THE SYNTHESIS OF ^{13}C-ENRICHED α-METHYLDOPA.

Matthew M. Ames and Neal Castagnoli,Jr.
Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.
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SUMMARY

Alpha-methyldopa hydrochloride, specifically labelled with ^{13}C in the benzylic position, was prepared for use in biotransformation studies. Carbonation of the lithio derivative of 3,4-dibenzyloxybenzene with $^{13}CO_2$ (64 atom percent) was followed by $LiAlH_4$ reduction of the resulting benzoic acid. Oxidation with CrO_3 provided the benzaldehyde which was condensed with nitroethane to form the phenylnitropropene. Reduction of this phenylnitropropene with Fe/HOAc gave the corresponding phenyl-2-propanone which was converted to its hydantoin derivative. Base hydrolysis of the hydantoin followed by debenzylation in concentrated hydrochloric acid gave the desired alpha-methyldopa hydrochloride in 16% overall yield.

INTRODUCTION

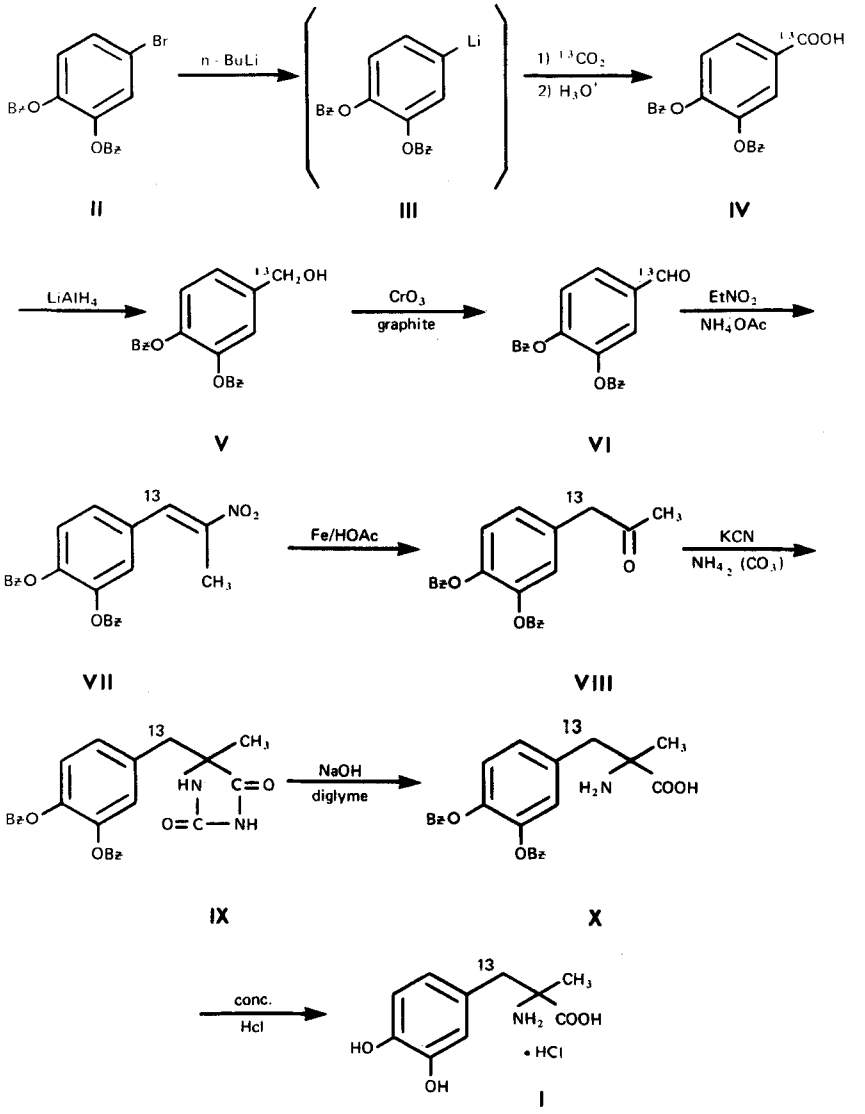
S(-)-Alpha-methyldopa[S(-)-3-(3,4-dihydroxyphenyl-2-methylalanine] (I) has been demonstrated to be an effective antihypertensive agent in man (1,2). The detailed mechanism by which this effect is achieved remains unresolved but presumably involves metabolism of the drug (3). Our interest in the biotransformation of (I) has led to the synthesis of the ^{13}C enriched drug, specifically labeled in the benzylic position. The benzylic position was selected in anticipation of the possible formation of metabolites derived from side chain cleavage.

