SYNTHESIS OF ^{2}H, ^{13}C, ^{15}N-ISOTOMERS OF BRANCHED-CHAIN AMINO ACIDS(1)

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SUMMARY

Synthetic schemes for 2(S,R)-amino-3-[D_3](R,S)methylpentanoic acid, 2(S,R)-amino-3-[^{13}C](R,S)methyl-[4-^{13}C]pentanoic acid (isoleucine) and [1-^{13}C,^{15}N](S)-leucine are described. The side chain labeled isoleucines were constructed by first alkylating 2,4,4-trimethyl-2-oxazoline with appropriate labeled alkyl halides and then reducing the resultant oxazolines to the aldehydes. Strecker synthesis yielded a mixture of isoleucine and alloisoleucine which was separated via the N-acetyl derivatives. In a separate sequence, Strecker synthesis using 3-methylbutanal, [^{13}C]cyanide and [^{15}N]ammonium hydroxide, followed by acid hydrolysis, gave [1-^{13}C,^{15}N]leucine amide. This amide was resolved by leucine aminopeptidase to produce [1-^{13}C,^{15}N](S)-leucine. Similar schemes were used for the synthesis of [1-^{13}C] or [^{15}N](S)-leucine and [1-^{13}C] or [^{15}N](S)-valine.

Key words: branched-chain amino acids, deuterium, carbon-13, nitrogen-15, 2,4,4-trimethyl-2-oxazoline, leucine aminopeptidase.

INTRODUCTION

The branched-chain amino acids isoleucine, leucine and valine are essential amino acids, and their isotomers have recently been used extensively in metabolic studies(2-6). In this paper, we present our synthetic efforts in producing various ^{2}H, ^{13}C and ^{15}N-labeled isotomers of isoleucine, leucine and valine. Our strategy for constructing the side chain of isoleucine is similar to the valine synthesis developed by Whaley et al.(7), which involves the double alkylation of 2,4,4-trimethyl-2-oxazoline to produce the requisite aldehyde precursor. This scheme is versatile enough to accommodate various labeling patterns. Strecker synthesis(7) was employed for the construction of amino acid functionalities but it was modified so as to conserve labeled cyanide and ammonia.

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RESULTS AND DISCUSSION

2,4,4-Trimethyl-2-oxazoline 1 was first alkylated(8) with [^{13}C]methyl iodide and then with [1-^{13}C]ethyl iodide to give the [$^{13}C_2$]oxazoline 2a in 90% yield. In a parallel sequence, 1 was first alkylated with [D_3]methyl iodide followed by ethyl iodide to yield [D_3]oxazoline 2b. These oxazolines were quaternized (90%), reduced (80%) and hydrolyzed (66%)(9) to give the aldehyde 3.

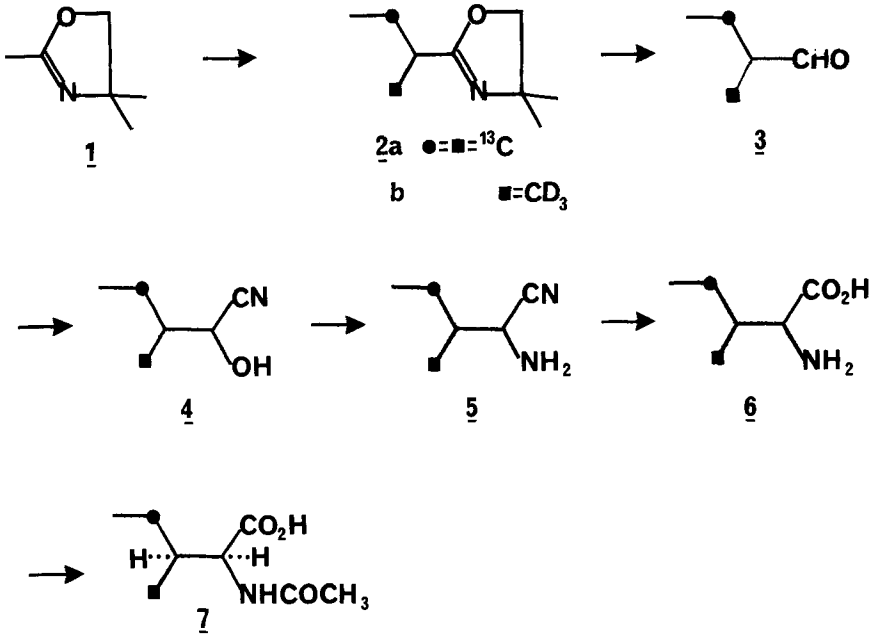
The aldehyde 3 was treated sequentially with 1.1 equivalent each of sodium bisulfite and sodium cyanide to give cyanohydrin 4 (85%), which was then stirred with 1.5 equivalent of ammonium hydroxide to yield the aminonitrile 5 (80%). Acid hydrolysis gave a diasteromeric mixture of labeled isoleucine and alloisoleucine 6 (60%). Fractionation of the mixture was accomplished via its N-acetyl derivative 7, using the published procedure(10a) (Scheme I) to give isoleucine in 50% yield.

