SYNTHESIS OF (S)-LEUCINE-$^{13}C_3$ AND ITS METABOLITES

Sun-Shine Yuan and Joseph Foos

KOR ISOTOPES
56 Rogers St., Cambridge, MA 02142 (USA)

SUMMARY

A synthesis for (S)-2-amino-4-methyl-^{13}C-pentanoic-2,5-$^{13}C_2$ acid ((S)-leucine-$^{13}C_3$) is described. The alkyl chain was constructed by condensing acetone-1,3-$^{13}C_2$ with triethyl phosphonacetate-1-^{13}C to form 3-methyl-^{13}C-2-butenoic-1,4-$^{13}C_2$ acid (beta-methylcrotonic-$^{13}C_3$ acid) and this was reduced to 3-methyl-^{13}C-butanal-1,4-$^{13}C_2$ (isovaleryl aldehyde-$^{13}C_3$). Conversion to (S)-leucine-$^{13}C_3$ was accomplished via the Strecker synthesis followed by enzymatic resolution.

Key words: Carbon-13, acetone, triethyl phosphonoacetate, beta-methylcrotonic acid, isovaleric acid, (S)-leucine.

INTRODUCTION

Maple syrup urine disease (branched chain ketonuria), isovaleric acidemia and beta-methylcrotonic aciduria are diseases caused by inborn errors of leucine catabolism (1). In order to obtain stable isotope labeled substrates for metabolic research in connection with these diseases, we have synthesized side chain $^{13}C_3$ labeled isotomers (isotopically different molecules, (2)) of (S)-leucine and its metabolites isovaleric acid and beta-methylcrotonic acid (cf. Tanaka et al., (2)). The synthesis was designed to alternate the position of ^{13}C-carbons in order to facilitate and simplify their identification by CMR spectroscopy. Thus, several ^{13}C synthons comprising the side chain were connected by a convergent sequence, and the racemic leucine product was resolved to give the natural (S) isomer. The scheme presented here is also versatile enough to allow for the labeling of

0362-4803/81/040563-07$01.00

Received October 9, 1979
Revised December 26, 1979

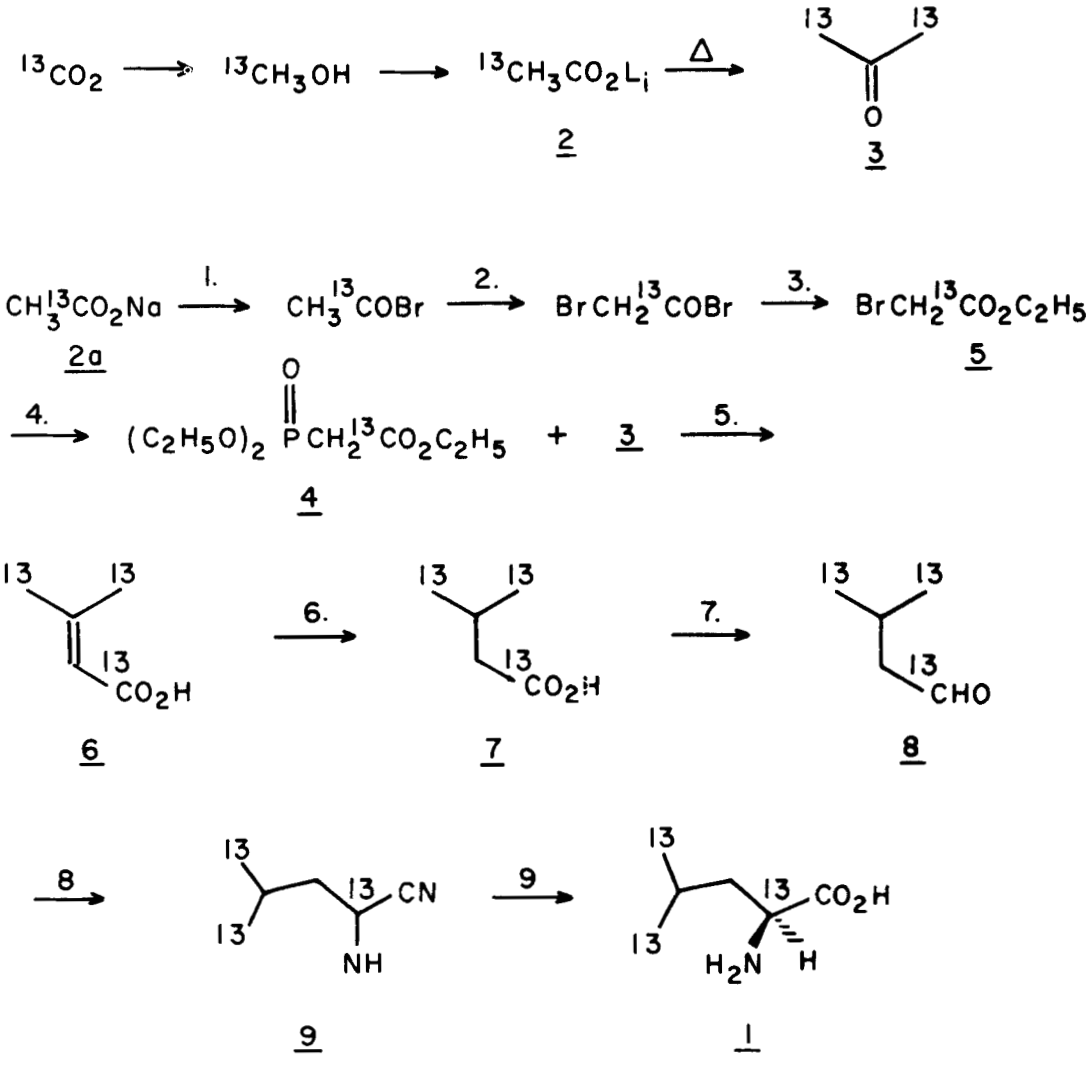
other positions in the amino acid.