Although previous studies (4,5) have failed to detect such metabolites, benzoic acid and its glycyl conjugate hippuric acid represent a major route in the metabolism of the structurally related amine amphetamine (6). Selection of ^{13}C as the label was based on stability relative to metabolic and chemical loss, safety in administration to human subjects, and the anticipated application of chemical ionization mass spectrometric techniques (7) in our biotransformation studies. Such techniques are currently being used to identify biotransformation products by the appearance of doublets or other characteristic patterns in the mass spectra of metabolites (8,9). In addition, with the aid of internal standards, stable isotopes have been employed in the quantitative estimation of metabolites (10,11). The ^{13}C-label may also prove of value in structural analysis by nmr.

DISCUSSION

The synthetic pathway devised (Scheme I) was based on carbonation with $^{13}CO_2$ (64 atom percent) of the lithio derivative (III) prepared from 3,4-dibenzyloxybromobenzene (II) and n-butyllithium after the method of Neish (12). The chemical ionization mass spectrum (cims) of the pure acid (IV) obtained in 50% yield verified the location of the label in that $^{13}C/^{12}C$ doublets were observed for the parent ions (MH^+ 336, 335) and for all appropriate fragments, while a singlet was observed at m/e 291 corresponding to the loss of CO_2.

The acid (IV) was reduced with lithium aluminum hydride (LAH) to the benzyl alcohol (V) in 90% crude yield. The pmr spectrum ($CDCl_3$) of the crude alcohol (V) displayed a signal for the two benzylic protons as a singlet at 4.53 δ (Ar-$^{12}\underline{CH}_2$-OH) and a doublet (Ar-$^{13}\underline{CH}_2$-OH, $\underline{J}$ = 144 Hz). The ratio of the integrated areas of the doublet to that of the singlet of the uncoupled proton signal confirmed the $^{13}C/^{12}C$ ratio of 64/36. The cims displayed the parent ions (MH^+ 322, 321) and fragment ions (m/e 320, 319) in the unexpected ratios of 12:23:23:9. We attributed this pattern to species MH^+, M^+, and $(M-H)^+$. This was verified by the cims of the ^{12}C compound which displayed ions at m/e 321(MH^+),


Br
n-BuLi
Li
1) $^{13}CO_2$
2) H_3O^+
$^{13}COOH$
BzO
OBz
II
III
IV
$LiAlH_4$
$^{13}CH_2OH$
CrO_3
graphite
^{13}CHO
$EtNO_2$
NH_4OAc
V
VI
13
NO_2
CH_3
Fe/HOAc
O
KCN
NH_4, (CO_3)
VII
VIII
HN
C=O
O=C
NH
NaOH
diglyme
H_2N
COOH
IX
X
conc.
Hcl
HO
OH
NH_2 COOH
• HCl
I

SCHEME 1

320 (M^+) and 319 $(M-H)^+$ in the appropriate ratios.

Oxidation of the alcohol (V) to 3,4-dibenzyloxybenz-7-^{13}C-aldehyde (VI) was first attempted with Jones Reagent (13), but this method gave inconsistent results (decomposition upon sublimation of the crude product). The reaction was successfully accomplished with chromium trioxide-graphite in refluxing toluene and gave the pure aldehyde (VI) in 82% yield starting from crude (V). The yield was not significantly improved when the purified alcohol was employed in model compound studies. The pmr spectrum of (VI) displayed the aldehyde proton signal as a singlet ($CDCl_3$) at 9.83 δ ($^{12}\underline{CH}O$) and as a doublet ($^{13}\underline{CH}O$, $\underline{J}$ = 174 Hz).

