mixture was stirred for an additional hour at -78°C, after which time it was allowed to warm to 10°C, and stirring was continued for 2 h at this temperature. The reaction mixture was cooled to -78°C, and butyl lithium (300 mL, 1.68 M in hexane, 0.50 mol) was added over a period of 1 h. After stirring for an additional 1.75 h at -78°C, methyl-^{13}C iodide (71 g, 0.50 mol) was added dropwise over a period of 45 min. The reaction mixture was allowed to warm to 10°C and was stirred for 45 min, after which it was poured over 1 kg of ice. The aqueous layer was adjusted to pH 2 while stirring vigorously, and the hexane-THF layer was separated. The aqueous layer was extracted with hexane (200 mL), and was then adjusted to pH 10 with 20% NaOH. The product 5 was extracted from the milky aqueous phase with hexane (4 x 200 mL), and the combined hexane extracts were dried over anhydrous K_2CO_3. Removal of the solvent and distillation of the residue afforded 56.4 g (86%) of 5 as a colorless liquid, bp 62-65°C at 15 torr. Ir (film): 1620 (C=N), 1455, 1365 and 1345 (iPr), 1030, 970, 900 cm^{-1}; cmr ($CDCl_3$): 176.8 (C=N),

64.0 ($\underline{C}H_2N$), 33.4 ($\underline{C}H_2$-S), 32.8 ($CH_3\underline{C}HCH_3$, $^1J_{CC}$=36 Hz), 20.7 ($\underline{C}H_3CH\underline{C}H_3$, $^1J_{CC}$=36 Hz) ppm.

2-(Isopropyl-$^{13}C_3$)thiazolidine ($\underline{6}$)--Aluminum foil (79 g), as 1.2-cm squares in a 5-L, round-bottom flask, was treated with 5% KOH (1 L) for 10 min, after which the basic solution was removed and the metal washed with H_2O (4 L). The aluminum was then treated with 0.5% $HgCl_2$ (900 mL). After 3 min, the solution was removed and a second treatment with aqueous $HgCl_2$ carried out. The foil was washed successively with H_2O (5 L), 95% EtOH (2 x 1 L), and ether (2 x 1.5 L). The flask containing the foil was fitted for reflux with an efficient condenser, and a solution of 2-(isopropyl-$^{13}C_3$)-2-thiazoline (56 g, 0.43 mol) in moist ether (2.9 L) was added. The reaction mixture was gently refluxed for 2 h; and, after cooling, the ether solution was filtered. The aluminum amalgam was washed with ether (2 x 500 mL), and the ether solutions were combined. After removal of the ether, the residual oily product was dried over anhydrous K_2CO_3. The drying agent was separated and washed with small amounts of ether. Distillation of the oil afforded 47.6 g (83%) of $\underline{6}$ as a colorless liquid, bp 76-79°C at 15 torr. Cmr ($CDCl_3$): 78.8 (N-$\underline{C}$H-S), 52.3 ($\underline{C}H_2$-N), 34.5 ($\underline{C}H_2$-S), 33.8 ($CH_3\underline{C}HCH_3$, $^1J_{CC}$=35 Hz), 20.4 ($\underline{C}H_3CHCH_3$, $^1J_{CC}$=35 Hz), 20.1 ($CH_3CH\underline{C}H_3$, $^1J_{CC}$=35 Hz) ppm.

2-Methyl-^{13}C-propanal-2,3-$^{13}C_2$ ($\underline{7}$)--A 1-L, flat-bottom flask equipped with a dropping funnel, magnetic stirrer, and condenser was charged with $HgCl_2$ (57.3 g, 0.211 mol), H_2O (48 mL), and acetonitrile (192 mL). To the stirred solution was added a solution of the thiazolidine $\underline{6}$ (26.79 g, 0.200 mol) in acetonitrile (60 mL) over a period of 40 min. During this time, a white precipitate separated. The reaction mixture was stirred for an additional 2 h, after which NaCl (100 g) and H_2O (190 mL) were added. The condenser was arranged for downward distillation, and the reaction mixture was heated with stirring. The distillate (bp 64-70°C), consisting of

H_2O, 7, and acetonitrile, was collected in a receiver connected to a Dry Ice-isopropyl alcohol trap. The combined distillate from the receiver and trap was dried over anhydrous K_2CO_3, and the acetonitrile solution of the aldehyde 7 was added to a stirred solution of $NaHSO_3$ (20.8 g, 0.200 mol) in H_2O (24 mL). The aldehyde-sodium bisulfite addition compound separated as a colorless solid, and the mixture was stirred overnight. The addition product was collected, washed with acetonitrile, and air-dried to give 35.63 g (99%) of 2-methyl-^{13}C-propanal-2,3-$^{13}C_2$ bisulfite addition product (8) as a colorless solid.

