STABLE ISOTOPE LABELED ALPHA-KETOACIDS

Sun-Shine Yuan and Alfred M. Ajami

Tracer Technologies, Inc.

225 Needham Street, Newton, MA 02164

SUMMARY

Alkylation of diethyl oxalpropionate with $[D_3]$methyl iodide followed by acid hydrolysis led to 3-$[D_3]$methyl-2-oxobutyric (α-ketoisovaleric) acid. Alkylation of N-t-butylpropylimine with $[D_3]$methyl iodide followed by hydrolysis gave 2-$[D_3]$methylpropanal. This was further alkylated with 2,2-difluoro-1-tosyloxyvinyllithium and hydrolyzed to afford 4-$[D_3]$methyl-2-oxo-pentanoic (α-ketoisocaproic) acid. Diazotization of $[1-^{13}C]$leucine ethyl ester with t-butylnitrite followed by peracid oxidation and hydrolysis gave $[1-^{13}C]$4-methyl-2-oxopentanoic acid. In each case, the α-ketoacids were isolated as sodium salts.


Key words: 3-$[D_3]$methyl-2-oxobutyric acid, 4-$[D_3]$methyl-2-oxopentanoic acid, N-t-butyl-2-$[D_3]$methylpropylimine, 2-$[D_3]$methylpropanal, $[1-^{13}C]$4-methyl-2-oxopentanoic acid, α-ketoacids.

INTRODUCTION

In their comprehensive review, Matthews and Bier (2) have described various tracer studies with stable isotope labeled amino acids in the field of

nutritional biochemistry. The role of branched chain amino acids in particular, and that of the α-ketoacids derived from them, is also receiving close attention (3,4). As a continuation of our earlier work (5,6) on improved synthetic methods for these types of compounds of biomedical interest, we now present two approaches for labeling branched-chain α-ketoacids.

Our first goal was to introduce three mass units into one of the metabolically less reactive sites of the ketoacids, in order to furnish the requisite tracers for plasma kinetics under investigation by GC-MS techniques. The candidate labeled compounds were 3-[D_3]methyl-2-oxobutyric (α-keto-isovaleric, KIV) acid and [D_3]methyl-2-oxopentanoic (α-ketoisocaproic, KIC) acid. Our second goal was to produce large quantities of [1-^{13}C]KIC for the study of decarboxylation kinetics, using the isotope ratio mass spectrometer to measure $^{13}CO_2$ in breath. We addressed these objectives by adapting known routes (7), heretofore untested on isotopically labeled synthons, with a view toward producing multigram quantities of end products.

RESULTS AND DISCUSSION

The [D_3]methyl group can be easily incorporated into KIV as shown in Scheme I. Diethyl oxalpropionate was alkylated with [D_3]methyl iodide, using potassium carbonate in dimethylformamide (8), to give 2 in 60-70% yield; and then 2 was hydrolyzed by dilute hydrochloric acid to give 3-[D_3]methyl-2-oxobutyric acid in 80% yield.

Scheme I

1   2   3a R=H
                                                             b R=Na

For the synthesis of deuterated KIC, we first attempted the Michael addition of methyl magnesium iodide-cuprous chloride (9) or of dimethyl copper lithium (10) to crotonic aldehyde. Unfortunately, in both cases the carbonyl group reacted preferentially. Our second approach was to construct 2-[D_3]methyl-propanal via direct alkylation of propanal (11). This route also was not successful because the volatility of the product made the final isolation too difficult. We then chose the N-t-butylpropylimine **4** as our starting material, and alkylation by [D_3]methyl iodide using lithium diisopropylamide as base gave imine **5** in 55% yield (12). Acid hydrolysis of **5** produced 2-[D_3]methylpropanal **6** (75%), and **6** was then transformed by addition of 2,2-difluoro-1-tosyloxyvinyl-lithium (13) to generate 4-[D_3]methyl-2-oxopentanoic acid (55%) as shown in Scheme II.