Condensation of the aldehyde (VI) with nitroethane gave the crude nitrostyrene (VII) in 85% yield. Studies on the model compound indicated that the purity of this product was satisfactory for the next step. The pmr spectrum ($CDCl_3$) displayed the methine proton signal as a singlet at 7.99 δ ($^{12}\underline{CH}$=) and as a doublet (^{13}CH=, $\underline{J}$ = 159 Hz). In addition, long range ^{13}C-proton coupling was observed for the <u>ortho</u> protons of the substituted benzene ring and for the terminal methyl group on the side chain.

Reduction of the nitrostyrene (VII) to the phenyl-2-propanone (VII) was first attempted with iron and hydrochloric acid and gave a brown oil which was difficult to purify. Although this method has been reported in the literature (14) for the preparation of ketone (VIII), the yield and characterization of the product were not given. We then attempted the reduction with iron and glacial acetic acid (15) and found this method to be quite satisfactory. The purified ketone (VIII, 86%) was a pale yellow oil which was characterized by nmr, cims and analysis. The pmr spectrum ($CDCl_3$) displayed the characterisric singlet for the methyl ketone protons at 2.03 δ while the two benzylic protons were observed as a singlet at 3.53 δ (Ar-$^{12}\underline{CH}_2$-CO) and as a doublet (Ar-$^{13}\underline{CH}_2$-CO, $\underline{J}$ = 128 Hz).

In studies with the model ^{12}C ketone, we found that if the oil was allowed

to stand in the presence of air, a white solid appeared after several days. This solid was collected, recrystallized and identified by nmr, cims, mp and glpc retention time of the methyl ester as 3,4-dibenzyloxybenzoic acid (IV). This was repeated several times, and failed only when the oil was protected from air by an atmosphere of nitrogen.

The ketone (VIII) was readily converted to the hydantoin (IX) with potassium cyanide and ammonium carbonate in refluxing aqueous ethanol (16) in 85% yield. The pmr spectrum (DMSO-d_6) displayed the signals of the two benzylic protons as an AB quartet with calculated chemical shifts of 2.72 and 2.91 δ (Ar-$^{12}\underline{CH}_2$-C, $\underline{J}_{AB}$ = 15 Hz), and as a doublet of that quartet (Ar-$^{13}\underline{CH}_2$-C, $\underline{J}$ = 128 Hz).Prior to the next step, a calculated amount of unenriched hydantoin (IX) was added to the ^{13}C-enriched compound so that the alpha-methyldopa hydrochloride finally obtained would display the parent ion as a 1:1 doublet.

To insure homogeneity, the mixture was recrystallized. Hydrolysis of the hydantoin (IX) was accomplished in a mixture of sodium hydroxide, water and diglyme (14) and gave the amino acid (X, 68%) as a white solid. Compound (X) was readily debenzylated in concentrated hydrochloric acid in the presence of benzene (17), yielding the hydrochloride of the desired ^{13}C-enriched alpha-methyldopa (I). After removing all volatiles, a white hygroscopic solid was obtained. The pmr spectrum was consistent with both the model compound prepared in the identical manner as the labeled compound, and with the spectrum of the hydrochloride of a commercial sample of alpha-methyldopa. The pmr spectrum of the labeled compound (I) displayed the signals of the two benzylic protons as an AB quartet with calculated chemical shifts of 3.32 and 3.12 δ (Ar-$^{12}\underline{CH}_2$-, $\underline{J}_{AB}$ = 15 Hz) and as a doublet of that quartet (Ar-$^{13}\underline{CH}_2$-, $\underline{J}$ = 133 Hz). The cims showed the parent ion (MH^+ 213, 212) as a doublet with the expected 1:1 ratio. The overall yield of the eight step synthesis was 16% (based on $^{13}CO_2$ as the limiting reagent).