DISCUSSION

Our synthetic route for (S)-2-amino-4-methyl-^{13}C-pentanoic-2,5-$^{13}C_2$ acid [(S)-leucine-$^{13}C_3$, 1] is outlined in Figure 1. Accordingly acetic-2-^{13}C acid was synthesized by the method of Ott et al. (4) and its lithium salt 2 was pyrolyzed (5) to give acetone-1,3-$^{13}C_2$ (3) in 85% yield. It should be noted that the PMR spectrum of this acetone-$^{13}C_2$ exhibited a complex splitting pattern (see experimental section) which becomes intelligible after consideration of Lynden-Bell and Sheppard's (6) analysis of acetylene-$^{13}C_2$. Triethyl phosphonoacetate-1-^{13}C (90% ^{13}C) (4) was synthesized from sodium acetate-1-^{13}C as follows: treatment in PBr_3 gave acetyl bromide-1-^{13}C, bromination produced bromoacetyl-1-^{13}C bromide, addition of ethanol generated ethyl bromoacetate-1-^{13}C (5) (67% overall yield), and Arbuzov condensation of 5 with triethyl phosphite afforded 4 in 80% yield.

The acetone (3) and triethyl phosphonoacetate (4) were mixed with sodium hydride in boiling dimethoxyethane (7) to produce the ethyl ester of 6. Saponification of this ester afforded 3-methyl-^{13}C-butenoic-1,4-$^{13}C_2$ acid (6; beta-methylcrotonic acid) in 75% yield. A solution of 6 in methanol was hydrogenated over platinum on charcoal to give 3-methyl-^{13}C-butyric-1,4-$^{13}C_2$ acid (7) (isovaleric acid) in quantitative yield. Isovaleric acid (7) was reduced with lithium aluminum hydride to give an intermediate 3-methyl-^{13}C-butanol-1,4-$^{13}C_2$ (80% yield) and the alcohol was then oxidized by chromium trioxide-pyridine complex (8) to yield 3-methyl-^{13}C-butanal-1,4-$^{13}C_2$ (8) (isovalerylaldehyde) in 52% yield.

