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SYNTHESIS OF CARBON-14- AND DEUTERIUM-LABELED TRIMEBUTINE AND METABOLITES

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Summary

Synthesis of 2-dimethylamino-2-phenylbutanol 3,4,5-trimethoxybenzoate (Trimebutine, I) labeled with carbon-14 and deuterium, and of its alcohol- and acid-moiety metabolites labeled with deuterium are described. Carbon-14-labeled I was obtained from $K^{14}CN$ in five steps with a radiochemical yield of 31.6% and a specific activity of 2.50 μCi/mg. I and its metabolites labeled with five deuterium atoms in the aromatic ring were synthesized from hexadeuterobenzene as a starting material. Metabolites labeled with three deuterium atoms on the 4-methyl group were obtained from trideuteromethyl iodide as a labeling material. The synthetic yields of $^{2}H_{5}$- and $^{2}H_{3}$-2-dimethylamino-2-phenylbutanol were 53.4% and 13.1% respectively, based on the deuterated starting materials. The acid moiety metabolite, 3,4,5-trimethoxybenzoic acid, labeled with nine deuterium atoms in the three methoxy groups was obtained from gallic acid and hexadeuterodimethylsulfate in 18.6% yield.

Key words: Trimebutine, metabolites, carbon-14, deuterium

Introduction

Trimebutine maleate (I), 2-dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate hydrogen maleate, is a drug having a modulator action on gastrointestinal tract motility (1,2). Pharmacokinetics of this compound was investigated

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by Astier *et al.* (3), who measured the unchanged form in plasma after intravenous administration of I to man, but little is known about its metabolic fate. We have investigated the metabolic fate of I in animals using carbon-14- and deuterium-labeled I and metabolites.

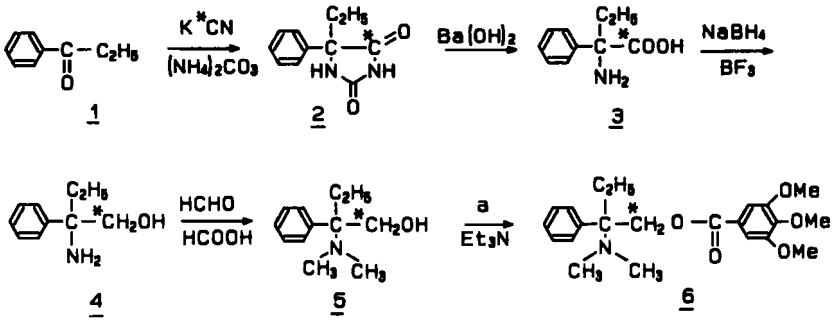
In this paper, we describe the synthesis of I labeled with carbon-14 (^{14}C-I) and five deuterium atoms in the 2-phenyl aromatic ring ($^{2}H_5$-I), and of the alcohol-moiety metabolites labeled with five deuterium atoms in the aromatic ring and those with three deuterium atoms in the 4-methyl group. The synthesis of the acid-moiety metabolite, 3,4,5-trimethoxybenzoic acid, labeled with nine deuterium atoms in the three methoxy groups is also described. The pentadeuterated alcohol-moiety metabolites labeled in the ring and the nonadeuterated acid-moiety metabolite were used as internal standards in GC/MS measurement. The alcohol-moiety metabolite, 2-dimethylamino-2-phenylbutanol (DPB), labeled with three deuterium atoms was used to assess the amounts of N-demethylated alcohol-moiety metabolites arising via DPB after simultaneous administration of trideuterated DPB and I in the same animal.

Methods and Results

The synthesis of I has been previously reported in patents by Viennosis (4), Miyoshi *et al.* (5) and Claude *et al.*(6). The synthesis of I and the alcohol-moiety metabolites labeled with carbon-14 and deuterium followed the same synthetic route.

The synthesis of ^{14}C-I provides carbon-14 at a metabolically inert site, i.e., at C-1 in 2-dimethylamino-2-phenylbutanol(5) as shown in Fig. 1. The synthesis began with a reaction of K^{14}CN with propiophenone and resultant 5-ethyl-5-phenylhydantoin-4-^{14}C (2) was then hydrolized in a barium hydroxide solution to 2-amino-2-phenylbutyric acid-1-^{14}C (3) according to the method of Viennosis (4). 3 was reduced with sodium borohydride in the presence of boron trifluoride etherate to 2-amino-2-phenyl-butanol-1-^{14}C (4), which was then methylated with formic acid and formaldehyde to 2-dimethylamino-2-phenyl-butanol-1-^{14}C (5). ^{14}C-I was obtained by the condensation of 5 with 3,4,5-trimethoxybenzoyl chloride. The overall radiochemical yield of 14C-I (6) from

$K^{14}CN$ was 31.6% and the specific activity of ^{14}C-I was 2.50 μCi/mg.