For the synthesis of [1-^{13}C], [^{15}N] or [1-^{13}C,^{15}N](S)-leucine and valine, we reacted the corresponding 3-methylbutanal or 2-methylpropanal with 1.1 equivalent each of sodium bisulfite and [^{13}C]sodium cyanide to give the cyanohydrin 9 (80-90%). (Scheme II). The resultant cyanohydrin was treated with 1.5 equivalent of [^{15}N]ammonium hydroxide to give the aminonitrile 10 (80-90%) and the excess ammonia (0.5 eq.) was recovered for recycling. The aminonitrile was hydrolyzed in cold conc. HCl to produce the aminoamide 11 (80%). The amide was then subjected to enzymatic resolution using leucine aminopeptidase(10b) to give ^{13}C and/or ^{15}N labeled (S)-leucine or (S)-valine. In order to increase the enantioneric purity of these products, on occasion it was necessary to treat them further with D-amino acid oxidase (10c). Residual (R) components, arising from excessive hydrolysis of the aminonitrile (10) into (R,S)amino acid prior to the aminopeptidase resolution of amide (11), could be removed easily by this simple, albeit additional, manipulation.

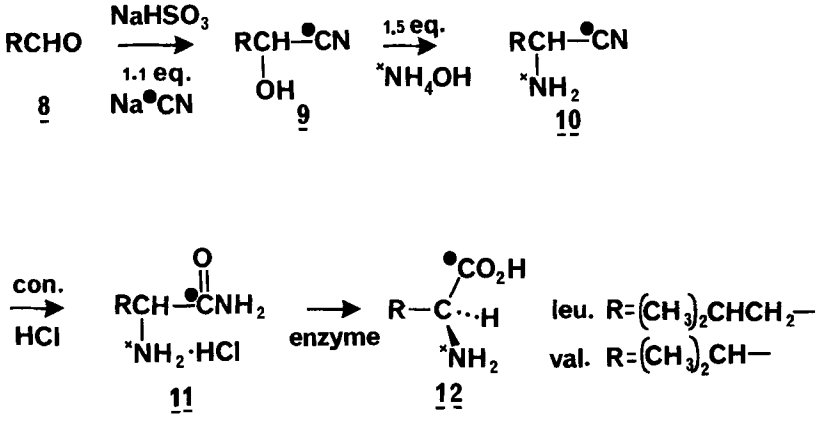
EXPERIMENTAL

General: Labeled starting materials were prepared according to published procedures(12,13). ^{1}H-NMR were recorded on a Varian EM 360A NMR spectrometer. GC analyses were performed on a Bendix-3000 gas chromatograph. Optical rotations were measured in a Rudolph-62 polarimeter (C=5, 1NHCl) and literature values were from US Pharmacopeia XX, 1980.

