Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 10

SYNTHESIS OF OPTICALLY PURE DEUTERIUM-LABELLED NICOTINE, NORNICOTINE AND COTININE

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SUMMARY

We describe methods for the synthesis of enantiomerically pure (S)-nicotine-3',3'-d_2, (S)-nornicotine-3',3'-d_2, and (S)-cotinine-4',4'-d_2. The key intermediate was 5-bromomyosmine, which underwent base catalyzed exchange with deuterium oxide to give 5-bromomyosmine-3',3'-d_2 with > 99% incorporation of label. This intermediate was reduced to (±)-5-bromonornicotine-3',3'-d_2 with sodium borohydride, resolved, and converted to (S)-nornicotine-3',3'-d_2 by reductive debromination with hydrogen and a palladium catalyst. Reductive alkylation with formaldehyde and sodium borohydride provided (S)-nicotine-3',3'-d_2, which was converted to (S)-cotinine-4',4'-d_2 by reaction with bromine followed by zinc reduction. The deuterium label is located at positions that are not attacked in the major routes of mammalian metabolism of these alkaloids. Syntheses of tetradeuterated analogs of nicotine and cotinine and a pentadeuterated analog of nicotine, in which additional deuterium atoms are incorporated in the methyl groups, are also reported.

Key Words: Nicotine, cotinine, nornicotine, deuterium, enantiomers

INTRODUCTION

Nicotine [3-(1-methyl-2-pyrrolidinyl)-pyridine] occurring in tobacco is largely, if not entirely, the levorotatory (S)-enantiomer [1]. Tobacco smoke contains a small amount (about 2-10% of the total nicotine) of (R)-nicotine, presumably formed by racemization during the combustion process [2,3]. Nornicotine [3-(2-pyrrolidinyl)-pyridine], a minor tobacco alkaloid, exists in the plant as an approximately 40:60 ratio (R):(S) enantiomers [4]. Nornicotine is also a mammalian metabolite of nicotine [5]. Presumably, the nornicotine formed metabolically from (S)-nicotine also has the (S)-configuration, but this remains to be verified experimentally. Metabolic formation of cotinine [1-methyl-5-(3-pyridyl)-2-pyrrolidinone], a major metabolite of nicotine, has been shown to occur without significant racemization [6].

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0362–4803/88/101117–12$06.00

Received August 17, 1987

Since enantiomers often differ in their metabolic profile and pharmacologic effects [7], it is important that biologic studies be carried out with stereochemically defined substances. To carry out metabolic studies in smokers, we required stereochemically pure deuterium-labelled tobacco alkaloids. In this paper we describe syntheses of the pure (S)-enantiomers of nicotine-3',3'-d_2, nornicotine-3',3'-d_2, and cotinine-4',4'-d_2. We also report methods for the synthesis of racemic tetradeuterated analogs of nicotine and cotinine and a pentadeuterated analog of nicotine which have been used as internal standards in quantitative mass spectrometric analyses.

RESULTS AND DISCUSSION

Syntheses of deuterium-labelled nicotine and cotinine with the label located at various positions of the pyrrolidine ring and on the methyl group have been reported [9,10,11]. The products of most of these syntheses were racemic, although (S)-cotinine labeled on the 3'-position, or (S)-nicotine labelled on either the 4'-position or the 5'-position may be prepared from readily available (S)-cotinine by known synthetic routes [9,11].

For studies of the pharmacokinetics and biotransformation pathways of tobacco alkaloids, we required sufficient quantities of specific deuterium-labeled nicotine, nornicotine, and cotinine to administer pharmacologically relevant doses to humans. We required (1) incorporation of the label in a position remote from major sites of metabolism, to avoid loss of label or a kinetic isotope effect; (2) a synthetic route to optically pure (R)- and (S)-enantiomers; (3) at least two deuterium atoms, to minimize interferences in mass spectrometric analysis caused by the natural abundance of carbon-13 in tobacco-derived nicotine, and (4) incorporation of the label in a portion of the molecule retained in a major ion produced in electron-impact mass spectrometry. The first three of these requirements could be met by incorporating the label on the pyridine ring or at the 3'-carbon of the pyrrolidine ring, since these positions remain intact in the primary nicotine metabolites and in most of the reported metabolites of cotinine. However, since the major electron impact mass spectral fragmentation pathways of nicotine and cotinine involve loss of the pyridine ring, the 3'-position of nicotine, which corresponds to the 4'-position of cotinine, was chosen for incorporation of two deuterium atoms, to meet the fourth requirement as well.