EXPERIMENTAL*

3,4-Dibenzyloxybenzoic-7-^{13}C- Acid (IV). Following the procedure of Neish (12), the lithio derivative (III) prepared from 3,4-dibenzyloxybromobenzene (II, 2.87 g, 7.8 mmoles) and n-butyllithium (2.41 ml of a 2.28 M solution, 5.50 mmoles) was converted to the acid (IV) by carbonation with $^{13}CO_2$ (64 atom percent) generated from barium carbonate (0.988 g, 5.00 mmoles). The average yield of crude acid (IV) from several runs was 1.15 g (67%, mp 175-180°). After combining the individual reaction products, recrystallization from ethyl acetate provided the pure acid (IV) in 50% yield: mp 183-185° (lit.[12] mp 186°); nmr (DMSO-d_6) δ 7.82-7.08 (m, 13, aromatic), 5.25 (s, 2, $C\underline{H}_2$-C_6H_5), and 5.22 ppm (s,2, $C\underline{H}_2$-C_6H_5); cims (300°) m/e (rel. intensity) 336 (89), 335 (50), 318 (17), 317 (11), 291 (50), 245 (33), 244 (56), 243 (34), 181 (22), 91 (100).

3,4-Dibenzyloxybenzyl-7-^{13}C Alcohol (V). The above acid (IV, 3.0 g, 9.0 mmoles) in dry tetrahydrofuran (THF, 90 ml) was added dropwise under nitrogen to an ice cold stirred solution of LAH (1.8 g, 48 mmoles) in 175 ml dry THF.

* Solvents were removed on a rotary evaporator under vacuum. Melting points were taken on a Thomas-Hoover Apparatus and are uncorrected. Literature melting points refer to the ^{12}C compounds. Nmr were recorded on a Varian A-60A instrument in the indicated solvent with TMS as the internal standard unless otherwise indicated. Chemical ionization mass spectra were taken on an Associated Electronics Incorporated Model MS 902 double focus mass spectrometer equipped with a direct inlet system and modified for chemical ionization mass spectrometry. The reagent gas was isobutane at a pressure of 0.5 to 1.0 torr, at the indicated source temperature. Glpc were run on a Varian Model 2100 with a U-shaped 2m x 2 mm Pyrex column packed with 3% OV-1 on acid-washed, DMCS-treated Chromasorb W. The temperature was 205°, chart speed 0.25 inches/min, and the attenuation 8 x 10^{-11}.

After stirring an additional 18 hr at room temperature, the reaction mixture was cooled again and treated with 1.9 ml water, 1.9 ml 15% aqueous sodium hydroxide, and 5.2 ml water sequentially (18). After 15 min stirring the solids were vacuum filtered and digested in boiling ether. The combined clear organic filtrates were dried ($MgSO_4$), filtered, and evaporated to yield a white solid (3.65 g, 95%): mp 66-68° (lit.[19] mp 71-72°); nmr ($CDCl_3$) δ 7.78-6.82 (m, 13, aromatic), 5.13 (s, 4, O-$\underline{CH_2}$-C_6H_5), 4.53 (s, 2, $^{12}\underline{CH_2}$-OH), 4.53 (d, 2, $\underline{J}$ = 144 Hz, $^{13}\underline{CH_2}$-OH), and 1.86 ppm (s, 1 -CH_2-$\underline{OH}$); cims (210°) m/e rel intensity) 322(12), 321(23), 320(23), 319(9), 304(60), 303(41), 291(100), 214(47), 213(35), 181(29), 91(41).

3,4-Dibenzylozybenz-7-^{13}C-aldehyde (VI). Without further purification, the crude alcohol (V, 3.8 g, 12.0 mmoles) and chromium trioxide-graphite mixture (Seloxcette, purchased from Alpha Products, Ventron Corporation, 5.0 g, 55% by weight, 27.5 mmoles)were heated under reflux in dry toluene (75 ml) with stirring and under nitrogen for 72 hr. The mixture was twice filtered through beds of magnesium sulphate, and after removing the solvent the residue was sublimed (120°, 50 μ) to yield a white solid (3.1 g, 81%); mp 84-86° (lit.[20] mp 86°); nmr ($CDCl_3$) 9.83 δ (s, 1, $^{12}\underline{CHO}$), 9.83 (d, 1, $\underline{J}$ = 174 Hz, $^{13}\underline{CHO}$) 7.70-6.88 (m, 13, aromatic), 5.23 (s, 2, O-$\underline{CH_2}$-C_6H_5), and 5.20 ppm (s, 2, O-$\underline{CH_2}$-C_6H_5; cims (200°) m/e (rel intensity) 320(100), 319(60), 230(50), 229(35), 228(28), 227(16), 91(12).