Finally, racemic leucine was synthesized from aldehyde 8 via a modified Strecker synthesis (9): aldehyde 8 was stirred with liquid hydrogen cyanide containing a catalytic amount of pyridine; the resultant cyanohydrin was aminated by heating with liquid ammonia in a stainless steel vessel to produce 2-amino-4-methyl-^{13}C-pentanonitrile-2,5-$^{13}C_2$ (9) (80% yield overall) and the aminonitrile 9 was in turn hydrolyzed in hydrochloric acid to give racemic leucine (80% yield). Resolution was accomplished by first making the



1. PBr_3; 2. Br_2; 3. C_2H_5OH; 4. $(C_2H_5O)_3P$; 5. NaH; NaOH;

6. H_2/Pt; 7. $LiAlH_4$; CrO_3 – Pyridine; 8. HCN; $NH_3(\ell)$;

9. Conc. HCl; trifluoroacetic anhydride; carboxypeptidase A.

Figure 1

N-trifluoroacetyl derivative with trifluoroacetic anhydride in cold trifluoroacetic acid and then hydrolyzing this derivative with carboxypeptidase A at pH 7 in a 38°C bath (10). (S)-leucine-$^{13}C_3$ (1) was obtained in 60% yield along with (R)-N-trifluoroacetyl leucine in 80% yield.

EXPERIMENTAL

Materials and Methods: Methanol-^{13}C (90% ^{13}C) and acetic-1-^{13}C acid or 2-^{13}C (90% ^{13}C) were prepared by the methods of D.G. Ott et al. (4). PMR and CMR spectra were recorded with a Varian EM 360A and a Brucker HFX-100 NMR spectrometer. Mass spectra were recorded with a Varian MAT-44 mass spectrometer. The identities, purities and yields of several intermediates were checked using a Bendix-3000 gas chromatograph.

Acetone-1,3-$^{13}C_2$(3): Aqueous lithium hydroxide solution (1 eq.) was added to acetic-2-^{13}C acid and the product was evaporated to dryness. Powdered lithium acetate-2-^{13}C was pyrolyzed in a Pyrex glass boat inside a Vycor tube at 400°C and a slow nitrogen flow carried the product acetone-1,3-$^{13}C_2$ to a Dry Ice acetone trap. The average yield of several runs was 85%. PMR ($CDCl_3$):δ 1.0 (relative integration = 14), 1.1 (70), 1.2 (15), 2.1 (24), 3.1 (16), 3.2 (70) and 3.3 ppm (13).

Ethyl bromoacetate-1-^{13}C (5): Aqueous sodium hydroxide solution (1 eq.) was added to acetic-1-^{13}C acid and the solution was evaporated to dryness. Under a nitrogen atmosphere, sodium acetate-1-^{13}C (70 g) was treated with 150 ml of phosphorus tribromide with vigorous stirring, and the acetyl bromide formed was distilled. Bromine (60 ml) was added to the acetyl bromide and the mixture was heated at reflux for 3 hr. The excess bromine and hydrogen bromide were removed in vacuo, and the crude bromoacetyl bromide was chilled to 0° C. Gradual admixture with 35 ml of ethanol followed by evaporation of excess alcohol and HBr gave 63 g (67% overall yield) of 5. PMR ($CDCl_3$): δ 1.3 (3H, t, CH_3CH_2O), 3.8 (2H, d, J_{CCH}=5 Hz, $Br^{13}CH_2$) and 4.2 ppm (2H, d of q, J_{COCH}=3 Hz, CH_3CH_2O).

Triethyl phosphonoacetate-1-^{13}C (4): In a three-neck flask equipped with a dropping funnel and a condenser, ethyl

bromoacetate-1-^{13}C (30 g, 0.18 mol) was added dropwise into 45 ml (0.22 mol) of triethyl phosphite. The mixture was heated at reflux for 9 hr (pot 170°C). Distillation gave 32 g (88% yield) of product 5, bp 105°C/0.5 Torr. PMR ($CDCl_3$): δ 1.3 (3H, t, $CH_3CH_2O_2C$), 1.4 (6H, t, CH_3CH_2OP), 2.9 (2H, dd, J_{PCH}=22 Hz, J_{CCH}=7 Hz, $PCH_2{}^{13}CO_2$) and 3.9-4.4 ppm (6 H, m, CH_3CH_2O).

