d,l-6a-^{13}C-Glaucine 1.5 Phosphate

Daniel R. Henton* and Christian T. Goralski

Pharmaceuticals Process Research, Michigan Applied Science and Technology Laboratories, The Dow Chemical Company Midland, Michigan 48674

Jerry P. Heeschen, Richard A. Nyquist, and Curtis D. Pfeiffer

Analytical Sciences, Michigan Applied Science and Technology Laboratories, The Dow Chemical Company, Midland, Michigan 48674

SUMMARY

d,l-Glaucine 1.5 H_3PO_4 is a non-narcotic antitussive for which a sample of carbon-13 labelled material was required for metabolism studies in humans. The sample of 99% enriched d,l-6a-^{13}C-glaucine 1.5 H_3PO_4 was prepared in 7 steps in 36% overall yield starting with purchased 99% enriched 1-^{13}C-papaverine. The papaverine was N-methylated with methyl iodide and the resulting methiodide reduced to d,l-1-^{13}C-laudanosine with sodium borohydride. The laudanosine was O-demethylated with 48% HBr and cyclized to d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine (THA) using aqueous ferric chloride/sodium acetate and isolated as the hydrochloride salt (THA·HCl). The THA·HCl was converted to the free base and remethylated with phenyltrimethylammonium hydroxide. The resulting d,l-6a-^{13}C-glaucine was purified by column chromatography and isolated as the hydrobromide salt. The d,l-6a-^{13}C-glaucine 1.5 H_3PO_4 was obtained by conversion of the hydrobromide to the free base and treatment with 85% phosphoric acid in ethanol.

Keywords: d,l-6a-^{13}C-glaucine, d,l-1-^{13}C-laudanosine, d,l-1-^{13}C-laudanosoline, d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine, phenolic coupling, O-methylation of phenols

INTRODUCTION

The aporphine alkaloid d,l-glaucine and its salts are known to possess analgesic (1) and antitussive (2) activity. Specifically, d,l-glaucine 1.5 phosphate (3,4) was chosen for clinical investigation (5) as a non-narcotic (6) antitussive agent. This paper describes the synthesis of d,l-6a-^{13}C-glaucine 1.5 phosphate (1) for use in metabolism and pharmacokinetic studies in humans.

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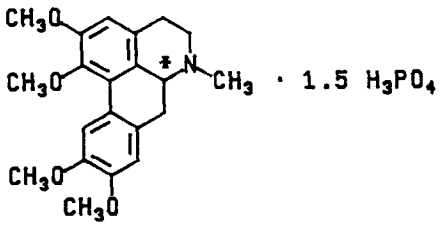