* denotes ^{14}C label, a: 3,4,5-trimethoxybenzoyl chrolide

Fig. 1 Reaction scheme for the synthesis of ^{14}C-trimebutine

Deuterium-labeled compounds are of necessity prepared from the commercially available, inexpensive reagents. I and its alcohol-moiety metabolites labeled with deuterium in the aromatic ring was synthesized from hexadeuterobenzene as a starting material and the synthetic route is shown in Fig. 2. 2-Methylamino-2-(pentadeuteropheny)butanol (12) was synthesized by reduction of 2-formamino-2-(pentadeuterophenyl)butyric acid (11), which was prepared by formylation of 2-amino-2-(pentadeuterophenyl)butyric acid (10) with acetic anhydride and formic acid. The overall yield of $^{2}H_5$-I (15) from hexadeuterobenzene was 33.6% and the deterium content was 91.1% d_5.

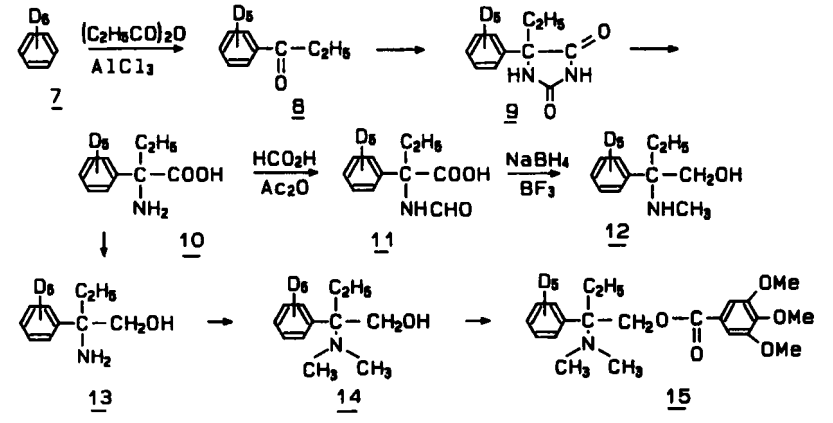


Fig. 2 Synthetic scheme for pentadeuterated trimebutine and metabolites

Trideuterated metabolites were synthesized as shown in Fig. 3. Trideuteropropiophenone (18) was prepared in two steps from CD_3I and ethyl benzoyl-

acetate. Reaction of CD_3I with ethyl benzoylacetate gave ethyl 2-benzoyl-propionate (17), which was then decarboethoxylated to 18 according to the method of Krapcho *et al.* (7). The trideuterated metabolites were synthesized from 18 by the same procedure as that for pentadeuterium-labeled metabolites. 2-Dimethylamino-2-phenyl-4,4,4-trideuterobutanol (24) was synthesized from CD_3I in 13.1% yield and 92.8% d_3 deutrium content.

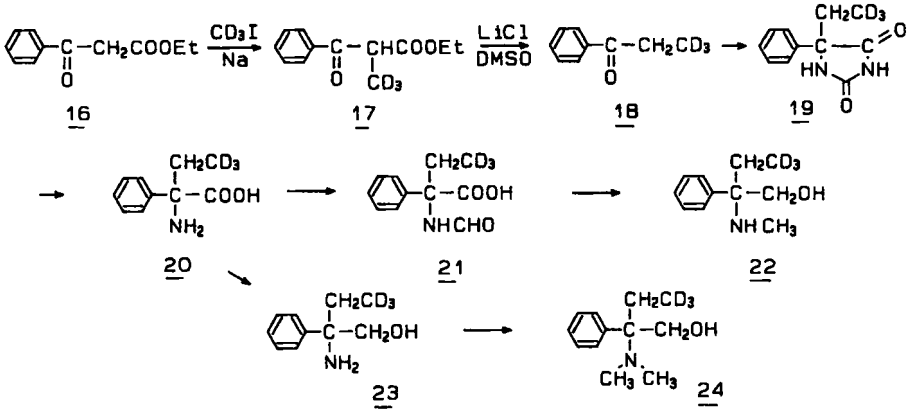


Fig. 3 Synthetic scheme for trideuterated metabolites

The acid-moiety metabolite, 3,4,5-trimethoxybenzoic acid, labeled with nine deuterium atoms in the three methoxy groups was prepared from gallic acid by methylation with hexadeuterodimethylsulfate according to the procedure by Mauthner (8) as shown in Fig.4.