1-3,4-Dibenzyloxyphenyl)-2-nitro-1-^{13}C-prop-1-ene (VII). A mixture of the aldehyde (VI, 10.0 g, 31 mmoles), ammonium acetate (1.2 g, 16 mmoles), and notroethane (85 ml) was held at reflux with stirring and under nitrogen for 18 hr. Upon cooling a solid was obtained which was crystallized from absolute ethanol to yield the crude nitrostyrene (VII, 9.64 g, 83%. A second crop (0.5, g, 5%) was obtained from the concentrated filtrate. The following data applies to the first crop: mp 107-109° (lit.[14] mp 117°); pmr ($CDCl_3$) 7.99 δ (broad singlet,

1, ^{12}C<u>H</u>=C, 7.99 (doublet of broad singlet, 1, <u>J</u> = 159 Hz, ^{13}C<u>H</u>=C), 768-7.27 (m, 10, aromatic from benzyl substituents), 7.03 (s, 3, aromatic from main phenyl ring in ^{12}C molecule), 7.03 (d, 2, J = 3 Hz, <u>ortho</u> protons of main ring split by benzylic ^{13}C), 7.03 (s, 1, <u>meta</u> proton of main ring not split by benzylic ^{13}C), 5.23 (s, 2, O-C<u>H</u>$_2$-C$_6$H$_5$), 5.21 (s, 2, O-C<u>H</u>$_2$-C$_6$H$_5$), 2.32 [d, 3, <u>J</u> = 1 Hz ^{12}CH=C(NO$_2$)C<u>H</u>$_3$] and 2.32 ppm [doublet of doublets, 3, <u>J</u> = 4 Hz, ^{13}CH=C(NO$_2$)C<u>H</u>$_3$]; cims (230°) m/e (rel intensity) 377(100), 376(58), 287(27), 286(17), 285(7), 284(5), 181(10), 91(25).

<u>1-(3,4-Dibenzyloxyphenyl)-1-^{13}C-2-propanone (VIII)</u>. A mixture of 20-mesh iron filings (12.55 g, 22.41 g atoms) and glacial acetic acid (40 ml) was heated under reflux and nitrogen with vigorous stirring until a grey milky color was observed (30 min) at which time the crude nitrostyrene (VII, 9.5 g, 2.5 mmoles) was added. After an additional 2 hr of heating and stirring the hot mixture was filtered through a bed of Celite and washed with hot glacial acetic acid (300 ml). Distilled water (300 ml) was added to the filtrate and the orange milky solution was extracted with methylene chloride (three 100-ml portions). The combined extracts were washed with five percent sodium bicarbonate (three 100-ml portions) and distilled water (100 ml). The reddish-yellow organic layer was dried ($MgSO_4$), the solvent removed, and the crude product 8.58 g (98%) purified by molecular distillation (160°, 50 μ) to give a pale yellow oil (7.5 g, 86%); (pmr ($CDCl_3$) 7.81-6.54 δ (m, 13, aromatic), 5.13 (s, 4, O-C<u>H</u>$_2$-C$_6$H$_5$) 3.53 (s, 2, ^{12}C<u>H</u>$_2$-CO), 3.53 (d, 2, <u>J</u> = 126 Hz, ^{13}C<u>H</u>$_2$-CO), and 2.03 ppm (s, 3, C<u>H</u>$_3$); cims (240°) m/e (rel intensity) 348(100), 347(64), 330(9), 329(6), 258(33), 257(22), 256(10), 255(6), 181(61), 180(47), 179(33), 91(39).

<u>Anal</u>. Calcd for $^{12}C_{22.36}$ $^{13}C_{64}H_{22}O_3$; C, 79.78; H, 6.39. Found: C, 79.54; H, 6.36.