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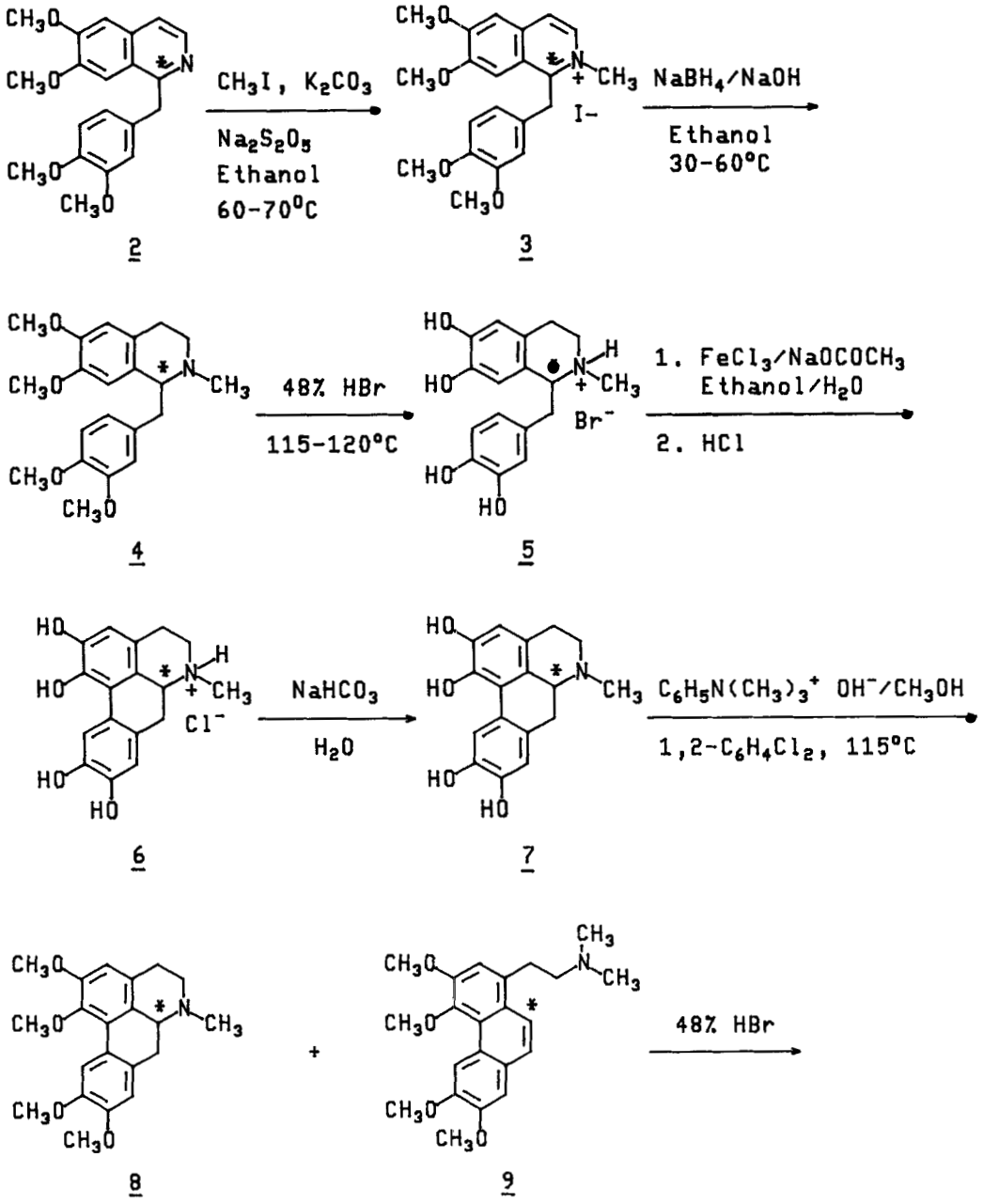
RESULTS AND DISCUSSION

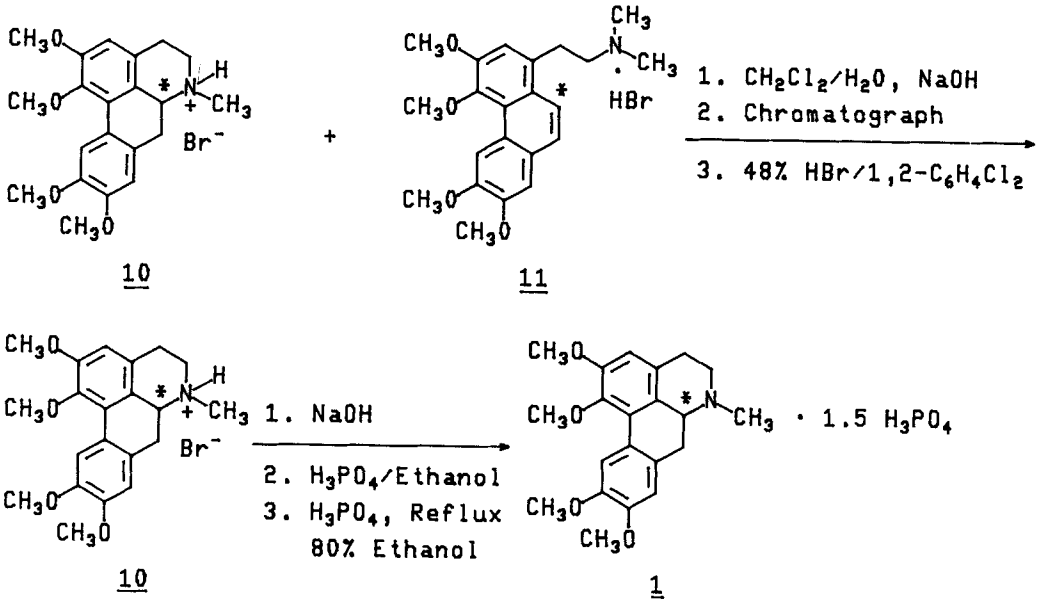
The reaction sequence employed for the synthesis of d,l-6a-^{13}C-glaucine 1.5 phosphate is an improved version of that employed by Maasboel (7) for the preparation of d,l-glaucine hydrobromide (Scheme I). The starting material for the synthesis, 1-^{13}C-papaverine (2), was purchased from Pathfinder Laboratories and contained 99% ^{13}C in the 1-position. The infrared spectrum (KBr) of 2 was essentially identical with that of the unlabelled compound, with the exception of some of the complex in-plane vibrations involving the ^{13}C-atom. Thus, unlabelled papaverine displayed a band at 1555 cm^{-1} which shifts to 1550 cm^{-1} in 2. The 15 MHz ^{13}C NMR spectrum ($CDCl_3$) of 2 displayed a strong signal at 157.78 ppm for the labelled carbon.

