Synthesis of L-[1-^{13}C]Isoleucine via Amidocarbonylation

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SUMMARY

Cobalt catalyzed amidocarbonylation of 2-methylbutanal with [^{13}C]carbon monoxide gave N-acetyl-D,L-[1-^{13}C]alloisoleucine and N-acetyl-D,L-[1-^{13}C]isoleucine. The former was separated by crystallization from acetone and resolved by renal acylase I to give L-[1-^{13}C]alloisoleucine (1) and N-acetyl-D-[1-^{13}C]alloisoleucine (2). Then 2 was epimerized and resolved to produce L-[1-^{13}C]isoleucine (3). The crystallization mother liquor was selectively resolved by Aspergillus acylase to yield 3.

Key words: carbon-13, carbon monoxide, cobalt catalysis, aldehyde, amidocarbonylation, enzymatic resolution, isoleucine, alloisoleucine.

INTRODUCTION

The classical methods for synthesizing carboxyl labelled amino acids involve either the Strecker synthesis or the Bucherer hydantoin modification in which the more expensive labelled cyanide is invariably used (2a).

For ^{13}C labelling, the amidocarbonylation reaction discovered by Wakamatsu *et al.* (3) affords a more economical means for obtaining N-acylamino acids, because the primary ^{13}C source of [^{13}C]carbon monoxide can be utilized directly and the product of this single-step procedure is amendable to enzymatic resolution immediately. We have explored this approach and report here the synthesis of isoleucine as an example.

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The branched-chain indispensable amino acid isoleucine possesses two asymmetric centers at the 2 and 3 carbon positions, so any ordinary synthetic approach will produce a mixture of four stereoisomers. Loftfield and Eigner (4) showed that the Bucherer hydantoin synthesis using (S)-2-methylbutanal gave D,L-[1-^{14}C]-isoleucine with very little loss of asymmetry at C-3. However, Pino et al. (5) found that the optical activity of the aldehyde was easily lost in the amidocarbonylation and that the product was 60% N-acetylated alloisoleucine and 40% isoleucine.

Fortunately, it is known (2b) that L-isoleucine can still be isolated cleanly from such a mixture, thereby justifying the basis for our synthetic scheme which relies on an achiral starting material.

RESULTS AND DISCUSSION

(RS)-2-Methylbutanal was amidocarbonylated by shaking at 120° for 8 hrs in an autoclave containing two moles of [^{13}C]carbon monoxide-hydrogen (1:1) mixture at 100 atm pressure, one mole of acetamide and 0.01 mole of cobalt acetate in ethyl acetate. This amount of cobalt was sufficient to catalyze the reaction while minimizing the loss of labeled carbon monoxide through the formation of hydridocobalt-[^{13}C]tetracarbonyl. Prior to workup, the excess gaseous reactants were vented into a resevoir and set aside for recycling in subsequent carbonalytions.

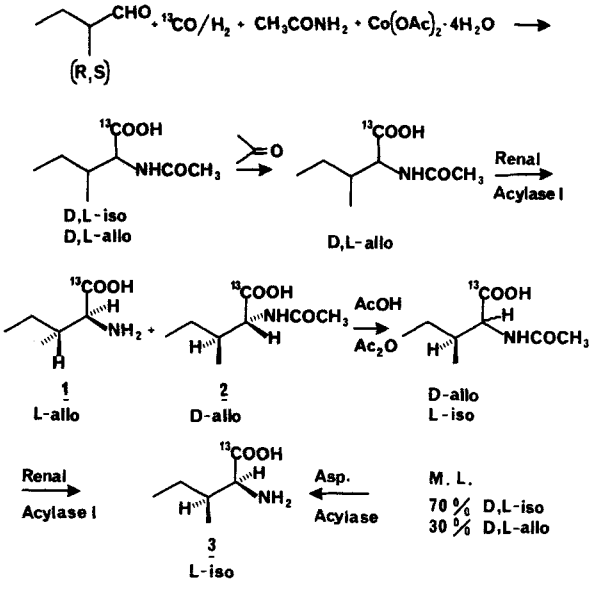
The product was isolated in 70% yield and was shown by GC on a chiral liquid phase to be a mixture of 55% N-acetyl-D,L-[1-^{13}C]alloisoleucine(2) and 45% N-acetyl-D,L-[1-^{13}C]isoleucine. Repeated crystallization from acetone afforded pure allo derivative in 40% yield. It was resolved by hog renal acylase I to give L-[1-^{13}C]alloisoleucine(1) (80%) and N-acetyl-D-[1-^{13}C]alloisoleucine(2) (90%). Compound 2 was then epimerized in acetic acid-acetic anhydride to give a mixture of N-acetyl-D-[1-^{13}C]-alloisoleucine and N-acetyl-L-[1-^{13}C]isoleucine which was resolved similarily to yield L-[1-^{13}C]isoleucine(3).