3-Methyl-^{13}C-2-butenoic-1,4-$^{13}C_2$ acid (6): Under nitrogen, triethyl phosphonate-1-^{13}C (61 g, 0.25 mol) in 50 ml of dry dimethoxyethane (DME) was added to a flask containing 6.2 g (0.26 mol) of sodium hydride and stirred overnight. Acetone-1,3-$^{13}C_2$ (15 g, 0.25 mole) in 50 ml of DME was added and the mixture was heated at reflux for 12 hr. After cooling, 100 ml of saturated sodium chloride solution was added and the product was extracted into ether. After drying (Na_2SO_4) and the removal of ether, the crude ester was heated with 52 g of sodium hydroxide in 400 ml of water for 6 hr. The solution was chilled, acidified with conc. HCl and extracted into ether. After drying (Na_2SO_4) and evaporation, 24 g of acid 6 was obtained (78% yield). PMR ($CDCl_3$):δ1.9 (3H, d of d, J_{CH}=126 Hz with 4 and 1 Hz splittings, $^{13}CH_3$), 2.1 (3H, d of d, J_{CH}=126 Hz with 4 and 1 Hz splittings, $^{13}CH_3$), 5.7 (1 H, m, CH=) and 8.9 ppm (1H, broad, $^{13}CO_2H$).

3-Methyl-^{13}C-butyric-1,4-$^{13}C_2$ acid(7): A solution of acid 6 (24 g) in 200 ml of methanol containing 4 g of 5% platinum on charcoal was hydrogenated for 4 hr under 1 atm of hydrogen at room temperature. After filtration and evaporation, 24 g of acid 7 was obtained (99% yield). PMR ($CDCl_3$): δ 1.0 (6H, m, J_{CH}=132 Hz, $(^{13}CH_3)_2CH$), 2.1 to 2.7 (3H, m, $CHCH_2CO_2H$, overlaps with $^{13}CH_3$) and 9.6 ppm (1H, d of t, J_{COH}=168 Hz, J_{HCOOH}=2 Hz, $^{13}CO_2H$).

3-Methyl-^{13}C-butanal-1,4-$^{13}C_2$ (8): A solution of acid 7 (24 g, 0.23 mol) in 100 ml of ether was added to a suspension of 13 g (0.35 mol) of lithium aluminum hydride in 400 ml of ether under nitrogen. The mixture was heated to reflux for 2 hr and then the excess hydride was destroyed by dropwise addition of an equivalent amount of 8 N NaOH. The solid was filtered and washed with ether. The ether solution was dried (Na_2SO_4) and evaporated. The residue was distilled to give 18 g (88% yield) of

3-methyl-^{13}C-butanol-1,4-$^{13}C_2$ (bp 130°C). PMR (CCl_4):δ 1.1 (6H, m, J_{CH}=124 Hz, $^{13}\underline{CH}_3$), 1.6 (3H, m, $(CH_3)_2\underline{CH}\underline{CH}_2$, overlaps with $^{13}CH_3$), 3.7 (2H, d of t, J_{CH}=140 Hz, J_{HCCH}=6 Hz $^{13}\underline{CH}_2OH$) and 3.7 ppm (1H, s, O<u>H</u>).

The alcohol thus obtained was added to 34 g of CrO_3-pyridine complex in 150 ml of ether and stirred for 1 hr. Ether (150 ml) was added and the supernatant was removed. The gummy residue was washed with more ether and the combined ether solutions were filtered through a pad of Florisil. After drying over Na_2SO_4-K_2CO_3, the solution was distilled using a 15 cm Vigreux column. The fraction distilling at 78-98°C was collected. It contained 4.5 g (52% yield) of predominantly aldehyde <u>8</u> with some ether. PMR (CCl_4): δ 1.1 (6H, m, J_{CH}=128 Hz, $^{13}\underline{CH}_3$), 2.1-2.5 (3H, m, $\underline{CH}\underline{CH}_2$, overlaps with $^{13}CH_3$) and 9.7 ppm (1H, d of t, J_{CH}=168 Hz, J_{HCCH}=2 Hz, $^{13}\underline{CH}O$).