The 2 was allowed to react, under nitrogen, with an excess of methyl iodide in ethanol at reflux to give a thick slurry of the methiodide, 3 (8). Potassium carbonate was added to the reaction to react with any hydrogen iodide which might have formed from solvolysis of the methyl iodide. A small amount of sodium metabisulfite was also added to prevent formation of a dark orange color which may have been caused by trace amounts of free iodine.

After removing excess methyl iodide and some ethanol, the methiodide, 3, was reduced to d,l-1-^{13}C-laudanosine (4) with a solution of sodium borohydride in aqueous sodium hydroxide (9). It was necessary to keep the temperature of the reaction below 40-45°C initially to prevent N-demethylation of 3 to regenerate 2. After partitioning the completed reaction between water and methylene chloride, removing the methylene chloride, and crystallizing from aqueous ethanol, 4 was obtained in 90.4% overall yield from 2. The 15 MHz ^{13}C NMR spectrum ($CDCl_3$) of 4 displayed a strong signal at 64.80 ppm for the labelled carbon.

SCHEME I


CH3I, K2CO3
Na2S2O5
Ethanol
60-70°C
NaBH4/NaOH
Ethanol
30-60°C
48% HBr
115-120°C
1. FeCl3/NaOCOCH3
Ethanol/H2O
2. HCl
NaHCO3
H2O
C6H5N(CH3)3+ OH-/CH3OH
1,2-C6H4Cl2, 115°C
48% HBr
2
3
4
5
6
7
8
9



10 11 10 1

Treatment of 4 with refluxing 48% hydrobromic acid afforded a 94.5% yield of d,l-1-^{13}C-laudanosoline hydrobromide (5) (8).

The 5 was dissolved in an acetate buffered mixture of ethanol and water, cooled to 5°C, and treated with an excess of ferric chloride in aqueous ethanol. The reaction was continued for 24 hrs and d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine hydrochloride (6) precipitated in 83.7% yield (10,11). The 6 was converted to d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine (7) with aqueous sodium bicarbonate in 87.9% yield. The infrared spectrum of 7 indicated that it existed in a zwitterionic form as a phenolate anion and a quaternary ammonium salt.

To effect methylation, the 7 was dissolved in a solution of 4.5 equivalents of phenyltrimethylammonium hydroxide in methanol and the resulting solution slowly added to hot (115°C) 1,2-dichlorobenzene. The methanol flash distilled leaving a solution of d,l-6a-^{13}C-glaucine (8) and 10-^{13}C-3,4,6,7-tetramethoxy-N,N-dimethyl-1-phenanthreneethanamine (9) in 1,2-dichlorobenzene. The crude product was precipitated as d,l-6a-^{13}C-glaucine hydrobromide (10) containing 5.5% 10-^{13}C-3,4,6,7-tetramethoxy-N,N-dimethyl-1-phenanthreneethanamine hydrobromide (11). The crude 10 was converted back to the free base and

chromatographed on silica gel using a methanol/chloroform eluent system. The fractions which contained pure 8, as determined by HPLC, were combined, concentrated, and treated with 48% hydrobromic acid to give d,l-6a-^{13}C-glaucine hydrobromide (10) of 98.9% purity in 62.7% yield from 7.