When checked by GC, mother liquors from the acetone crystallization consisted of 70% N-acetyl-D,L-[1-^{13}C]isoleucine and 30% D,L or racemic allo derivative. Chibata et al. (6) have shown that

the acylase isolated from various species of Aspergillus will resolve a mixture of N-acetylisoleucine and alloisoleucine to give L-isoleucine preferentially. So, this mother liquor fraction was resolved accordingly to produce another batch of pure 3.

Both end-products prepared in this manner were exhaustively analyzed by capillary gas-chromatography and found to contain < 1% diastereomeric and enantiomeric cross-contamination. Mass fragmentographic analyses, in another laboratory, confirmed these findings and further verified that the structure and isotopic labeling sites of both stereoisomers conformed to theoretical predictions (7).

This type of catalytic ^{13}CO incorporation illustrates the utility of carbonylamidation in the preparation of labeled essential amino acids for nutritional research (8). We have utilized the same reaction scheme in the 100 gram scale synthesis of several other amino acids, including leucine concurrently labelled with ^{13}C and ^{15}N--a benchmark tracer for the study of protein dynamics (9). In each instance, amidocarbonylation has proven more cost effective than corresponding preparations with ^{13}C-cyanide synthons, especially since unconverted, excess monoxide can be recycled or processed into other useful intermediates (10) without incident.



In the specific context of work presented here, the economical utilization of tracer afforded large quantities of intermediates, thereby permitting the convenient separation of diastereomers by recrystallization. Equivalent amounts of end products could not have been prepared at a comparable cost via labeled cyanide, except perhaps by resorting to a more laborious chromatographic method for the isolation of allo- and isoleucine diastereomers (11).

General: Reagents were purchased from Sigma-Aldrich and [^{13}C]carbon monoxide at >99% ^{13}C enrichment was supplied by Isotec, Inc.. Routine GC analyses were performed on Carle instruments equipped with a flame ionization detector and either 6 ft packed Supelco SP-300 columns or 150 ft coated capillaries as described by Smith and Wonnacott (12). NMR spectra were recorded using an IBM NR80-F spectrometer, and chemical shifts were reported as ppm from TMS.

Amidocarbonylation of (RS)-2-methylbutanal:

A one liter rocking stainless-steel autoclave was charged with 0.5 mole of (RS)-2-methylbutanal, 0.5 mole of acetamide, 0.005 mole of cobalt (II) acetate tetrahydrate and 400 ml of ethyl acetate. The void space was evacuated and then 50 atm of [^{13}C] carbon monoxide was added, followed by 50 atm of hydrogen. The autoclave was heated to 120^{o}C with rocking for 8 hours or until gas uptake had leveled off as determined by monitoring the internal pressure. The autoclave was cooled, and the excess gas transferred into a spare container immersed in liquid nitrogen. The residual solution was chilled in a refrigerator, and the crystalline product was isolated by filtration (0.35 moles, 70% yield). A further 5% of the product was isolated by base extraction and reacidification of the mother liquid.