For the synthesis of carboxyl labeled KIC, as shown in Scheme III, [1-^{13}C]leucine ethyl ester was diazotized using t-butylnitrite in acetic acid, and the diazo compound was oxidized by m-chloroperoxybenzoic acid (14) to produce the desired ethyl [1-^{13}C]4-methyl-2-oxopentanoate in 45-60% yield;

Scheme II

Scheme III
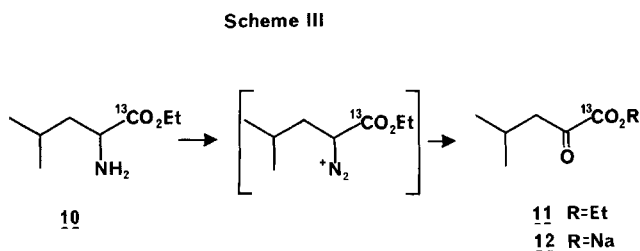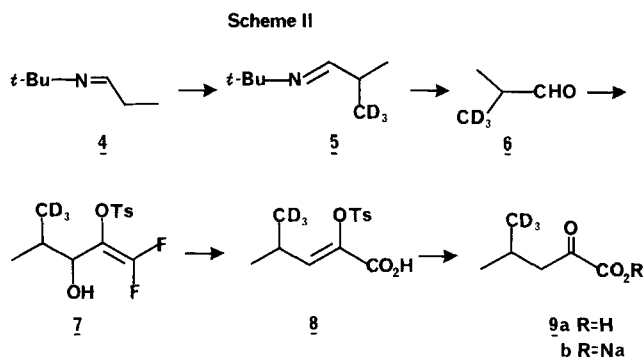
saponification with 1 equivalent of base gave the sodium salt (15). We chose this degradative approach in preference to a constructive synthesis using labeled acyl synthons because racemic [1-^{13}C]leucine and (R)-[1-^{13}C]leucine are readily available intermediates in the routine manufacture of (S)-[1-^{13}C]-leucine, thereby affording a greater degree of cost effective process integration.

The ketoacids produced in this study were all at least 99% chemically pure as judged by glc retention times and mass spectral analyses of their bis-o-trimethylsilyl quinoxalinols (16). The physical characteristics and NMR spectra were indistinguishable from those of standard materials (Sigma), except for the spectral features attributable to the isotopic labels; and biological assay of sterile solutions showed them to be pyrogen-free.

EXPERIMENTAL

General: ^{1}H-NMR spectra were recorded on a Varian EM 360A or on an IBM NR80F spectrometer. GC analyses were performed on a Bendix-3000 gas chromatograph or on a Carle AGC-111 fitted with 6 ft packed columns. Mass spectral analyses were performed on a Hewlett Packard 5985B GC/MS system and are reported with the relative intensity in parentheses after each major fragment. All reagents were obtained from Aldrich or Sigma Chemical companies and were used without purification.

3-[D$_3$]methyl-2-oxobutyric acid, sodium salt (3b): Diethyl oxalpropionate (31g, 0.15 mole) was stirred with potassium carbonate (21g, 0.15 mole) in 35 ml of dry dimethylformamide for 1 hr. [D$_3$]methyl iodide (99 mol % D) was then added, and the mixture was stirred at room temperature for 16 hr. Addition of chloroform (150 ml) and filtration of the suspension afforded crude product after solvent evaporation. The oily residue was distilled to give 23 g of 2

(72%), bp 80-90°C (1 torr). NMR(CDCl$_3$): δ 1.2 (t, J=6 Hz, 3H), 1.3 (t, J=6 Hz, 3H), 1.4 (s, 3H), 4.2 (q, J=6 Hz, 2H) and 4.3 ppm (q, J=6 Hz, 2H). To effect decarboxylation and hydrolysis, the diester 2 was heated to reflux in 100 ml of 4 N HCl for 24 hr, and the resultant free acid 3a was extracted into ether. Drying (MgSO$_4$) and evaporating the solvent gave an oily residue which was distilled to yield 9.6g of 3a (83%), bp 45-50°C (1.2 torr). NMR(CDCl$_3$): δ 1.2 (d, J=6 Hz, 3H, 4-CH$_3$) and 3.4 ppm (q, J=6 Hz, 1H, 3-CH). One equivalent of NaOH (2N) was added to the acid by the literature procedure (15), and the resultant sodium salt 3b was recrystallized from aqueous acetone, mp 230°C with decomposition (236°C dec., Sigma standard). EIMS of silyl quinoxalinol (16): 263 (M$^+$, 93.7), 248 (100), 73 (69.1). Analysis for species abundance showed the presence of 99.1 mol % D. GLC of silyl quinoxalinol (16): Rt 30 min, 3% OV-1 on 100-200 Gas-Chrom Q, 50-180°C at 2°C/min (lit. 26 min).