The 10 was converted to crude d,l-6a-^{13}C-glaucine phosphate by conversion to the free base and treatment with 85% phosphoric acid in ethanol (3). The crude phosphate salt was crystal digested (4) in refluxing 80% aqueous ethanol containing 25 wt % 85% phosphoric acid to give d,l-6a-^{13}C-glaucine 1.5 phosphate (1) of 99.9% purity (HPLC) in 92.7% yield from 10. The overall yield of 1 from 2 was 36.5%. The 15 MHz ^{13}C NMR spectrum (DMSO-d_6) of 1 displayed a strong signal at 60.91 ppm for the labelled carbon.

EXPERIMENTAL SECTION

General. All melting points are uncorrected. The infrared spectra were recorded as KBr pellets with a Perkin Elmer Model 180 spectrometer. The nuclear magnetic resonance spectra were recorded with a Jeol FX spectrometer. The elemental analyses were performed by the Analytical Laboratory, Michigan Applied Science and Technology Laboratories, The Dow Chemical Company.

1-^{13}C-1-((3,4-Dimethoxyphenyl)methyl)-6,7-dimethoxyisoquinoline (1-^{13}C-Papaverine, 2). The 99% 1-^{13}C-papaverine was obtained from Pathfinder Laboratories, Inc. (Lot #90815), mp 145-147°C (Lit. (12) mp 147°C).

d,l-1-^{13}C-1-((3,4-Dimethoxyphenyl)methyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (d,l-1-^{13}C-Laudanosine, 4). A 100 mL, three-neck flask equipped with a magnetic stirrer, a thermometer, and a reflux condenser was flushed with nitrogen and charged with 10.00 g (29.4 mmol) of 1-^{13}C-papaverine, 1.0 g of potassium carbonate, 50 mg of sodium metabisulfite, 30 mL of absolute ethanol, and 7.2 mL (116 mmol) of methyl iodide. The reaction mixture was heated to reflux (pot temperature 59°C). The papaverine dissolved giving a solution which was light yellow/orange in color. Within 1.5 hrs a heavy slurry of the methiodide had formed. The mixture was heated at reflux for a total of 21 hrs. The progress of the reaction was monitored by HPLC (Partisil^R 10 SCX column; 9:1 CH_3OH/0.5 M $NaClO_4$ eluent; 2.0 mL/min; 280 nm

detection). The excess methyl iodide and some ethanol were distilled off. An additional 10 mL of ethanol was added and distilled off the ensure complete removal of the methyl iodide. The mixture was cooled to 30°C and a solution of 1.12 g of sodium borohydride in 2.5 g of 50% aqueous sodium hydroxide, diluted to 10 mL with water, was slowly added. The addition required 15 min. A red/orange color developed throughout the slurry, and the mixture foamed vigorously. The reaction temperature slowly rose to 50-55°C. At the end of the addition, the papaverine methiodide had dissolved and the reaction solution was a very pale green color. The disappearance of the red/orange color corresponded to the completion of the reduction (approximately 8 mL of the sodium borohydride solution had been added at that time) as determined by HPLC analysis. The solution was cooled and diluted with 25 mL each of methylene chloride and water. The aqueous phase was further extracted with two 10 mL portions of methylene chloride. The methylene chloride layers were combined and washed with 10 mL of water. The methylene chloride solution was dried over sodium sulfate and concentrated to a volume of approximately 20 mL, giving a bright red solution. The solution was treated with 5 mL of ethanol and 20 mL of water and the remainder of the methylene chloride was distilled off. After all of the methylene chloride was gone, the temperature of the distillation flask was raised to 75°C and 5-10 mL of ethanol was added. The mixture was then cooled to 5-10°C and the contents of the flask became a solid mass. The solid was washed out with several small portions of water and separated by filtration. The resulting light pink solid was thoroughly washed with two 15 mL portions of water and vacuum dried at 60°C for 16 hrs to give 9.52 g (90.4% yield) of d,l-1-^{13}C-laudanosine. A 114 mg sample was removed for ^{1}H and ^{13}C NMR analysis.