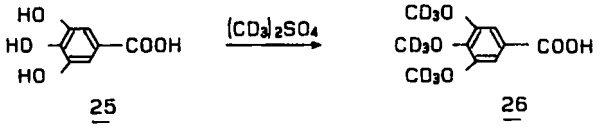


Fig. 4 Synthetic sheme for nonadeuterated 3,4,5-trimethoxybenzoic acid

Experimental

All boiling and melting points are uncorrected. Radioactivity was determined in a liquid scintillation spectrometer Aloka LSC-652 using Triton scintillator (dimethyl POPOP 0.1g, PPO 4g, Triton 320ml, toluene 640ml). Thin layer chromatography was developed on Silica gel $60F_{254}$ plates (0.25mm, Merck) and a radiochromatograms were obtained using a thin layer chromatogram scanner Aloka TRM-1B. Isotopic purity was determined by mass spectrometry.Mass spectra

of solid compounds were obtained with a Jeol JMS-HX100 mass spectrometer equipped with a DA-5000 computer. Mass spectra of liquid compounds were obtained with a GC/MS Shimadzu 6020 equipped with a glass column (2 mm i.d.x 1 m) packed with 3% OV-1 Gas Chrome Q and SCAP 1123 computer.

Potassium [^{14}C]cyanide (56.3 mCi/mmol) was purchased from Amersham Corp. Hexadeuterobenzene (deuterium purity >99%), trideuteromethyl iodide (deuterium purity >99%) and hexadeuterodimethylsulfate (deuterium purity >99) were obtained from E. Merck Co.. Ethyl benzoylacetate, propionic anhydride, gallic acid and 3,4,5-trimethoxybenzoic acid were obtained from Tokyo Kasai Kogyo and propiophenone from Nakarai Kagaku Co.. All other solvents and reagents were of reagent grade and obtained from readily available commercial sources.

5-Phenyl-5-ethylhydantoin-4-^{14}C (2)

To a solution of propiophenone (520 mg, 3.88 mmol) in 7 ml of 50% ethanol were added KCN (K^{14}CN 4.76 mg, 0.07 mmol, 4 mCi, unlabeled 238 mg; total 3.73 mmol) and $(NH_4)_2CO_3$ (1.2 g, 12.5 mmol). The mixture was warmed at 60° for 60 hr with stirring. The solution was concentrated to about a half volume under reduced pressure and acidified with 2N HCl. The precipitated crystalline product was filtered and dried to yield 2 (642 mg, 5.25 çCi/mg) in 84.3 % radiochemical yield. The TLC radiochromatogram showed one peak of 2 at Rf 0.37 ($CHCl_3$:EtOH=10:1 v/v).

2-Amino-2-phenylbutyric acid-1-^{14}C (3)

2 (640 mg, 3.14 mmol) was placed along with $Ba(OH)_2.8H_2O$ (3.2 g, 10.2 mmol) suspended in 10 ml of H_2O to a 100 ml stainless autoclave. The autoclave was heated at 160°(bath temp.) for 2.5 hr with stirring. After standing over night, the reaction mixture was transferred into a beaker and then carbon dioxide was introduced into the mixture. The precipitate was filtered off and the filtrate was evaporated under reduced pressure to give 3 as a powder (569 mg,6.11 μCi/mg). The TLC radiochromatogram showed two peaks at Rf 0.43 (3) and 0.74 (2) (BuOH:AcOH:H_2O= 4:1:2 v/v). The peak of 3 at Rf 0.43 comprised 90 % of radioactivity of the product. The crude product was used for the following reaction without purification.