We have previously reported a method for the synthesis of (R)- and (S)-nornicotine of high enantiomeric purity [8]. The key step in the synthesis was resolution of (±)-5-bromonornicotine, which had been prepared by reduction of 5-bromomyosmine. Myosmine is known to undergo base-catalyzed exchange of hydrogen in the 3'-position with D_2O to give myosmine-3'-3'-d_2 [9]. This suggested that 5-bromomyosmine would also undergo hydrogen-deuterium exchange, providing a synthetic route to the desired deuterium-labeled tobacco alkaloids. Refluxing 5-bromomyosmine in D_2O containing potassium carbonate resulted in hydrogen-deuterium exchange at the desired position (Fig. 1). Isolating the product and repeating the exchange process three times yielded 5-bromomyosmine-3',3'-d_2 which was > 99% d_2. Reduction

with sodium borohydride in 90:10 methanol-acetic acid at -78°C yielded (±)-5-bromonornicotine-3',3'-d_2 without appreciable loss of label. It was found to be important to carry out the reduction at low temperature since small scale reductions at room temperature resulted in significant loss of deuterium.

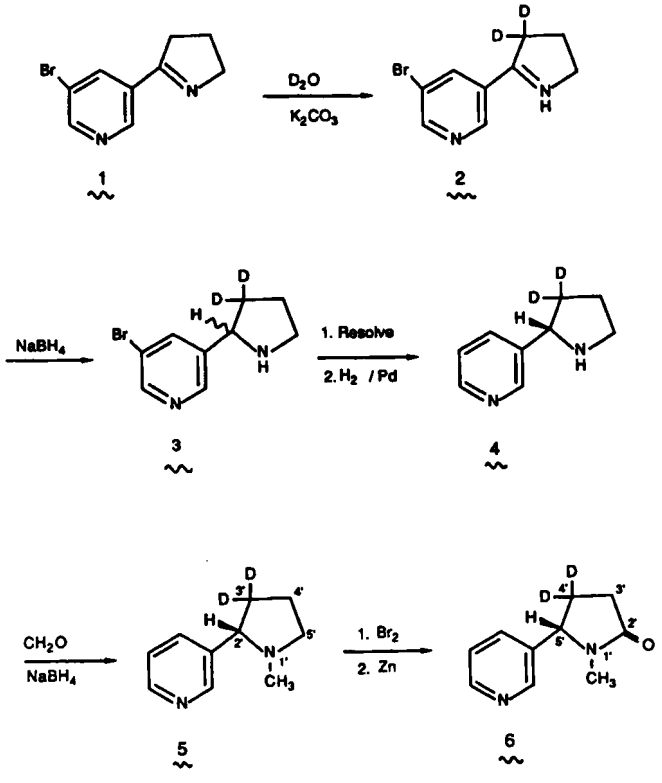

Figure 1. Synthesis of (S)-nornicotine-3',3'-d_2 (4), (S)-nicotine-3',3'-d_2 (5), and (S)-cotinine-4',4'-d_2 (6).

Resolution of (±)-5-bromonornicotine-3',3'-d_2 with (+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) gave, after four recrystallizations from acetonitrile, (S)-5-bromonornicotine-3',3'-d_2 which was >99.5% optically pure, by capillary GC analysis of the diasteriomeric (-)-camphanic acid amide derivative. Conversion of (S)-5-bromonornicotine-3',3'-d_2 to (S)-nornicotine-3',3'-d_2 by reductive debromination paralleled our previously reported synthesis of unlabelled nornicotine enantiomers [8]. Methylation with formaldehyde and sodium borohydride provided (S)-nicotine-3',3'-d_2, which was purified by distillation and converted to the crystalline tartrate salt. The electron impact mass spectrum displayed a molecular ion, m/z 164 (14%), and base peak m/z 86 which are shifted by 2 mass units from the corresponding ions, m/z 162 and 84 of natural nicotine (Fig. 2). Due to the abundant M-1 peak, the fragment ion of m/z 86 which results from loss of the pyridine ring, rather than the molecular ion, was used to assess the isotopic purity. The m/z 85 ion was not detectable (relative abundance < 1%), verify-

ing that no loss of deuterium had occurred during the reductive debromination and methylation steps.