The crude crystalline mass was dissolved in a minimum amount of hot acetone (5X); and, upon cooling to 25^{o}, the first crop was harvested. This step was repeated twice to give N-acetyl-(2RS,3SR)-[1-^{13}C]alloisoleucine in 40% yield. GC analysis of its methyl ester on a SP-300 column showed the absence of any isoleucine. ^{1}H-NMR (CD_3COCD_3): 0.8 (3H,d,J=6Hz, $C\underline{H}_3CH$), 0.9 (3H,t,J=6Hz $C\underline{H}_3CH_2$), 1.2 (2H,m,CH_2), 1.8-2.1 (1H,m,$CH_2C\underline{H}$), 2.0 (3H,s,$COCH_3$), 4.4-4.6 (1H,m, α-CH) and 7.0 (1H,bs,NH).

L-[1-^{13}C]Alloisoleucine(1): N-Acetyl-D,L-[1-^{13}C]alloisoleucine (0.1 mole) was suspended in 500 mL of water and titrated with NH_4OH to pH 7.2. Hog renal acylase I (50 mg) was added, and the solution was kept at 37°C for 24 hours. The pH was adjusted to 5.5 by acetic acid, and the solution was filtered with charcoal then evaporated to a syrup, after addition of a few drops of n-octanol to supress foaming. The syrup was triturated with absolute alcohol (500 mL), and the precipitate was isolated by filtration to give 5.8 g (44 mmole) of 1. GC analysis demonstrated its chemical and optical purity to be >99%. ^{1}H-NMR(DC1-D_2O): 1.1 (3H,d,J=6Hz,C$\underline{H}_3$CH), 1.2 (3H,t,J=6Hz, C$\underline{H}_3CH_2$), 1.5 (2H,m, C$\underline{H}_2$), 2.1 (1H,m,CH_2C$\underline{H}$) and 4.0 (1H,q,J=6Hz, J_{CCH}=6Hz,α-CH). ^{13}C-NMR: 12.6 ($\underline{C}H_3CH_2$), 14.9 ($\underline{C}H_3CH$), 27.0(CH_2), 37.1 ($CH_2\underline{C}H$) 59.7 (α-CH,J_{CC}=53.3 Hz) and 175.7 (CO_2H). The alcoholic filtrate, obtained from the isolation of 1 as just described, was concentrated to a syrup and titrated with HCl to pH 2. We isolated a second crystalline product, N-acetyl-D-[1-^{13}C]alloisoleucine(2) in >90% yield after filtration.

L-[1-^{13}C]Isoleucine(3):(a). Compound 2 from previous preparation was dissolved in 250 ml of boiling acetic acid and 75 mL of acetic anhydride was added slowly to effect racemizaiton. After 3 min, the solution was chilled, and the excess acid was removed in vacuo. Resolution in the same manner with renal acylase, gave 3. GC analysis showed >99% chemical and optical purity. ^{1}H-NMR(DC1-D_2O):1.1 (3H,t,J=6Hz, C$\underline{H}_3CH_2$), 1.2 (3H,d,J=6Hz, C$\underline{H}_3$CH), 1.5 (2H,q,CH_2), 2.1 (1H,M,CH_2C$\underline{H}$) and 3.9 (1H,q,J=6Hz, J_{CCH}=6Hz, α-CH). ^{13}C-NMR: 12.5 ($\underline{C}H_3CH_2$), 16.2 ($\underline{C}H_3CH$), 25.9 (CH_3CH_2), 37.3 ($CH_3\underline{C}H$), 61.0 (α-CH, J_{CC}=53.4 Hz) and 175.2 (CO_2H).

(b). The acetone mother liquors from the initial isolation of N-acetyl-D,L-[1-^{13}C]alloisoleucine were evaporated to dryness. The solids were suspended in water (500 ml per 0.1 mole) and titrated to pH 7.2 with NH_4OH. Resolution was accomplished as described above with Aspergillus acylase (Sigma A2156, 100 mg per 0.1 mole of substrate) instead of hog renal acylase. The free amino acid 3 was recovered in 60% yield. Its purity and spectral characteristics were identical to those of material prepared by route (a).

NOTES AND REFERENCES

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