<u>2-Amino-4-methyl-^{13}C-pentanonitrile-2,5-^{13}C (9)</u>: The crude aldehyde <u>8</u> (9 g, 0.1 mole) was added to liquid HCN (generated by adding 29 g of KCN to dil. sulfuric acid) followed by one drop of pyridine catalyst. After stirring for 2 hr, the formation of cyanohydrin was complete. PMR (neat):δ 1.0 (6H, m, J_{CH}=125 Hz $^{13}\underline{CH}_3$), 1.8 (3H, m, $\underline{CH}\underline{CH}_2$), 4.5 (1H, t, J_{CH}=150 Hz, J_{HCCH}=7 Hz, $^{13}\underline{CH}OH$) and 4.3 ppm (1H, s, OH). This was added to 50 ml of liquid ammonia in a stainless steel vessel. The vessel was sealed and heated at 110°C for 30 min to an internal pressure of 500-600 psi. Upon cooling, excess ammonia was evaporated and an oily aminonitrile <u>9</u> was obtained (8.6 g, 80% yield). PMR (neat):δ 1.0 (6H, m, J_{CH}=122 Hz, $^{13}\underline{CH}_3$), 1.7 (3H, m, $\underline{CH}\underline{CH}_2$), 3.8 (1H, d of t, J_{CH}=143 Hz, J_{HCCH}=7 Hz, $^{13}\underline{CH}NH_2$) and 4.3 ppm (2H, broad, $\underline{NH}_2$).

<u>(S)-Leucine-2,5,4-methyl-$^{13}C_3$ (1)</u>: The aminonitrile <u>9</u> was dissolved in 100 ml of 20% hydrochloric acid and heated at reflux for 5 hr. The solution was evaporated to dryness and the residue was dissolved in 50 ml of water. Sodium hydroxide solution (5 N) was added to this solution until it reached pH 4. The racemic leucine precipitated and was collected by filtration to yield 8.6 g of product (80% yield).

The resolution was performed according to Turk <u>et al.</u> (10): 3

g (60%) of (S)-leucine 1, $[\alpha]^{25^{\circ}}$ = 14.1° (c=0.6 in 6 N HCl) vs authentic sample from Sigma +14.3°, plus 5 g (81% of (R)-N-trifluoroacetyl leucine (N-TFA-leucine) were obtained. PMR of 1 (D_2O, DCl): δ 1.1 (6H, m, J_{CH}=125 Hz, $^{13}\underline{CH}_3$), 1.9 (3H, m, $\underline{CH}\underline{CH}_2$), 4.2 (1H, d of t, J_{CH}=148 Hz, J_{HCCH}=6 Hz, $^{13}\underline{CH}NH_2$) and 5.4 ppm (3H, s, $N\underline{H}_2$ and $CO_2\underline{H}$). CMR of 1 (D_2O, DCl): 22.3 (d, J_{CCC}=15 Hz, $\underline{C}H_3$). 22.4 (d, J_{CCC}=15 HZ, $\underline{C}H_3$) and 52.7 ppm (s, $\underline{C}HNH_2$) from TMS. Mass spectrum of (R)-N-TFA of 1: m/e 185 (loss of CO_2H), 141 (loss of CO_2H and $(CH_3)_2CH$) and 72 (loss of CO_2H and N-TFA).

ACKNOWLEDGEMENT

We are grateful to Drs. J.C. Bradley and A.M. Ajami for helpful discussions and to Mr. D.E. Hart and Ms. J.M. Pang for technical assistance.

REFERENCES

1. J. Dancis & M. Levitz in J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson, Editors: The metabolic basis of inherited disease, 4th Edition, McGraw-Hill, N.Y., 1978 p. 397.

2. S. Kimel and S. Speiser, Chem. Rev. 77:437 (1977).

3. K. Tanaka, T.M. Armitage, H.S. Ramsdale, Y.E. Hsia, S.R. Lipsky and L.E. Rosenberg, Proc. Nat. Acad. Sci. U.S. 72:3692 (1975).

4. D.G. Ott, V.N. Kerr, T.W. Whaley, T. Benziger and R.K. Rohwer, J. Labelled Compds. 10:315 (1974) and D.G. Ott and V.N. Kerr, Ibid 12:119 (1976).

5. I. Zabin and K. Bloch, J. Biol. Chem. 185:117 (1950).

6. R.M. Lynden-Bell and N. Sheppard, Proc. Royal Soc., London, Ser. A 269:385 (1962).

7. W.S. Wadsworth, Jr., and W.D. Emmons, J. Amer. Chem. Soc. 83:1733 (1961).

8. J.C. Collins, W.W. Hess and F.J. Frank, Tetrahedron Letters 3363 (1968).

9. W.F. Gresham and C.E. Schweitzer, U.S. Patent #2,520,312 (1950).

10. J. Turk, G.T. Panse and G.R. Marshall, J. Org. Chem. 40:953 (1975).