d,l-1-^{13}C-Laudanosoline Hydrobromide (d,l-1-^{13}C-1-((3,4-Dihydroxyphenyl)methyl)-2-methyl-1,2,3,4-tetrahydro-6,7-isoquinolinediol Hydrobromide, 5). The solid d,l-1-^{13}C-laudanosine (4, 9.41 g, 0.0263 mol) was charged to a 100 mL, three-neck flask flushed with nitrogen and equipped with a distillation head, a thermometer, and a magnetic stirrer. To the solid, 28 mL of 49% hydrobromic acid was added and the mixture heated at 118°C for 14 hrs as excess water distilled off. The product precipitated as a grey/white solid. The slurry was cooled below 10°C and the product separated by filtration. The

product was washed with several small portions of acetone, air-dried, and vacuum dried at 60°C for 1.5 hrs to give 9.507 g (94.5% yield) of d,l-1-^{13}C-laudanosoline hydrobromide, mp 233-236°C (Lit. (13) 230-232°C).

Analysis. Calculated for $^{13}CC_{16}H_{20}O_4 \cdot HBr$: C, 53.54; H, 5.26; N, 3.65. Found: C, 53.30; H, 5.35; N, 3.71.

d,l-6a-^{13}C-1,2,9,10-Tetrahydroxyaporphine Hydrochloride (d,l-6a-^{13}C-6-Methyl-5,6,6a,7-tetrahydro-1,2,9,10-tetrahydroxy-4H-dibenzo(de,g)quinoline Hydrochloride, 6). A 250 mL, three-neck flask flushed with nitrogen and equipped with a distillation head, a thermometer, and a magnetic stirrer was charged with 9.41 g (0.0263 mol) of d,l-1-^{13}C-laudanosoline hydrobromide (5), 22.5 mL of ethanol, and 17 mL of water. The temperature of the mixture was raised to 80°C and a homogeneous solution formed. To this solution, 2.08 g (0.0254 mol) of sodium acetate was added and the solution became pale yellow in color. The solution was cooled to 3°C and a cooled (4°C) solution of ferric chloride (22.5 mL 42° Baume', 0.0761 mol) diluted with 17 mL of ethanol was quickly added. The stirrer was then started, and a black color developed throughout the solution as the temperature rose to 9°C. The mixture was allowed to stir for 24 hrs while slowly warming to room temperature. The mixture was cooled with an ice bath and 12 mL (0.139 mol) of concentrated hydrochloric acid was added. After stirring for 1 hr with ice bath cooling, the precipitated 6 was separated by filtration and washed with acetone. The solid was vacuum dried at 60°C for 40 hrs to give 6.983 g (83.7% yield) of d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine hydrochloride, mp 237°C (dec.) (Lit. (11) mp 239-242°C).

d,l-6a-^{13}C-1,2,9,10-Tetrahydroxyaporphine (d,l-6a-^{13}C-6-Methyl-5,6,6a,7-tetrahydro-1,2,9,10-tetrahydroxy-4H-dibenzo(de,g)quinoline, 7). A 250 mL, three-neck flask equipped with a magnetic stirrer, a thermometer, and a reflux condenser fitted with a nitrogen bubbler was charged with 2.60 g (0.031 mol) of sodium bicarbonate and 68 mL of deionized water. To the resulting solution, the 6.983 g (0.0220 mol) of d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine hydrochloride was slowly added. Some foaming occurred, and the solid aporphine became light purple in color. The slurry was then heated to reflux and allowed

to cool slowly to room temperature while stirring for 15 hrs. The resulting dark purple solid was separated by filtration, washed with two 10 mL portions of deionized water and 10 mL of methanol. The solid was air-dried and vacuum dried at 60°C for 3 hrs to give 5.458 g (87.9% yield) of d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine, mp 223.5°C (dec)(Lit. (11) mp 225-227°C).

