SYNTHESIS OF (2RS)-[3-^{13}C]- and (2RS)-[3-^{14}C]LEUCINE

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SUMMARY

(2RS)-[3-^{13}C]- and (2RS)-[3-^{14}C]Leucine have been prepared *via* carbonation of the Grignard reagent 2 from 2-bromopropane, with $^{13}CO_2$ or $^{14}CO_2$. Reduction of the labelled isobutyric acid, displacement of the derived isobutyl brosylate 5 with acetamidomalonic ester and hydrolysis afforded (2RS)-[3-*C]leucine with 55-65% incorporation of label from Ba*CO_3 on a scale of 5-25 mmol.

Key Words: [3-^{13}C]leucine, [3-^{14}C]leucine, Ba$^{13}CO_3$, Ba$^{14}CO_3$, Grignard, acetamido-malonic ester.

Specifically ^{13}C-labelled precursors have played a leading role in recent years in the study of metabolic and biosynthetic pathways. They become the agents of choice when incorporations are sufficiently high for label to be detected in the metabolic product by ^{13}C NMR spectroscopy.[1-3] In the course of metabolic studies with branched-chain amino acids in plant tissue cultures,[4] we required [3-^{13}C]- and [3-^{14}C]leucine. A preparation of [3-^{14}C]leucine analogous to that described below has been published in an inaccessible Russian journal,[5] but experimental details are lacking and the yields appear to be moderate. We here report syntheses of [3-^{13}C]- and [3-^{14}C]leucine based on the inexpensive barium [^{13}C]carbonate and sodium [^{14}C]carbonate. By careful attention to the practical details outlined, we have consistently obtained overall incorporations of 55-65% of the Ba*CO_3 label into leucine on a scale varying from 5 to 25 mmolar. The sequence of reactions used is outlined in the Scheme.

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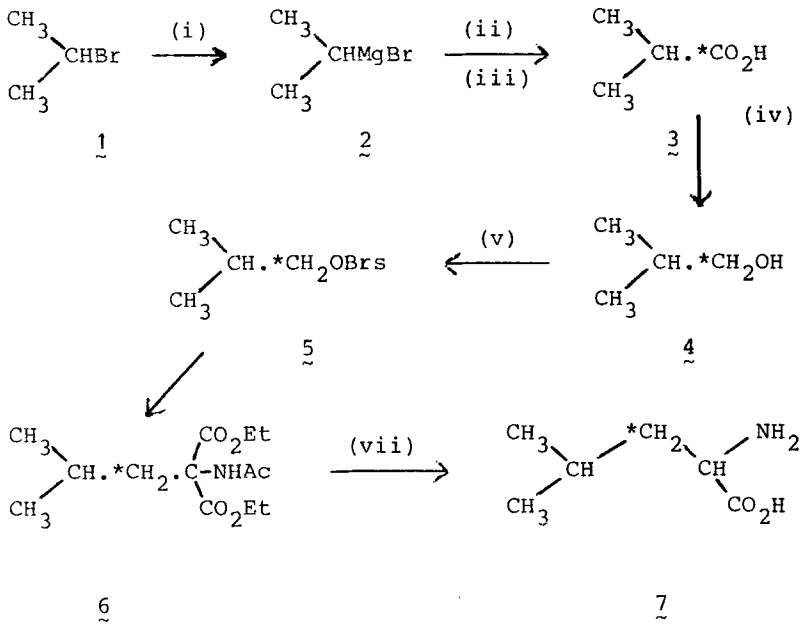


SCHEME

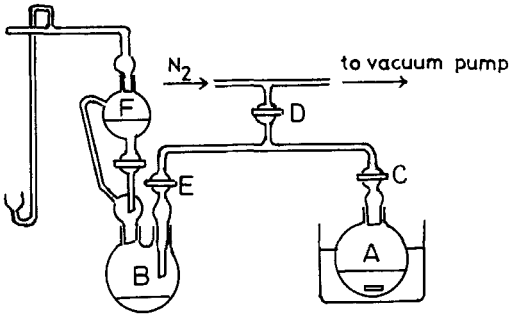
(i) Mg/Et_2O; (ii) Ba^*CO_3/conc. H_2SO_4; (iii) 6N HCl; (iv) $LiAlH_4/Et_2O$; (v) BrsCl/pyridine; (vi) $AcNH.CH(CO_2Et)_2/NaH$; (vii) 6N HCl

EXPERIMENTAL

Materials and Methods. Barium [^{13}C]carbonate (90 atom% ^{13}C) was purchased from Prochem (BOC Ltd., London, now Amersham International plc), sodium [^{14}C]carbonate (50 mCi/mmol) from Amersham International plc. PMR and CMR spectra were obtained with Varian XL100 and Bruker WP200SY spectrometers, mass spectra with a Kratos-VG MS902 mass spectrometer.

