PREPARATION OF D- AND L-ALANINE-2,3-$^{13}C_2$

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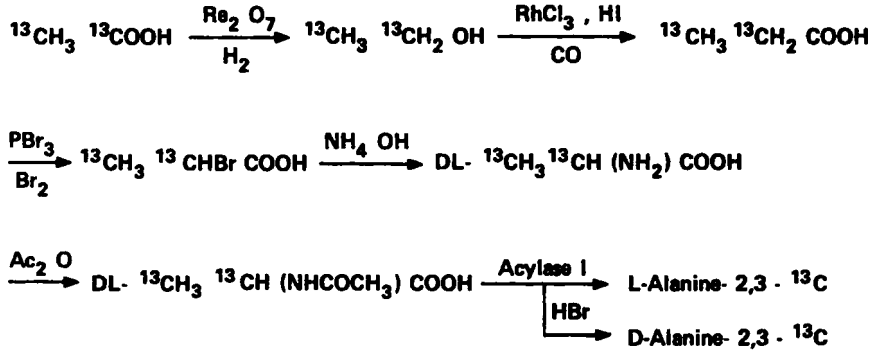
SUMMARY

Specifically labeled, optical isomers of alanine were synthesized from acetic-$^{13}C_2$ acid by a six-step procedure with yields of 37-38% of the optically active material.

Key Words: Ethanol-$^{13}C_2$, Propionic-2,3-$^{13}C_2$ acid, 2-Bromopropionic-2,3-$^{13}C_2$ acid, DL-Alanine-2,3-$^{13}C_2$, L-Alanine-2,3-$^{13}C_2$

INTRODUCTION

Stable isotope-labeled materials are being used in medical research and clinical diagnosis to extend tracer techniques beyond those currently in use with radioisotopes (1). Amino acids, sugars, and fatty acids, with carbon-13, have been used to study metabolic disorders such as diabetes (2) and cystic fibrosis (3). L-Alanine is one simple, easily synthesized, amino acid which has been needed in several research studies. Highly labeled L-alanine-^{13}C was produced in a series of reactions beginning with acetic-$^{13}C_2$ acid (4) as shown in Scheme 1.



Scheme 1

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DISCUSSION

Acetic acid was reduced to ethanol by a catalytic method (5) rather than by chemical reduction. The choice was made in favor of a method which would use readily available reactants and which had advantages of purification. Although hydrogenation with rhenium heptoxide is slow, it is essentially quantitative. While used to advantage here, carbonylation (6) is not a universal technique for formation of all labeled acids. Other work within this Laboratory has established that, with ethanol containing a single ^{13}C label, a rearrangement takes place during the reaction, giving rise to a mixture of isotopic isomers, probably through ethylene. The bromo acid has been synthesized by action of phosphorous tribromide and bromine in carbon tetrachloride on propionic acid (7) and by reaction of propionic anhydride with bromine alone. The small amount of side product, dibromopropionic acid, is not removed prior to synthesis of the amino acid and apparently does not give a product in the reaction as shown by a sequential nmr analysis. An efficient formation of the racemic amino acid takes place using a large excess of ammonium hydroxide with the halogenated acid.

Resolution of the racemic alanine was accomplished by an enzymatic method (8) and the procedures of Baker and Sober (9); however, even with the prior work, proper conditions for enzymatic bond cleavage had to be determined by many trial runs to yield a product in a reasonable time with a reasonable amount of enzyme. The overall yield of optically active products through the six steps was 37-38%.

EXPERIMENTAL

Materials and Methods--Melting points were taken on a Fischer-Johns apparatus and were uncorrected. The infrared spectra were measured on a Perkin-Elmer Model 710, and only those wavelengths were reported where significant differences were seen in comparison with material of natural abundance (parenthetical frequencies are natural abundance). Specific gravities were determined by weighing in a pycnometer (ca. 5 ml). Optical rotations were read with a Hilger standard polarimeter, and refractive indices were determined with a Bausch and Lomb

precision refractometer. Pmr spectra were taken on a Perkin-Elmer Hitachi R-24 spectrometer using either sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or tetramethylsilane TMS as internal standards.