2-[D$_3$]methylpropanal (6): Propanal (29g, 0.5 mole) was added to t-butylamine stirred at ice-bath temperature. After 1 hr, we added potassium carbonate (10g). The supernatant organic phase was decanted and distilled to give 35g (61%) of imine 4, bp 95-102°C. NMR(CDCl$_3$): δ 7.4 (t, J=4 Hz, 1H, CH=N), 2.3 (m, 2H), 1.1 (s, 9H) and 0.9 ppm (d, J=6 Hz, 3H). The imine 4 was alkylated as described by House and co-workers (12). A cold (-40°C) solution of lithium diisopropylamide (0.6 mole) in 450 ml of dry THF was treated with 4 (67.8g, 0.6 mole) by slow addition. The resulting solution of the lithiated imine was warmed to 20°C over a period of 1.5 hours. We then added dropwise a solution of [D$_3$]methyl iodide (95.7g, 0.66 mole) in 50 ml THF with vigorous stirring while taking care to maintain the reaction temperature in the 20-40°C range by means of external cooling. When the addition was complete, the reaction mixture was stirred for 4 hours at RT, at which time NMR analysis of an aliquot showed complete disappearance of lithiated imine species. After admixture and partition with saturated brine, the organic phase was decanted; and the aqueous phase extracted twice with ether. The combined organic layers were dried (MgSO$_4$) and fractionally distilled promptly to give 5 in 50-55% yield, bp

105-110°C. NMR(CDCl$_3$):δ1.0 (d, J=6 Hz, 3H), 1.1 (s, 9H), 2.4 (m, 1H) and 7.3

ppm (d, J=6 Hz, 1H, C$\underline{H}$=N). Aqueous acetic acid hydrolysis (12) of $\underline{5}$ gave

2-[D$_3$]methylpropanal in 75% yield, bp 60-63°C. NMR(CDCl$_3$): δ 1.1 (d, J=6 Hz,

3H), 2.4 (dq, J=6 Hz and 1 Hz, 1H) and 9.6 ppm (d, J=1 Hz, 1H).

4-[D$_3$]methyl-2-oxopentanoic sodium salt (9b): Aldehyde $\underline{6}$ (22g, 0.3 mole) was

added slowly by syringe transfer under argon to a stirred solution of 2,2-

difluoro-1-tosyloxyvinyllithium (13) (0.3 molar in THF) at -78°C, and the

resulting blue solution allowed to warm to 0°C. It was treated with 500 ml of

saturated brine and acidified with HCl to pH 4. After decantation of the

organic layer, the remaining aqueous emulsion was extracted twice with 9:1

ether-ethanol. Concentration of the combined organic layers, after drying

(MgSO$_4$), gave 82 g of the crude tosyl carbinol $\underline{7}$. It was chilled to below 0°C

and treated with 150 ml of ice cold 95% H$_2$SO$_4$, added in portions and with

vigorous stirring so as to maintain a reaction temperature below 4°C with the

aid of external cooling (ice-salt slush). Thirty minutes after addition of acid

was completed, the dark brown reaction mixture was poured into 600 g of crushed

ice and extracted into ether. Concentration afforded the unsaturated tosyloxy-

acid $\underline{8}$ (85 g) NMR(CDCl$_3$): δ 1.1 (d, J=6Hz, 5-C$\underline{H}_3$), 2.4 (s, ArC$\underline{H}_3$), 2.8 (m,

4-C$\underline{H}$), 6.6 (d, J=10 Hz, vinyl C$\underline{H}$), 7.2-8.0 (dd, aromatic) and 8.8 ppm (s,

CO$_2\underline{H}$). Hydrolysis to KIC was effected by refluxing $\underline{8}$ in 300 ml of 10% NaOH

(w/v) for 3 hours. Acidification of the cooled reaction mixture with HCl to pH

4, extraction into diethyl ether, and fractional distillation after solvent

evaporation, gave $\underline{9a}$ in 55% yield overall, bp 75-80°C (0.5 torr). NMR (CDCl$_3$):δ

1.9 (d, J=6 Hz, 3H), 2.2 (m, 1H), 2.8 (d, J=6 Hz, 2H) and 7.7 ppm (s, 1H). We

then followed the literature procedure (15) in order to isolate the pure sodium

salt $\underline{9b}$, mp 272°C with decomposition (lit. 280°C dec., Sigma standard). EIMS of

silyl quinoxalinol (16):277 (M$^+$, 91.3), 262 (100), 73 (67). Analysis for

species abundance showed the presence of 98.7 mol % D. GLC of silylquinoxalinol

(16): Rt 35 min, same conditions as for $\underline{3b}$ (lit. 31 min).