[1-^{13}C]Isobutyric Acid 3. The apparatus used was a simplified version of that described by Dauben[6,7] (see Figure). The entire assembly was checked for leaks by evacuating it to 0.1 mm Hg. The

Grignard reagent 2 was prepared in a 100 ml flask, A, by reacting together isopropyl bromide (2.53 g, 20.6 mmol) and magnesium turnings (500 mg, 20.6 mmol) in dry ether (50 ml). $Ba^{13}CO_3$ (1.00 g, 5.05 mmol, 90.3 atom % ^{13}C) was placed in B and F charged with conc. H_2SO_4 (10 ml, degassed). The apparatus was then assembled as in the Figure, and evacuated to 0.1 mm Hg with taps D and E open. Nitrogen was then carefully admitted via D to restore atmospheric pressure and the cycle of evacuation and nitrogen admission repeated twice more. During the last evacuation B and F were gently warmed, using a hot air gun, to remove residual gases. Flask A was next cooled in liquid N_2 and evacuated separately by closing E and opening C and D. After 10 min. E was opened and the entire assembly evacuated. Tap D was then closed and H_2SO_4 from F added as far as possible down the side of flask B on to the $BaCO_3$, at first very cautiously so as to moderate the initial evolution of CO_2 and avoid expulsion of $BaCO_3$ from B, and then at such a rate as to keep the pressure constant. Tap C was then closed and the contents of A allowed to warm to -10°C. Vigorous stirring in A was started as soon as the ether melted and was continued at -10°C for 15 min. Flask A was again cooled in liquid N_2, C opened and any residual



FIGURE

CO_2 in the rest of the system displaced towards A by heating externally with a hot air gun. Tap C was closed once more and the contents of A allowed to reach room temperature with stirring which was continued for 2 h. more. Tap C was then opened and air admitted via D. Flask A was detached, cooled to 0°C and acidified with 6N HCl, cautiously from a dropping funnel. The ether layer was separated, the aqueous layer washed with ether (4 x 35 ml), the combined ether extracts washed with brine and dried over anhydrous Na_2SO_4. This solution (ca. 200 ml) was used direct in the next step.

Note. Provided a constant vacuum was maintained throughout the carbonation, yields of isobutyl alcohol in Step (iv) were in excess of 80%, as determined by quantitative g.l.c. analysis (OV-17, 6 ft.; R.T. 1.125 min. at 30°, N_2 pressure 5 lb./sq. in.). Fluctuations in pressure during Step (ii) resulted in reduced yields.

[1-^{13}C]Isobutyl Alcohol 4. The solution of [^{13}C]isobutyric acid in ether (200 ml), obtained above, was added dropwise to a stirred suspension of $LiAlH_4$ (400 mg, 10 mmol) in ether (20 ml) in an argon atmosphere, and then the solution was heated at reflux for 3 h. After cooling saturated aqueous Na_2SO_4 was added dropwise and with stirring until a white gelatinous precipitate formed. This was removed by filtration and the ethereal solution was dried over anhydrous Na_2SO_4. G.l.c. analysis of the ether solution (OV-17, 30°C), showed a single peak, R.T. 1.125 min., coincident with authentic iso-butanol, in a yield of 87 ±2%.

[1-^{13}C]Isobutyl Brosylate 5. The ether solution of [^{13}C]isobutanol, obtained above, was carefully reduced to a volume of about 5 ml, by evaporation at 760 mm and 45°C (bath temperature). Dry

pyridine (25 ml) was added, the mixture cooled to 0°C, followed by p-bromobenzenesulphonyl chloride (BrsCl) (3.07 g, 12 mmol), and the mixture stored for 16 h. at 0°C. The reaction mixture (containing separated pyridine HCl) was poured into ice cold water (50 g) and extracted with ether (5 x 50 ml), the combined extracts washed with brine and dried over anhydrous Na_2SO_4. Removal of the ether at 20°C gave a pale yellow oil (1.136 g), whose ^{1}H NMR spectrum was identical with that of crystalline brosylate 5 (see below). The oil was dissolved in the minimum volume of light petroleum (40-60°). Cooling to -78° and magnetic stirring of the solution induced separation of the crystalline brosylate 5, m.p. 29-30°; (0.876 g; 59% based on $BaCO_3$). ^{1}H NMR: δ ($CDCl_3$) 0.87 (6H, dd, $^3J_{CH}$ = 6Hz, J_{HH} = 7Hz, $(CH_3)_2C$); 1.92 (1H, m, $CH(CH_3)_2$); 3.81 (2H, dd, $^1J_{CH}$ = 150Hz, J_{HH} = 6Hz, CH_2OBrs); 7.71 (4H, m, aromatic H's). MS: M^+ 294.9784 (calc. for $C_9{}^*CH_{13}O_3S^{81}Br$ 294.9872; peak corresponding to $C_9{}^*CH_{13}O_3S^{79}Br$ not measurable because of overlap with reference peak); 237.9127/235.9149 ($M^+ - C_4H_8$); 220.9098/218.9118 ($M^+ - C_4H_9O$); 156.9477/154.9498 ($M^+ - C_4H_9O_3S$).