Ethanol-$^{13}C_2$--In a typical preparation, 1 mol of acetic-$^{13}C_2$ acid (4) was placed in a 1-liter stainless steel autoclave with 150 ml of water and 2 g of rhenium heptoxide. A Teflon-covered magnetic stirring bar was inserted, and the autoclave was sealed and pressured to 700 psi with hydrogen. Stirring was commenced, and the autoclave was heated to 180°C. Over a period of 7 days, the pressure was maintained between 700 and 1000 psi by addition of hydrogen and, when no further pressure drop was observed, heating was terminated. The cooled autoclave was opened, and the product mixture was transferred to a glass flask fitted for distillation. The fraction distilling between 72 and 93°C (580 torr) was collected to give 107 g of distillate [59% water and 41% ethanol, by pmr spectroscopy (44 g, 0.92 mol, 92% yield)]. Anhydrous ethanol was produced by treating the aqueous material with 3A molecular sieve and subsequent bulb-to-bulb vacuum transfer. d_4^{20} 0.822; bp 71-71.5°C (590 torr); n_D^{21} 1.36107; nmr (CCl_4) δ 1.16 (m, 3H, CH_3, $J_{^{13}C-H}$ 128 Hz, $J_{^{13}C-C-H}$ 4.3 Hz), 3.59 (m, 2H, CH_2, $J_{^{13}C-H}$ 147 Hz, $J_{^{13}C-C-H}$ 2.0 Hz); ir (neat) 1880 (1920), 1365 (1380), 1070 (1085), 1020 (1040), 860 (870) cm^{-1}.

Propionic-2,3-$^{13}C_2$ Acid--Into a 1-liter stirred autoclave were placed 0.48 mol of aqueous ethanol-$^{13}C_2$, 0.8 g of rhodium trichloride, and 10 ml of 57% hydriodic acid. The autoclave was sealed, carbon monoxide (500 psi, 1.5 mol) was admitted, stirring was started, and the reaction mixture was brought to 200°C. During 10 hr of reaction time, the internal pressure was decreased by 290 psi (represents 0.52 mol of carbon monoxide). After cooling, the reaction mixture was drawn from the autoclave, and 0.5 ml of phosphorous acid was added to prevent formation of elemental iodine. The product was distilled and collected as the

aqueous solution. A second distillation was made from silver sulfate, and the distillate was neutralized with standardized sodium hydroxide. Evaporation gave 41.1 g (88%) of sodium propionate-2,3-$^{13}C_2$ as a white solid. Anhydrous propionic-2,3-$^{13}C_2$ acid was obtained from treatment of the sodium propionate with 85% phosphoric acid and a subsequent bulb-to-bulb vacuum transfer of the acid from the inorganic salt. d_4^{20} 1.017; bp 133-133.5°C (590 torr); n_D^{22} 1.38629; nmr ($CDCl_3$) δ 1.18 (m, 3H, CH_3, $J_{^{13}C-H}$ 127 Hz, $J_{^{13}C-C-H}$ 3.8 Hz), 2.30 (m, 2H, CH_2, $J_{^{13}C-H}$ 130 Hz, $J_{^{13}C-C-H}$ 3.5 Hz); ir (neat) 1410 (1415), 1050 (1974), cm^{-1}.

DL-2-Bromopropionic-2,3-$^{13}C_2$ Acid--Aqueous propionic-2,3-$^{13}C_2$ acid (0.39 mol) was dried *in situ* by azeotropic distillation with carbon tetrachloride. To this solution was added 13.5 ml (0.07 mol) of phosphorous tribromide, and heat was applied to give a gentle reflux. Over the next 11 hr, a solution of 81 g (0.51 mol) of bromine in 20 ml of carbon tetrachloride was added. Reflux was continued for another 12 hr; then the colorless solution was cooled, 4 ml of water was added, and the mixture was refluxed for 20 min. After cooling, the reaction flask was fitted for distillation, carbon tetrachloride was removed, and the product was distilled at reduced pressure. bp 116-118°C (20 torr); yield 57 g (0.37 mol), 94%; nmr ($CDCl_3$) δ 1.86 (m, 3H, CH_3, $J_{^{13}C-H}$ 132 Hz, $J_{^{13}C-C-H}$ 4.8 Hz), 4.48 (m, 1H, CH, $J_{^{13}C-H}$ 152 Hz, $J_{^{13}C-C-H}$ 2.0 Hz); ir (neat) 960 (975) cm^{-1}.

DL-Alanine-2,3-$^{13}C_2$--A mixture of 53 g (0.34 mol) of DL-2-bromopropionic-2,3-$^{13}C_2$ acid and 10 ml of water was placed in an addition funnel. Over a period of 2 hr, the acid mixture was added dropwise to a stirred 2 liters of cold concentrated ammonium hydroxide. After 3 days at room temperature, the solution was reduced to about 100 ml under reduced pressure. All solid material in the mixture was brought into solution by heating; then 400 ml of hot methyl alcohol was added. The crystals of product were filtered from the chilled solution after standing overnight. A total of 75 ml of cold methyl alcohol was used, in several portions, to wash the product. Combined first and second crops of product weighed 23.8 g, 71%; mp sublimes *ca.* 250°C.