d,l-6a-^{13}C-Glaucine Hydrobromide (d,l-6a-^{13}C-6-Methyl-5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-4H-dibenzo(de,g)quinoline Hydrobromide, 10). A solution of 5.34 g (0.0818 mol) of potassium hydroxide in 15.5 mL of methanol was prepared. Similarly, a solution of 14.04 g (0.0818 mol) of phenyltrimethylammonium chloride in 17.5 mL of methanol was prepared. The solutions were mixed, and the resulting slurry was cooled with an ice bath. The solid potassium chloride was separated by filtration and washed with two 5 mL portions of methanol. The combined filtrates were placed in a 50 mL addition funnel and the 5.458 g (0.0182 mol) of d,l-6a-^{13}C-1,2,9,10-tetrahydroxyaporphine was added and the mixture shaken until a homogeneous solution resulted. The addition funnel was attached to the center neck of a 500 mL, three-neck flask equipped with a magnetic stirrer, a thermometer, and a distillation head which was flushed with nitrogen and contained 300 mL of 1,2-dichlorobenzene which had been heated to 120°C. The dark blue solution of the aporphine was dripped slowly into the hot 1,2-dichlorobenzene at such a rate that the temperature did not fall below 113°C. Generally, the temperature ranged between 115°C and 118°C and the addition required 0.5 hrs. The mixture was heated to 123°C when the addition was complete, and it was then cooled below 30°C. The solution was filtered to remove a small amount of black solid which had separated. The reaction flask was washed with three 5 mL portions of acetone and the washes were added to the 1,2-dichlorobenzene solution. The solution of crude d,l-6a-^{13}C-glaucine was analyzed by HPLC which indicated that the level of side product 10-^{13}C-3,4,6,7-tetramethoxy-N,N-dimethyl-1-phenanthreneethanamine (9) was 5.5%. The crude 8 was precipitated as the hydrobromide salt by adding 2.5 mL of 49% hydrobromic acid to the 1,2-dichlorobenzene solution and stirring the resulting mixture for 16 hrs. The resulting solid was separated by filtration, washed with several portions of acetone, air-dried, and vacuum dried at 60°C for 3 hrs to give 6.18 (77.7% yield) of crude 10 as a grey solid. The crude 10 was

purified by conversion to the free base form (a mixture of 8 and 9) and chromatographed on silica gel according to the procedure described below.

A 125 mL separatory funnel was charged with 5 mL of 50% sodium hydroxide solution and 20 mL of deionized water. The crude 10 was added to the sodium hydroxide solution and rinsed in with 30 mL of methylene chloride. The mixture was shaken until all of the solid dissolved. The layers were separated, and the aqueous layer was extracted with 10 mL of methylene chloride. The methylene chloride layers were combined, washed with 10 mL of water, and dried over sodium sulfate. The methylene chloride solution was concentrated to a volume of approximately 15 mL. This solution was placed on top of a column of silica gel (300 g) slurry packed with 2% methanol in chloroform. The elution proceeded as follows: 1.6 L 2% $CH_3OH/CHCl_3$, nothing; 400 mL 2% $CH_3OH/CHCl_3$, trace of d,l-glaucine and a higher R_f impurity (by HPLC); 900 mL 2% $CH_3OH/CHCl_3$ and 400 mL 4% $CH_3OH/CHCl_3$, pure d,l-glaucine; 700 mL 4% $CH_3OH/CHCl_3$, d,l-glaucine plus a lower R_f impurity (by HPLC); 100 mL 10% $CH_3OH/CHCl_3$, traces of d,l-glaucine, an impurity, and 3,4,6,7-tetramethoxy-N,N-dimethyl-1-phenanthreneethanamine (9); 1.25 L 10% $CH_3OH/CHCl_3$ and 350 mL of 20% $CH_3OH/CHCl_3$, pure 9.