<u>5-Methyl-5-(3,4-dibenzyloxy-7-^{13}C-benzyl)hydantoin (IX)</u>. A mixture of the ketone (VIII, 7.5 g, 21.7 mmoles), absolute ethanol (150 ml), distilled water

(40 ml), potassium cyanide (1.8 g, 28 mmoles) and ammonium carbonate (18.25 g, 190 mmoles) was heated under reflux with stirring for 6.5 hr. During this peroid solids which formed in the condenser were washed down with additional distilled water (10 ml). After standing overnight at room temperature additional distilled water (20 ml) was added and then most of the ethanol was removed by evaporation. The white solids were vacuum filtered, washed with distilled water, and dried for 12 hr on the vacuum filter to give the crude product (IX, 8.57 g, 95%); mp 182-184° (lit[14] mp 188°); nmr (DMSO-d_6) 7.98 δ (s, 1, CO-NH-CO), 7.72-6.60 (m, 13, aromatic), 5.12 (s, 4, O-CH_2-C_6H_5), 2.91 and 2.72 (AB quartet, 2, J_{AB} = 15 Hz, Ar-$^{12}CH_2$-), 2.92 and 2.72 (doublet of AB quartet, 2, J = 128 Hz Ar-$^{13}CH_2$-) and 1.37 ppm (s, 3, -CH_3). The crude hydantoin was then diluted by the formula of 72.5% crude ^{13}C-enriched hydantoin and 27.5% recrystallized ^{12}C material. This mixture was then recrystallized from absolute ethanol with a recovery of 97%, for an estimated yield of pure ^{13}C-enriched hydantoin of 82%: cims (250°) m/e (rel intensity) 418(100), 417(100), 328(43), 327(46), 326(17), 325(16), 250(30), 249(30), 238(26), 237(27), 181(46), 91(46).

3-(3,4-Dibenzyloxyphenyl)-3-^{13}C-2-methylalanine (X). A mixture of the hydantoin (IX, 0.87 g, 2.1 mmoles) in distilled water (4 ml) and diglyme (2 ml) containing sodium hydroxide (2.0 g, 50 mmoles) was heated under reflux with stirring at 125° for 36 hr. The hot, heterogeneous reaction mixture was transferred to a 30-ml separatory funnel, and allowed to cool to approximately 40°. The upper yellow organic layer was drawn off and after cooling the white solid which formed was suspended in ether (20 ml), filtered, and washed with additional ether (10 ml) and then dissolved in distilled water (30 ml) at a temperature of 45°. After filtering the pH was adjusted to 5.5 with five percent acetic acid. The thick white solids were cooled, filtered and washed with cold distilled water (10 ml). The solids were then mixed with absolute ethanol (30 ml) which was heated until the solids were well dispersed and then cooled on an ice bath.

The white crystals were vacuum filtered, and washed with small amounts of cold ether and absolute ethanol, and dried on the vacuum filter to yield 0.56 g (68%) of the colorless amino acid (X): mp (with decomposition) 219-231° (lit.[14] mp 220-230° with decomposition): cims (285°) m/e (rel intensity) 393(100), 392(93), 257(15), 256(19), 255(11), 254(8).

3-(3,4-Dihydroxyphenyl)-3-^{13}C-2-methylalanine Hydrochloride (I). Into a 3-neck 50-ml flask equipped with magnetic stirrer and a nitrogen gas inlet was placed the amino acid (X, 0.56 g, 1.4 mmoles), benzene (10 ml), and concentrated hydrochloride acid (10 ml). This system was purged with nitrogen gas, and the heterogeneous reaction mixture vigorously stirred at room temperature for 18 hr. The solvents were then removed, and the white solids left under vacuum (1 mm) for 72 hr to give an extremely hygroscopic white solid (0.35 g, 100%): nmr (D_2O-DSS) 7.23-6.67 δ (m,3, aromatic), 3.32 and 3.12 (AB quartet, 2, J_{AB} = 15 Hz, Ar-$^{12}CH_2$), 3.32 and 3.12 (doublet of AB quartet, 2, J = 133 Hz, Ar-$^{13}CH_2$-), and 1.77 ppm (s, 3, -CH_3); cims (290°) m/e (real intensity) 213(100), 212(100), 167(42), 166(44), 124(36), 123(38).

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