2-Amino-2-phenylbutanol-1-^{14}C (4)

To a mixture of crude 3 (565 mg, 3.16 mmol), sodium borohydride (150 mg, 3.95 mmol) and 7 ml dry tetrahydrofurane (THF) was added boron trifluoride etherate (600 mg, 4.23 mmol) dropwise with stirring at a temperature below 10°. Stirring was continued for one hour and then the mixture was heated to reflux for 20 hr with stirring. It was then cooled in an ice bath and 0.5 ml of water was added to decompose the residual reagent. The solvent was removed under reduced pressure and the residual mixture was extracted with benzene (10 ml x 3) after addition of an aqueous K_2CO_3 solution. The extract was washed with a saturated K_2CO_3 solution, dried over $MgSO_4$ and evaporated to leave 4 as an oil (448 mg, 6.07 μCi/mg) in 78.8% radiochemical yield. The TLC radiochromatogram showed one peak of 4 at Rf 0.49 ($BuOH:AcOH:H_2O$ =4:1:2 v/v).

2-Dimethylamino-2-phenylbutanol-1-^{14}C (5)

A mixture of crude 4 (446 mg, 2.70 mmol), 37% formaldehyde (0.5 g, 6.17 mmol) and 98% formic acid(0.5 g, 10.7 mmol) was heated at 70° for 24 hr with stirring. The reaction mixture was made alkaline by addition of an aqueous K_2CO_3 solution and extracted with benzene (10 ml x 3). The benzene extract was dried over $MgSO_4$ and evaporated. The residual oil was converted to its hydrochlolide with 4% $HCl-CH_3OH$ and crystallized from ethanol-ether to give 5 as colorless prisms (460 mg, 4.59 μCi/mg) in 77.9% of radiochemical yield. The TLC radiochromatogram showed one peak at Rf 0.38 ($BuOH:AcOH:H_2O$=4:1:2 v/v).

2-Dimethylamino-2-phenylbutyl-1-^{14}C 3,4,5-trimethoxybenzoate (6)

The hydrochloride salt of 5 (451 mg, 1.97 mmol) was dissolved in water and the solution was made alkaline with aqueous K_2CO_3 and then extracted with benzene (10 ml x 2). The benzene extract was evaporated and the residual oil was dissolved in 5 ml of dry THF. To this solution was added triethylamine (395 mg, 3.91 mmol) and then 7 ml of a THF solution of 3,4,5-trimethoxybenzoyl chloride (900 mg, 3.90 mmol), prepared from 3,4,5-trimethoxybenzoic acid and thionyl chloride, dropwise with stirring at a temperature below 10°. Stirring was continued for 3 hr at room temperature and the precipitates were filtered out and then the filtrate was evaporated. The residual mixture was dissolved

in benzene (20 ml) and extracted with 0.1N HCl (10 ml x 3). The aqueous phase was extracted with $CHCl_3$ (10 ml x 3) and the $CHCl_3$ phase was washed and evaporated to dryness under reduced pressure. The residue was recrystallized from acetone-ether as a hydrochloride to give 6 (505 mg, 2.50 μCi/mg) in 61.0% radiochemical yield. The overall yield of ^{14}C-I from $K^{14}CN$ was 31.6% based on radioactivity. The radiochemical purity was 98.7% as determined by TLC ($CHCl_3$: AcOH:CH_3OH=10:1:1 v/v, Rf=0.49). The TLC radiochromatogram showed one peak.

1-(Pentadeuterophenyl)-1-propanone (8)

Anhydrous alminium chloride (74.6 g, 0.559 mol) was added to a solution of hexadeuterobenzene (21.3 g, 0.254 mol) in 100 ml of dry carbone disulfide and to this mixture was added propionic anhydride (32.9 g, 0.559 mol) dropwise with stirring. The reaction mixture was refluxed for 16 hr. After removal of the solvent, the reaction mixture was carefully poured over cracked ice to which hydrochloric acid had been added. The reaction mixture was extracted with ether (50 ml x 2). The extract was washed with water, 10% NaOH and water successively,and dried over Na_2SO_4. After the solvent was removed, the product was distilled under reduced pressure at 92°/13 mmHg to yield 33.1 g (94.0% yield based on the amount of hexadeuterobenzene).

5-Ethyl-5-(pentadeuterophenyl)hydantoin (9)

To a solution of 8 (10.5 g, 75.5 mmol) in 100 ml of 50% ethanol were added potassium cyanide (6.5 g, 100 mmol) and $(NH_4)_2CO_3$ (24 g, 250 mmol), and then the mixture was warmed at 60° for 50 hr with srirring. The solution was concentrated and adjusted to pH 2 with 2N HCl. The precipitates were filtered and recrystallized from aqueous ethanol. The yield was 15.3 g of 9 melting at 194-196° in 96.8% yield.