[1-^{13}C]4-methyl-2-oxopentanoic acid (12): Racemic [1-^{13}C]leucine (90 mol % ^{13}C) was prepared by the Strecker synthesis, and (R)-[1-^{13}C]leucine was obtained by hydrolysis of the N-acetyl derivative recovered from resolution reactions with amino acid acylase, as reported previously (5,6). The ethyl ester 10 was prepared and transformed as described in general by Takamura et al. (17) into the α-diazoester 11, but using t-butylnitrite in the presence of acetic acid according to Thorsett (14). Thus, in a one pot sequence, the cooled diazotization mixture of 10 (30g, 0.2 mole) and t-butylnitrite (24g, 0.23 mole) in CHCl$_3$ (500 ml) was treated with acetic acid (1.8 ml) and m-chloroperoxy-benzoic acid (35g, 0.2 mole), added in portions at 10°C. Vigorous nitrogen evolution persisted over one hour while a precipitate formed, and the reaction temperature reached 25°C. We concentrated the mixture under reduced pressure, slurried it with 200 ml of pentane, and filtered to obtain a clear yellow solution which was washed with aqueous bicarbonate, dried (Na$_2$SO$_4$) and concentrated to 50 ml. Fractional distillation afforded the ketoester 11 in 60% yield, bp 85-95°C (0.15 torr). NMR (CDCl$_3$): δ 0.9 (d, J=6 Hz, 6H), 1.3 (t, J=6 Hz, 3H), 1.7-2.3 (m, 1H), 2.7 (d, J=6 Hz, 2H) and 4.3 ppm (dq, J=6 Hz, $J_{^{13}COCH}$=2 Hz). The ester was saponified over 16 hr at room temperature by stirring with one equivalent of NaOH (2N); and the sodium salt 12 was recovered by precipitation with acetone according to the literature procedure (15), mp 278°C with decomposition (lit. 280°C dec., Sigma standard). EIMS of silyl quinoxalinol (16):275 (M^{+}, 90.8), 260 (100), 73 (69). Analysis for species abundance showed the presence of 89.7 mol % ^{13}C. GLC of silylquinoxalinol (16): Rt 36 min, same conditions as for 9b.

REFERENCES

1.   Contribution No. 19 from this laboratory.
2.   Matthews D.E. and Bier D.M.--Ann. Rev. Nutr. 3:309 (1983).
3.   Miles J.M., Nissen S.V., Rizza R.A., Gerich J.E. and Haymond M.W.--Diabetes 32:197 (1983).
4.   Walser M.--Clin. Sci. 66:1 (1984).
5.   Yuan, S.-S. and Foos J.--J. Lab. Comp. Radiopharm. 18:563 (1981).
6.   Yuan S.-S.--J. Lab. Comp. Radiopharm. 20:173 (1983).

7.   Cooper A.J.L., Ginos J.Z. and Meister A.--Chem. Rev. <u>83</u>:321 (1983).
8.   White D.A.-Synth. Comm. <u>7</u>:559 (1977).
9.   Naef F. and Decorzant R.--Helv. Chim. Acta. <u>57</u>:1317 (1974).
10.   Chuit C., Foulon J.P. and Normant J.F.-Tetrahedron <u>36</u>:2306 (1980).
11.   Groenewegen P., Kallenberg H. and van der Gen A.--Tet. Letts. 491 (1978).
12.   House H.O., Liang W.C. and Weeks P.D.--J. Org. Chem. <u>39</u>:3102 (1974).
13.   Tanaka K., Nakai T. and Ishidawa N.--Tet. Letts. 4809 (1978).
14.   Thorsett E.D.--Tet. Letts. 1875 (1982).
15.   Meister A.--Biochem. Prep. 3:66 (1953).
16.   Langenbeck U., Mohring H.U. and Dieckman K.-P.--J. Chromatog. <u>115</u>:65 (1975).
17.   Takamura N., Mizoguchi T., Koga K. and Yamada S.--Tetrahedron <u>31</u>:227 (1975).