DL-N-Acetylalanine-2,3-$^{13}C_2$--A suspension of 21.8 g (0.24 mol) of DL-alanine-2,3-$^{13}C_2$ in 100 ml of glacial acetic acid in a 250-ml round-bottom flask was stirred rapidly and heated to 108°C. Heating was discontinued and, when the temperature had fallen to 100°C, 31 ml (0.33 mol) of acetic anhydride was added. The temperature rose rapidly to 110°C, and the reaction was held at that temperature for 1 min (all solid material going into solution). The reaction was immediately quenched with 500 ml of cold water, and this aqueous solution was subsequently evaporated to dryness under reduced pressure on a rotary evaporator to give 32.5 g, 97% of product; mp 134-136°C (lit. mp 137-138°C).

L-Alanine-2,3-$^{13}C_2$--To a solution of 31.4 g (0.24 mol) of DL-acetylalanine-2,3-$^{13}C_2$ in 200 ml of 1.5 N ammonium hydroxide was added 1.77 g of hog kidney acylase (Sigma Chemical Co., Grade II). After vigorous mixing, the mixture was held at 38°C on a water bath for 23 hr. The enzyme was deactivated (coagulated) by boiling the solution for several minutes; Norit was added, and the solution was filtered through a bed of Celite and then concentrated under reduced pressure on a rotary evaporator. With 100-125 ml of solution remaining, 50 ml of ethanol was added, and the solution was filtered to remove traces of protein. After further vacuum evaporation, a residual thick slurry was washed into a 500-ml Erlenmeyer flask with several portions of boiling ethanol to give a total volume of 320 ml. The slurry was boiled to reduce the volume of solvent to approximately 200 ml and to drive off all residual ammonia. After cooling the mixture in an ice bath, the crystals of L-alanine were collected on a filter and dried (8.2 g). One recrystallization from water-ethanol (120 ml of boiling ethanol added to 55 ml of hot aqueous solution) gave 6.8 g, plus a second crop of 0.7 g. The combined yield of product was 70%; mp sublimes ca. 250°C; $[\alpha]_D^{22}$ = +13.86 (9.02% 6 N HCl); nmr (D_2O) δ 1.47 (m, 3H, CH_3, $J_{^{13}C-H}$ 130 Hz, $J_{^{13}C-C-H}$ 4.1 Hz), 3.81 (m, 1H, CH, $J_{^{13}C-H}$ 134 Hz, $J_{^{13}C-C-H}$ 4.2 Hz); nmr (NaOD), δ 1.21 (m, 3H, CH_3, $J_{^{13}C-H}$ 129 Hz, $J_{^{13}C-C-H}$ 4.0 Hz), δ 3.30 (m, 1H, CH, $J_{^{13}C-H}$ 133 Hz, $J_{^{13}C-C-H}$ 4.0 Hz); ir (KBr) 1345 (1355), 1290 (1300), 1085 (1100), 990 (1000) cm^{-1}.

D-N-Acetylalanine-2,3-$^{13}C_2$--The mother liquor from the isolation of crude L-alanine-2,3-$^{13}C_2$ (0.12 mol) was applied to a Dowex 50W X-4 [H^+] column containing 370 ml (444 meq) of resin. Water (1 liter) was used to elute the acetyl compound from the column, and this solution was taken to dryness on a rotary evaporator at 25 torr pressure. The product weighed 15.3 g. This residue was taken up in boiling acetone (60 ml), the solution was cooled, and the resultant crystals were collected on a filter and air-dried to give 14.8 g of product; mp 127.5-128.5°C; $[\alpha]_D^{26}$ = +44.77 (8.98% 6 N HCl).

D-Alanine-2,3-$^{13}C_2$--A solution of 5.3 g (0.04 mol) of D-N-acetylalanine-2,3-$^{13}C_2$ in 75 ml of 2 N HCl was placed in a 250-ml round-bottom flask fitted with a reflux condenser. Refluxing was carried out for 4 hr; then the solution was cooled and taken to dryness on a rotary evaporator. The residue was twice taken up in water, and the solvent was evaporated to remove acetic acid traces. The process was repeated twice more with 10-15 ml of toluene. A final residue was then mixed with 4 ml (0.043 mol) of freshly distilled aniline and 200 ml of water. The solution was heated, filtered, and diluted with 53 ml of ethyl alcohol; then, upon cooling, crystals of the product formed slowly on the walls of the flask. After several days of standing in the cold, the crystals were filtered from the solution and washed with cold ethyl alcohol. The product weighed 2.65 g, 73%; mp sublimes; $[\alpha]_D^{22}$ = 13.38 (9.04% in 6 N HCl).

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