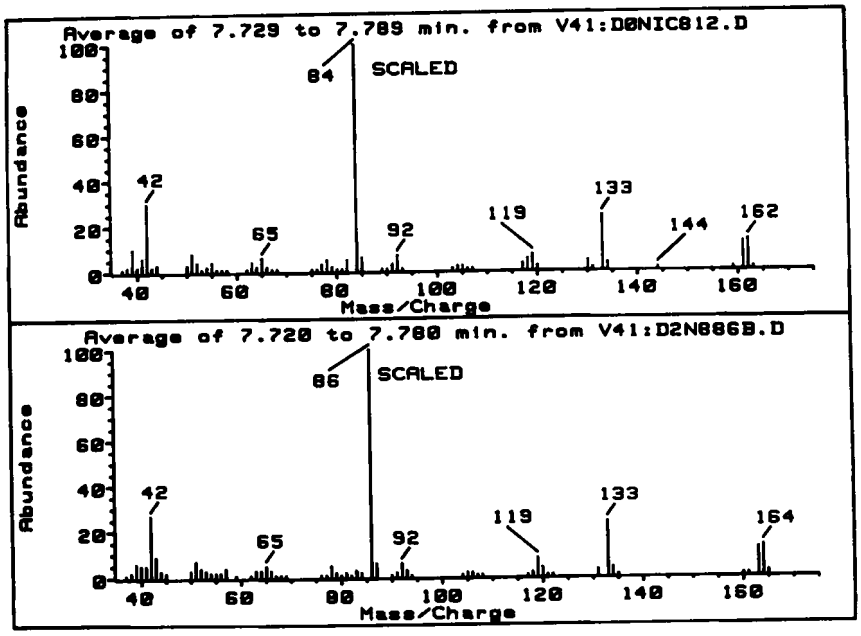

Figure 2. Electron impact mass spectra of (S)-nicotine (upper panel) and (S)-nicotine-3',3'-d_2 (lower panel).

Although unlikely, the possibility of some racemization occurring during the hydrogenolysis and methylation could not be ruled out a priori. Consequently, we developed a chromatographic method for determining nicotine optical purity. This involved demethylation to nornicotine via oxidation to nicotine-N'-oxide followed by treatment with ferrous sulfate [12]. The resulting nornicotine was converted to the amide with (-)-camphanic acid chloride and assayed for optical purity by capillary GC-MS. The ion chromatogram of m/z 330 (molecular ion) displayed one peak, retention time 16.30 min. Under these conditions, the diasteriomeric (-)-camphanic acid amides of racemic nornicotine-d_0 were well separated, the retention times being 16.09 and 16.30 minutes (Fig. 3). Consequently, the (S)-nicotine-3',3'-d_2 synthesized by our method has both high optical and isotopic purity. We should point out that (-)-camphanic acid chloride is superior to the trifluroacetylprolyl chloride which we used in previous studies [8] for assessing nornicotine optical purity since, unlike the latter reagent, commercially available (-)-camphanic acid chloride is optically pure.