DL-2-Hydroxy-3-methyl-^{13}C-butanenitrile-3,4-$^{13}C_2$ (9)--A 1-L, round-bottom flask was charged with 8 (35.63 g, 0.2 mol) and H_2O (79 mL). To this cooled mixture (ice bath) was added dropwise a solution of KCN (26.85 g, 97%, 0.400 mol) in H_2O (55 mL). After the addition was complete (10 min), the reaction mixture was stirred at room temperature for 2 h. The cyanohydrin 9 was extracted from the aqueous solution with CH_2Cl_2 (5 x 50 mL). The combined CH_2Cl_2 extracts were dried over anhydrous $MgSO_4$ and filtered. The solvent was removed on a rotary evaporator to give 16.34 g (80%) of the cyanohydrin 9 as a colorless oil. Cmr ($CDCl_3$): 119.1 (CN), 66.6 (CHCN), 32.7 (CH_3CHCH_3, $^1J_{CC}$=35 Hz), 17.5 (CH_3CHCH_3, $^1J_{CC}$=35 Hz, 17.0 (CH_3CHCH_3, $^1J_{CC}$=35 Hz) ppm.

DL-2-Amino-3-methyl-^{13}C-butanenitrile-3,4-$^{13}C_2$ Hydrochloride (10)--The cyanohydrin 9 (16.34 g, 0.160 mol) was added dropwise to conc. aqueous NH_3 (51 mL) in an Erlenmeyer flask with stirring. The reaction mixture turned golden yellow and, after 15 min of stirring, became cloudy. After stirring for 6 h at room temperature, the reaction mixture was extracted with CH_2Cl_2 (5 x 30 mL). The combined CH_2Cl_2 extracts were extracted with 10% aqueous HCl (4 x 55 mL). The aqueous extracts were combined and rotary evaporated to give 21.34 g (97%) of the aminonitrile hydrochloride 10 which was directly used in the next step (8).

DL-2-Amino-3-methyl-^{13}C-butanoic-3,4-$^{13}C_2$ Acid Hydrochloride (1)--A solution of the hydrochloride 10 (26.86 g, 0.196 mol) in conc. HCl (142 mL) was allowed to stand at room temperature for 24 h. The reaction mixture was then heated at 90-98°C (reflux) for 7 h. The reaction mixture was cooled to room temperature, and colorless crystals separated. After further cooling in an ice bath, more crystals formed. The crystals were collected, washed with cold conc. HCl, and dried to give 16.33 g of a mixture of DL-valine-$^{13}C_3$ hydrochloride (1) and NH_4Cl. The filtrate and washings were combined and evaporated to dryness on a rotary evaporator for an additional 23.27 g of solid. These combined solids were extracted with hot ethanol and filtered to remove NH_4Cl (9.94 g, 95%). The ethanol extracts were rotary evaporated to precipitate the hydrochloride 1 as a colorless crystalline solid. The solid was collected, washed with acetonitrile, and dried to give 27.83 g (91%) of 1, which was shown by titration to contain a small amount of NH_4Cl. An additional 1.10 g of solid was obtained from the mother liquor. The initial crop was triturated with absolute ethanol (105 mL) and the insoluble solid (NH_4Cl, 0.96 g) removed. The filtrate was rotary evaporated to a syrup (30 g), 75 mL of acetonitrile was added, and the mixture was cooled in an ice bath, whereupon the hydrochloride 1 crystallized to give 26.07 g (85%) of 1 as colorless crystals. An additional 9.43 g (2%) was obtained from the mother liquor. Cmr (D_2O): 172 (COOH), 58.7 (CH-N), 29.4 (CH_3CHCH_3, $^1J_{CC}$=34 Hz), 17.8 (CH_3CHCH_3, $^1J_{CC}$=34 Hz), 17.3 (CH_3CHCH_3, $^1J_{CC}$=34 Hz)·ppm.

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8. An alternate procedure, in which the hydrochloride 10 was isolated by precipitation from the CH_2Cl_2 solution with HCl gas, was suitable also.