Scheme I



Scheme II



4,4-Dimethyl-2-(1-[D_3]methylpropyl)-2-oxazoline (2b): Alkylation was carried out according to Whaley et al.(7) using 0.1 mole of 1, 2x0.11 mole of butylithium, 0.11 mole of deuteromethyl iodide (99 mol % D) and 0.11 mole of ethyl iodide. GC analysis of distilled 2b (bp 155-160°C, 90% yield) showed 5% each of monomethylated and monoethylated oxazoline. ^{1}H-NMR ($CDCl_3$): δ0.9 (t, J=7 Hz, 4'-CH_3), 1.28 (s, 4-CH_3), 1.5 (m, 3'-CH_2), 2.35 (t, J=6 Hz, 2'-CH) and 3.9 ppm (s, CH_2O).

4,4-dimethyl-2-(1-[^{13}C]methyl[3-^{13}C]propyl)-2-oxazoline (2a): Reagents were 0.2 mole of 1, 2x0.22 mole of butyllithium, 0.22 mole of [^{13}C]methyl iodide and 0.22 mole of [1-^{13}C]ethyl iodide (90 mol % ^{13}C). ^{1}H-NMR ($CDCl_3$): δ0.9 (q, J=7 Hz, J_{CCH}=7 Hz, 4'-CH_3), 1.1 (ddd, J=5 Hz, J_{CH}=130 Hz, J_{CCCH}=4 Hz, $^{13}CH_3$), 1.3 (s, 4,4-$(CH_3)_2$), 1.5 (dm, J_{CH}=125 Hz, $^{13}CH_2$), 2.3 (m, 2'-CH) and 3.9 ppm (s, CH_2O).

2-[D_3]Methylbutanal and 2-[^{13}C]Methyl[3-^{13}C]butanal (3): The deuterated or [$^{13}C_2$]oxazoline was quarternized with methyl iodide (90%) and then reduced by $NaBH_4$ to oxazolidine (80%)(9). Hydrolysis by aqueous oxalic acid (66%)(14) furnished the free aldehyde 3. Fractions boiling at 70-95°C were collected. ^{1}H-NMR ($CDCl_3$): 3b: δ0.9 (t, J=6 Hz, CH_3), 1.4 (m, CH_2), 2.3 (dt, J=6 and 2 Hz) and 9.6 ppm (d, J=2 Hz, CHO). 3a: δ0.9 (q, J_{CH}=7 Hz, J_{CCH}=7 Hz), 1.1 (dm, J_{CH}=130 Hz, $^{13}CH_3$), 1.4 (dm, J_{CH}=130 Hz, $^{13}CH_2$), 2.3 (m, 2-CH) and 9.6 ppm (bs, CHO).

2-Hydroxy-3-[D_3]methylpentanonitrile and 2-Hydroxy-3-[^{13}C]methyl[4-^{13}C]pentanonitrile (4): Cyanohydrin formation, using the procedure of (7), gave 4 in 80 to 90% yield. ^{1}H-NMR ($CDCl_3$): 4a: δ0.9 (q, J=7 Hz, J_{CCH}=7 Hz, CH_3), 1.1 (ddd, J=6 Hz, J_{CH}=130 Hz, J_{CCCH}=4 Hz, $^{13}CH_3$), 1.4 (dm, J_{CH}=125 Hz, $^{13}CH_2$), 1.7 (m, 3-CH), 3.6 (m, OH) and 4.3 ppm (m, 2-CH). 4b: δ0.9 (t, J=6 Hz, CH_3), 1.2-1.8 (m, CH_2 and 3-CH), 2.8 (m, OH) and 4.3 ppm (d, J=4 Hz, 2-CH).

2-Amino-3-[D_3]methylpentanonitrile and 2-Amino-3-[^{13}C]methyl[4-^{13}C]pentanonitrile (5): This was obtained in 80-90% yield(7). ^{1}H-NMR ($CDCl_3$): 5a: δ 1.0 (q, J=7 Hz, J_{CCH}=7 Hz, CH_3), 1.1 (ddd, J=6 Hz, J_{CH}130 Hz,, $^{13}CH_3$), 1.5 (dm, J_{CH}=125 Hz, $^{13}CH_2$), 1.4-1.8 (m, 3-CH and NH_2) and 3.6 ppm (m, 2-CH). 5b: δ 0.95 (t, J=7 Hz, CH_3), 1.2-1.8 (m, CH_2, 3-CH and NH_2) and 3.6 ppm (m, 2-CH).