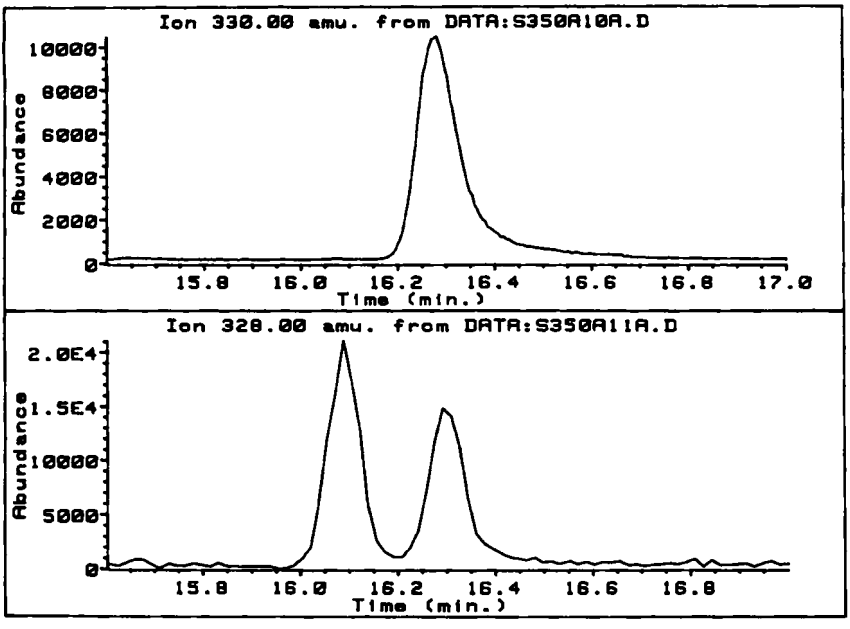

Figure 3. Determination of optical purity of (S)-nicotine-3',3'-d_2. Upper panel is the ion chromatogram of mz 330 (molecular ion) of the (-)-camphanic acid amide derived from (S)-nicotine-3',3'-d_2. The lower panel is the m/z 228 ion chromatogram of the (-)-camphanic acid amide derivative of racemic nornicotine-d_0.

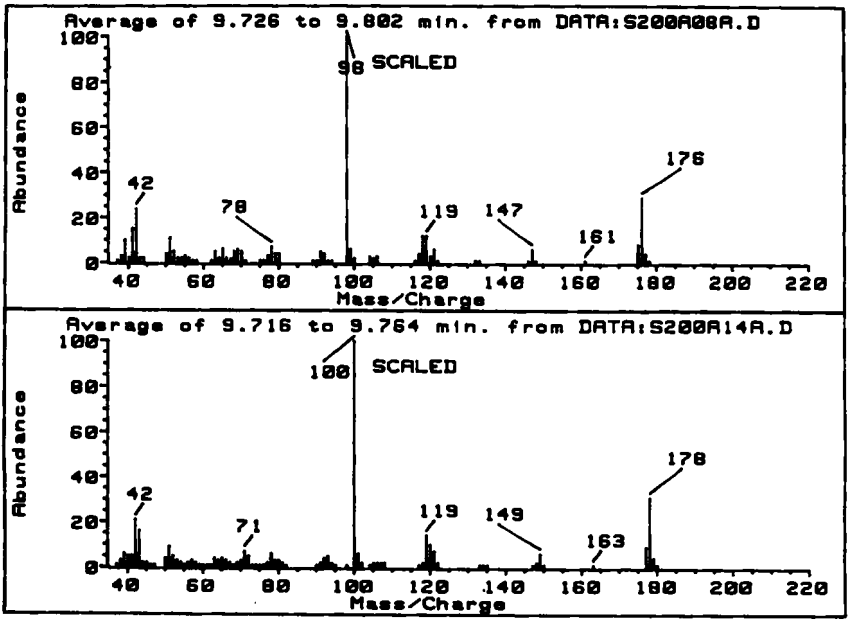

Figure 4. Electron impact mass spectra of (S)-cotinine (upper panel) and (S)-cotinine-4',4'-d_2 (lower panel).

Cotinine-4',4'-d_2 was synthesized from nicotine-3',3'-d_2 by bromination followed by zinc reduction (Fig. 1), as previously described for unlabelled cotinine [13]. This transformation has been shown to occur without significant loss of optical purity [14]. Isotopic purity was found to be >99% by mass spectral analysis (Fig. 4). We also synthesized tetradeuterated analogs of nicotine and cotinine for use as internal standards in quantitative GC-MS assays (Fig. 5). This involved synthesis of (±)-nornicotine-3',3'-d_2 as peviously described [9], which was converted to the N'-formyl derivative by heating with n-butyl formate. Reduction with lithium aluminum deuteride provided (±)-nicotine-3',3'-d_2-N-methyl-d_2. Conversion to the corresponding tetradeuterated analog of cotinine was carried out as described above. Although these syntheses were carried out on racemic materials, it is apparent that optically pure analogs could be obtained by using optically pure nornicotine-d_2. A pentadeuterated analog of (S)-nicotine (11) was prepared by reacting (S)-nornicotine sequentially with butyllithium and trideuteromethyl iodide [10].