2-Amino-2-(pentadeuterophenyl)butyric acid (10)

In a 300 ml autoclave were placed 9 (15 g, 71.7 mmol) and $Ba(OH)_2.8H_2O$ (67.5 g, 214 mmol) dissolved in 210 ml of water, and the autoclave was heated at 150° for 2 hr with stirring. The reaction mixture was transferred into a beaker and carbon dioxide was introduced into the mixture. The precipitated barium carbonate was filtered off and the filtrate was evaporated to dryness

under reduced pressure. The yield was 12.4 g of 10 decomosing at 268° in 93.9% yield. TLC showed one spot of 10 at Rf 0.43 (BuOH:AcOH:H_2O=4:1:2 v/v). The crude product was used for the following reaction without purification.

2-Formamino-2-(pentadeuterophenyl)butyric acid (11)

To a solution of 10 (1.84 g, 10.0 mmol) in 98% formic acid (19ml, 0.5 mol) was added acetic anhydride (7 ml, 74.1 mmol) dropwise with stirring at a temperature of 0-5°. The mixture was stirred for 2 hr at room temperature and then 5 ml of water was added to this solution to decompose the redidual reagent. The reaction mixture was poured to cold water and the precipitates were filtered and then recrystallized from aqueous ethanol to yield 1.21 g of 11 melting at 193-195° in 57.1% yield.

2-Methylamino-2-(pentadeuterophenyl)butanol (12)

To a suspension of 11 (1.21 g, 5.71 mmol) in 15 ml of dry THF was added sodium borohydride (0.9 g, 23.7 mmol) and then to this mixture was added boron trifluoride etherate (4.5 g, 32.0 mmol) at a temperature below 10° with stirring. Stirring was continued for one hour and the solution was refluxed with stirring for 20 hr. To the cooled mixture was added 1 ml of water and the solvent was removed under reduced pressure. After addition of 10 ml of an aqueous K_2CO_3 solution, the reaction mixture was extracted with benzene (20 ml x 3). The extract was washed with a saturated K_2CO_3 solution and dried over $MgSO_4$.After removing the solvent the residual oil was distilled in a bulb-tube at 130° (bath temp.)/2 mmHg to give 0.71 g in 67.6% yield. The hydrochloric acid salt was recrystallized from ethanol-ether to give colorless prisms melting at 168-170°. The mass spectrum (CI/isobutane) of the HCl salt showed ion peaks at m/z 185(MH^+), 167($MH-H_2O)^+$, 154($MH-CH_3NH_2)^+$ and 153($MH-CH_3OH)^+$. The isotopic composition was 89.2% d_5, 7.5% d_4, 2.6% d_3, 0.4% d_2 and 0.3% d_1.

2-Amino-2-(pentadeuterophenyl)butanol (13)

This was prepared from 10 (1.84 g, 10.0 mmol), sodium borohydride (0.41 g, 11 mmol) and boron trifluoride etherate (1.7 g, 12 mmol) by the same procedure as that for the preparation of 12 from 11. The product was distilled in a bulb-tube at 140° (bath temp.)/5 mmHg to give 1.19 g of 13 in 70.0% yield.

The hydrochloride was recrystallized from ethanol-ether to give colorless needles melting at 165-8°. The mass spectrum (CI/isobutane) of the HCl salt showed ion peaks at m/z 171(MH^+), 154($MH-NH_3$)$^+$ and 139($MH-CH_3OH$)$^+$. The isotopic composition was 91.8% d_5, 6.7% d_4, 1.1% d_3 and 0.3% d_2 and 0.1% d_1.

2-Dimethylamino-2-(pentadeuterophenyl)butanol (14)

A mixture of 13 (0.5 g, 2.94 mmol), 37% formaldehyde (0.5 g, 6.17 mmol) and 98% formic acid (0.5 g, 10.7 mmol) was heated at 70° for 60 hr with stirring. To the cooled mixture was added an aqueous K_2CO_3 solution, and then the mixture was extracted with benzene (10 ml x 3). The extract was washed with a saturated solution of NaCl and dried over Na_2SO_4. The solvent was evaporated and the residual oil was distilled in a bulb-tube at 150°(bath temp.)/5 mmHg to yield 0.52 g in 89.3% yield. The base was converted to the hydrochloride and recrystallized from ethanol-ether to give colorless prisms melting at 155-6°. The mass spectrum (CI/isobutane) of the HCl salt showed ion peaks at m/z 199(MH^+), 181($MH-H_2O$)$^+$, 167($MH-CH_3OH$)$^+$ and 154($MH-CH_3NHCH_3$)$^+$. The isotopic composition was 89.2% d_5, 7.5% d_4, 2.6% d_3, 0.4% d_2 and 0.3% d_1.