2-Amino-3-[D_3]methylpentanoic acid and 2-Amino-3-[^{13}C]methyl[4-^{13}C]pentanoic acid (isoleucine) (6): The aminonitrile (4.3 g, 40 mmol) was hydrolyzed by conc. HCl(7) and the solution was then evaporated. The residue was dissolved in about 100 mL of water and its pH was adjusted to 5.9 with LiOH. An equal volume of ethanol was then added and the amino acid was isolated by filtration (2.8 g, 65% yield). ^{1}H-NMR (D_2O): 6a: δ 0.9 (q, J=7 Hz, J_{CCH}=7 Hz, CH_3), 1.0 (dm, J_{CH}=130 Hz, $^{13}CH_3$), 1.3 (dm, J_{CH}=130 Hz, $^{13}CH_2$), 1.3 (m, 3-CH) and 3.7 ppm (m, 2-CH). 6b: δ 0.9 (t, J=6 Hz, CH_3), 1.2-1.6 (m, CH_2), 1.9 (m, 3-CH) and 3.8 ppm (m, 2-CH). Fractionation of the diasteromeric mixture was carried out according to (10a) to give 710 mg of isoleucine (50% yield).

Strecker synthesis (7): For leucine and valine, the aldehydes (0.2 mole) were converted first to the bisulfite adducts using 1.1 equivalent of $NaHSO_3$ and then 1.1 equivalent of NaCN or $Na^{13}CN$ (90 mol % ^{13}C) was added. Stirring continued for 4 h. The cyanohydrin (9) was extracted into CH_2Cl_2 in 80-90% yield. ^{1}H-NMR ($CDCl_3$): 9b: δ 0.95 (d, J=6 Hz, CH_3), 1.5-1.8 (m, 4-CH and CH_2), 3.5 (m, OH) and 4.5 ppm (q, J=6 Hz, J_{CCH}=6 Hz, 2-CH). 9c: δ 1.1 (d, J=6 Hz, CH_3), 2.0 (m, 3-CH), 3.3 (d, J_{HCOH}=5 Hz) and 4.3 ppm (q, J=5 Hz, J_{CCH}=5 Hz, 2-CH). These cyanohydrins were then stirred with 1.5 equivalents of NH_4OH or $^{15}NH_4OH$ (99 mol % ^{15}N) for 4 h and the aminohydrin isolated by CH_2Cl_2 extraction to give 10 in 80% yield. The aqueous layer, containing the excess $^{15}NH_4OH$ (0.5 eq.), was set aside for future recycling. ^{1}H-NMR: Most peaks unchanged, α-H became a multiplet and shifted to δ 3.7. No ^{15}N coupling could be detected. Hydrolysis of the aminonitriles was carried out with conc. HCl at 0-5°C for 24 h. Excess HCl was removed using high vacuum evaporation and heating to 40°C. The aminoamides were neutralized with LiOH and resolved using procedures given in (10b) (60-65% yield). Cytosol leucine aminopeptidase, Type III-cp (porcine kidney) and D-amino acid oxidase from Sigma Chemical Co. were used in the resolution. ^{1}H-NMR (D_2O-DCl): [1-^{13}C, ^{15}N] (S)-leucine: δ 0.9 (d, J=5 Hz, CH_3), 1.6-2.0 (m, CH_2 and 4-CH) and 4.0 ppm (q, J=5 Hz, J_{CCH}=5 Hz, 2-CH). GC-MS of this material (3) showed the presence of 92% 1-^{13}C and 99% ^{15}N. $[\alpha]_D^{25°}$ = +15°, (Lit. +15°). [1-^{13}C, ^{15}N] (S)-valine (D_2O): δ 1.1 (dd, J=7 Hz and 3 Hz, CH_3), 2.4 (m, 3-CH) and 3.8 ppm (t, J=5 Hz, J_{CCH}=5 Hz) (^{15}N coupling was not detectable in ^{15}N-valine). $[\alpha]_D^{25°}$ = +27° (Lit. +27.5°).

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