Filtration of the mother liquors through a short silica gel column produced a further quantity (0.16 g, 11%) of brosylate 5, identical in its NMR spectrum with that of crystalline material. Although this resisted efforts to crystallise it, it was no less effective than crystalline material in the next step.

Diethyl-[1-^{13}C]isobutyl-acetamidomalonate 6. Sodium hydride (0.38 g, 16 mmol) was suspended in 1,3-dimethyl-2-imidazolidinone (Aldrich; 16 ml)* and to the stirred suspension under argon was added diethyl acetamidomalonate (3.47 g, 16 mmol). Stirring was

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* Hexamethylphosphoramide (HMPT) was used in earlier runs. The use of 1,3-dimethyl-2-imidazolidinone resulted in improved yield (from 70 to 90%) and marked ease of isolation.

continued at 20° until the solution became clear and it was then heated at 60-70° for 2.5 h. The solution was cooled to 20°, [^{13}C]brosylate 5 (2.32 g, 7.93 mmol) in 1,3-dimethyl-imidazolidinone (2 ml) was added and the reaction stirred at 20° for 16 h. and then at 60-70° for 130 h. more. The cooled reaction was mixed with ice cold water (50 g) and extracted with ethyl acetate (5 x 40 ml). The combined extracts were washed with 4N NaOH (4 x 50 ml), ice cold water (4 x 50 ml) and then dried with anhydrous $MgSO_4$. Solvent removal in vacuo afforded the product 6 (1.95 g, 90%) as a crystalline solid whose ^{1}H NMR spectrum was identical with that of recrystallised material, and was satisfactory for use in the next stage. Recrystallisation from ether-hexane afforded diethyl-[1-^{13}C]isobutyl acetamido malonate 6 (1.3 g), m.p. 83-84° (Lit.[8] 84°). ^{1}H NMR: δ ($CDCl_3$) 0.86 (6H, m, $(CH_3)_2C$); 1.21 (6H, t, ester CH_3); 1.99 (3H, s, $NHCOCH_3$); 2.03 (1H, m, $CH(CH_3)_2$);2.29 (2H, dd, $^1J_{CH}$ = 132Hz; J_{HH} = 7Hz, C-3 H's); 4.23 (4H, q, ester CH_2); 6.84 (1H, bs, NH, D_2O-exchangeable). MS: M^+ 274.1615 (calc. for $C_{12}{}^*CH_{23}NO_5$ 274.1609); 201.1329 (M^+ -CO_2Et); 159.1220 (M^+ -CO_2Et-CH_2CO); 85.0845 (M^+ -2(CO_2Et)-CH_2CO).

(2RS)-[3-^{13}C]Leucine 7. Diethyl-[1-^{13}C]isobutyl-acetamido-malonate (0.766 g, 2.80 mmol) was hydrolysed by heating it under reflux with 6N HCl (10 ml) for 24 h. The solution was taken to dryness in vacuo on a rotary evaporator, dissolved in water (10 ml) and again taken to dryness, then dissolved in water (2 ml) and placed on an ion-exchange column (Dowex 50W-X8; standard H^+, 20 ml). Washing with water (until free of Cl^-) and then elution with 2N NH_4OH (until negative to ninhydrin) afforded (2RS)-[3-^{13}C]leucine, 7 (339 mg, 92%), m.p. 292-297° (sealed tube). ^{1}H NMR: δ (D_2O) 1.13 (6H, dd, $^3J_{CH}$ = 6Hz, J_{HH} = 7Hz; CCH_3); 1.95 (1H, m, C-3H); 1.98 (2H, double triplet of doublets, $^1J_{CH}$ = 134Hz; C-3 H's); 4.00

(1H, m, C-1 H). MS: M^+ 132.0978 (calc. for $C_5{}^*CH_{13}NO_2$ 132.0979), 87.1009 ($M^+ - CO_2H$), 74.0245 ($M^+ - C_4H_9$), 45.0531 (CO_2H^+).

(2RS)-[3-^{14}C]Leucine was synthesised by the same procedure, except that $Ba^{14}CO_3$ was prepared from $Na_2{}^{14}CO_3$ as follows: $BaCl_2$ (2.08 g, 10 mmol) in degassed water (3 ml) was added to $Na_2{}^{14}CO_3$ (250 µCi) and Na_2CO_3 (1 g, 9.4 mmol) in degassed water (2.25 ml). The precipitated $Ba^{14}CO_3$ was filtered on a sintered glass crucible and dried in vacuo at 120°C overnight, affording 1.85 g $Ba^{14}CO_3$. (2RS)-[3-^{14}C]leucine obtained from this by the procedure used above for (2RS)-[3-^{13}C]leucine, and purified by ion exchange chromatography as above, had a final specific activity of 26.2 µCi/mmol.

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