2-Dimethylamino-2-(pentadeuterophenyl)butyl 3,4,5-trimethoxybenzoate (15)

This compound was prepared from the base of 14 (0.39 g, 1.97 mmol), triethylamine (0.30 g, 2.97 mmol) and 3,4,5-trimethoxybenzoylchloride (0.68 g, 2.95 mmol) by the same procedure as that for the preparation of 6.The hydrogen maleic acid salt was prepared by dissolving the crude base(0.69 g) in CH_3OH and adding equimolar maleic acid (0.20 g). CH_3OH was removed and the residual solid was recrystallized from CH_3OH-ether to give 15 (0.65 g) as colorless prisms melting at 111-114° in 63.0% yield. The mass spectrum (CI/isobutane) showed ion peaks at m/z 393(MH^+), 212, 181(MH-3,4,5-trimethoxybenzoic acid)$^+$ and 167(MH - methyl 3,4,5-trimethoxybenzoate)$^+$. The isotopic composition was 91.1% d_5, 6.3% d_4, 1.8% d_3 and 0.4% d_2 and 0.4% d_1.

Ethyl 2-benzoyl-3,3,3-trideuteropropionate (17)

To a solution of ethyl sodiobenzoylacetate prepared from sodium (1.8 g, 78.3 mmol), 40 ml of absolute ethanol and ethyl benzoylacetate (13.7 g, 71.4 mmol) was added trideuteromethyl iodide (11.3 g, 77.9 mmol) dropwise with

stirring at a temperature below 10°. Stirring was continued for one hour at room temperature and then the mixture was refluxed for 2 hr. The solvent was removed and the residual mixture was extracted with ether after addition of about 10 ml of water. The ether extract was washed with a satulated solution of NaCl, dried over $MgSO_4$ and fractionlly distilled. The yield of 17 collected at 112-118°/2 mmHg was 12.4 g in 76.1% yield based on the amount of trideuteromethyl iodide. The mass spectrum(EI) showed ion peaks at m/z 209(M^+), 164 $(M-OC_2H_5)^+$, 136$(M-COOC_2H_5)^+$, 105$(C_6H_5CO^+)$ and 77$(C_6H_5^+)$.

1-Phenyl-3,3,3-trideutero-1-propanone (18)

17 (12.3 g, 58.9 mmol), 1 ml of water and 5 g of lithium chloride were dissolved in 50 ml of dimethyl sulfoxide and then the mixture was heated to reflux for 5 hr. After cooling to room temperature, the mixture was poured into chilled water. The mixture was then extracted with three 50 ml portions of ether and the combined extracts were washed with a saturated solution of NaCl and dried over $MgSO_4$. After the solvent was removed, the residual oil was then fractionally distilled at atmospheric pressure. The yield of 18 collected at bp 207-215° was 4.2 g (52.0%). The mass spectrum(EI) of 18 showed ion peaks at m/z 137(M^+) and 105$(M-CH_2CD_3)^+$.The isotopic composition was 94.7% d_3 and 5.3% d_2.

5-Phenyl-5-(2,2,2-trideuteroethyl)hydantoin (19)

This compound was prepared from 18 (4 g, 29.2 mmol), KCN (2.5 g, 38.5 mmol) and $(NH_4)_2CO_3$(9.5 g, 99.0 mmol) in 73.3% yield by the same procedure descrived above for 9. Recrystallization from aqueous ethanol gave 4.43 g of 19 as colorless needles melting at 193-194°. The mass spectrum (EI) showed ion peaks at m/z 207(M^+), 175(M-32) and 104(M-103).The isotopic distribution was 97.8% d_3 and 2.2% d_2.

2-Amino-2-phenyl-4,4,4-trideuterobutyric acid (20)

20 was obtained from 19 (4.2 g, 20.3 mmol) and a 56 ml solution containing $Ba(OH)_2.8H_2O$ (19.2 g, 61.0 mmol) by the procedure described above for 10. The product decomposing at 260° was 3.3 g in weight and 89.4% in yield. The mass spectrum (CI/isobutane) showed ion peaks at m/z 183(MH^+), 166$(MH-NH_3)^+$

and 137(MH-HCOOH)$^+$. The isotopic composition was 97.4% d_3, 1.2% d_2,1.0% d_1 and 0.5% d_0.