The fractions containing the pure 8 were concentrated by distillation to a volume of approximately 15 mL and 60 mL of 1,2-dichlorobenzene was added. The remaining $CH_3OH/CHCl_3$ was then distilled off. The resulting solution was cooled to room temperature and the pure d,l-6a-^{13}C-glaucine hydrobromide (10) was precipitated by adding 2 mL of 49% hydrobromic acid and stirring for 40 hrs. The precipitated solid was separated by filtration, washed with several portions of acetone, and vacuum dried at 60° for 3.5 hrs to give 4.99 g (80.7% yield, based on crude 8) of d,l-6a-^{13}C-glaucine hydrobromide. The 10-^{13}C-3,4,6,7-tetramethoxy-N,N-dimethyl-1-phenanthrenethanamine hydrobromide (11) was isolated in a similar fashion to give 165 mg. The purified 10 contained less than 0.05% 11 (by area) and was approximately 98.9% pure by HPLC (the major impurities appeared to be low levels of incompletely methylated hydroxyaporphines).

d,l-6a-^{13}C-Glaucine 1.5 Phosphate (d,l-6a-^{13}C-6-Methyl-5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-4H-dibenzo(de,g)quinoline 1.5 Phosphate, 1). A 125 mL separatory funnel was charged with 4.99 g (0.0114 mol) of d,l-6a-^{13}C-glaucine

hydrobromide, 16 mL of deionized water, 4mL of 50% sodium hydroxide, and 20 mL of methylene chloride. The funnel was stoppered and shaken until all of the solid went into solution. The layers were allowed to separate, and the methylene chloride layer was drained off and retained. The aqueous layer was extracted with 15 mL of methylene chloride. The methylene chloride layers were combined and returned to the separatory funnel after draining out the aqueous layer. The methylene chloride was washed with 15 mL of deionized water, filtered, and transferred *via* pipet to a 100 mL, three-neck flask equipped with a magnetic stirrer, a thermometer, a distillation head, and a heating mantle. The methylene chloride solution was concentrated to a volume of 13.5 mL. The distillation head was then replaced by a reflux condenser fitted with a nitrogen bubbler. The solution was cooled to room temperature and diluted with 14.6 mL of toluene-denatured, absolute ethanol. To the resulting solution, 1.6 mL (2.7 g, 0.024 mol) of 85% phosphoric acid and 4.2 mL of toluene-denatured, absolute ethanol were added. A heavy, white slurry formed. The slurry was stirred slowly under a nitrogen atmosphere for 20 hrs. The solid was separated by filtration, washed with several portions (total 120 mL) of toluene-denatured, absolute ethanol, and air-dried for 60 hrs. The dried solid was charged to a 100 mL, three-neck flask equipped with a magnetic stirrer, a thermometer, a reflux condenser fitted with a nitrogen bubbler, and a heating mantle. To the solid were added 40 mL of toluene-denatured, absolute ethanol, 10 mL of deionized water, and 0.8 mL of 85% phosphoric acid. The mixture was heated to reflux (78-80°C) and held there for 6 hrs. The slurry was allowed to cool to 29°C over 2 hrs, and then was cooled to 20°C with an ice/water bath. The solid was separated by filtration, washed with two 15 mL portions of toluene-denatured, absolute ethanol, air-dried, and vacuum dried at 50-55°C for 16 hrs to give 5.32 g (92.7% yield) of d,l-6a-^{13}C-glaucine phosphate (1), mp (one single endotherm by Differential Scanning Calorimetry) 255°C. (Lit. (4) 255°C). The purity of this material was determined to be 99.9% (area) by HPLC (Partisil^{R} ODS column; 4.6 x 250 mm; 55/45 water/acetonitrile/0.03M ammonium formate; 2.0 mL/min; 280 nm detection).

Analysis. Calc'd for $^{13}CC_{20}H_{25}NO_4 \cdot 1.5\ H_3PO_4$: C, 50.30; H, 5.91; N, 2.78. Found: C, 50.60; H, 5.89; N, 2.76.

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