2-Formamino-2-phenyl-4,4,4-trideuterobutyric acid (21)

21 was prepared from 20 (0.7 g, 3.85 mmol), 10 ml of formic acid and 3.5 ml of acetic anhydride by the procedure descrived above for 11. Recrystallization from aqueous ethanol gave 21 (350 mg) as colorless needles decomposing at 188° in 43.3% yield.

2-Methylamino-2-phenyl-4,4,4,trideuterobutanol (22)

This compound was obtained from 21 (0.35 g, 1.67 mmol), $NaBH_4$ (0.3 g, 7.89 mmol) and boron trifluoride (1.4 g, 9.86 mmol) by the same procedure as descrived for 12. Recrystallization of the HCl salt from ethanol-ether gave 0.19 g of 22 as colorless needles melting at 168-171° in 62.5% yield. The mass spectrum (CI/isobutane) showed ion peakes at 183(MH^+), 165(MH-H_2O)$^+$, 152(MH-CH_3NH_2)$^+$ and 151(MH-CH_3OH)$^+$. The isotopic composition was 97.5% d_3, 0.8% d_2, and 1.6% d_1.

2-Amino-2-phenyl-4,4,4-trideuterobutaol (23)

This compound was prepared from 20 (3.3 g,18.1 mmol), $NaBH_4$ (0.76 g, 20 mmol) and boron trifluoride (3.1 g, 21.8 mmol) by the same procedure as that for 12. The crude product (2.2 g) was distilled in a Claisen flask at 113°/3 mmHg to give a colorless oil of 23 (1.65 g) in 60.7% yield. The hydrochloride was recrystallized from ethanol-ether to give colorless needles melting at 168 -170°. The mass spectrum of the HCl salt (CI/isobutane) showed ion peaks at m/z 169(MH^+), 152(MH-NH_3)$^+$, and 137(MH-CH_3OH)$^+$. The isotopic composition was 98.0% d_3, 1.0% d_2, and 1.0% d_1.

2-Dimethylamino-2-phenyl-4,4,4-trideuterobutanol (24)

This compound was prepared from 23 (1.5 g, 8.93 mmol), 37% formaldehyde (1.5 g) and 98% formic acid (1.5 g) by the procedure described above for 14. The product was distilled in a bulb-tube at 125°/1.5 mmHg to give 1.63 g of 24 in 93.1% yield. The hydrochloric acid salt was recrystallized from ethanol-ether to give colorless prisms melting at 153-155°. The mass spectrum (CI/isobutane) showed ion peaks at m/z 197(MH^+), 179(MH-H_2O)$^+$, 165(MH-CH_3OH)$^+$ and 152

$(MH-CH_3NHCH_3)^+$. The isotopic composition was 93.7% d_3, 0.8% d_2, and 5.5% d_1.

3,4,5-Tri(trideuteromethoxy)benzoic acid (26)

This compound was prepared from gallic acid (0.55 g, 3.24 mmol), hexadeuterodimethylsulfate (2.02 g, 15.3 mmol) and 6 ml of 15% NaOH by the procedure for synthesis of the unlabeled compound(8). The product (0.30 g) recrystallized from water showed two spots on TLC($CHCl_3$:CH_3OH:AcOH=10:1:1 v/v). This product was esterified with 10 ml of a 3% HCl-CH_3OH solution and the resultant methyl ester was chromatographed on an aluminum oxide column (1 x 10 cm) in benzene-AcOEt(4:1 v/v) to yield the pure product.The purified ester was hydrolyzed with 20% sodium hydroxide in water-methanol(5:1 v/v) the resultant acid was recrystallized from benzene-petroleum ether to give 26(0.21 g, 18.6% yield based on hexadeuterodimethylsulfate) as colorless needles melting at 167-168°. This product showed one spot at Rf 0.58 ($CHCl_3$:AcOH:CH_3OH =10:1:1 v/v). The mass spectrum (EI) showed ion peaks at m/z 221(M^+), 203$(M-CD_3)^+$, 175$(M-CD_3CO)^+$ and 145$[M-(CD_3CO, CO)]^+$. The isotopic composition was 94.3% d_9 and 5.7% d_8.

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