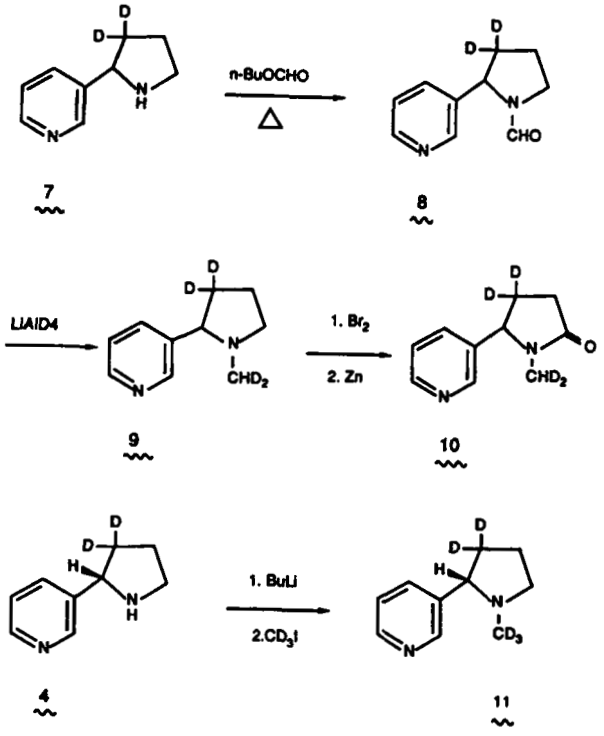

Figure 5. Synthesis of (±)-nicotine-3',3'-d_2-N-methyl-d_2 (9), (±)-cotinine-4',4'-d_2-N-methyl-d_2 (10), and (S)-nicotine-3',3'-d_2-N-methyl-d_3.

In conclusion, methods are described for the synthesis of optically pure, deuterium-labelled analogs of (S)-nicotine, (S)-nornicotine, and (S)-cotinine. Although we synthesized only the (S)-isomers, this methodology could be utilized for the synthesis of the (R)-isomers as well simply by substituting the resolving agent of the opposite configuration [8]. In addition, the

methodology described in this paper in combination with previously described methods [9-11] should be applicable to the synthesis of a wide variety of optically pure, isotopically labelled tobacco alkaloids and their metabolites. Studies of the metabolic disposition of (S)-nicotine-3',3'-d_2 in humans are in progress.

EXPERIMENTAL

Melting points were taken on a Laboratory Devices Mel-Temp apparatus and are uncorrected. Gas chromatographic determinations were carried out with a Hewlett Packard 5880A instrument equipped with a capillary inlet system, nitrogen-phosphorus detector, and Level IV computing integrator. Mass spectral analyses were carried out by GC-MS with electron impact ionization using a Hewlett Packard 5890 gas chromatograph with a capillary inlet system and capillary direct interface to a quadropole mass spectrometer, Hewlett Packard Model 5970B. Electron impact mass spectra (EIMS) and ion chromatograms were obtained using a Hewlett Packard 59970 MS Chem Station interfaced with the mass spectrometer and a 7475 graphics plotter. Microanalyses were carried out by Galbraith Laboratories, Knoxville, Tennessee.

5-Bromomyosmine-3',3'-d_2 (2). A mixture of 40 g (180 mmole) of 5-bromomyosmine (1) [8] and 50 g (2.5 mol) of deuterium oxide (99.8% d, Aldrich) containing anhydrous potassium carbonate (2 g) was heated under reflex overnight. The mixture was cooled and extracted twice with 50 mL portions of methylene chloride. The combined extracts were evaporated on a rotary evaporator, and then subjected to additional exchanges with 50 g portions of D_2O and 2 g of anhydrous potassium carbonate. Prior to the final exchange, the potassium carbonate was dried by heating in a test tube with a Bunsen burner and then cooling under dry nitrogen. After the fourth exchange, extraction with methylene chloride and evaporation of the solvent yielded 38.2 g of product. The course of the deuterium exchange was best carried out by reducing a small aliquot with sodium borohydride at low temperature (see below) and GC-MS analysis of the resulting 5-bromonornicotine, since the mass spectrum of 5-bromomyosmine is complicated by the presence of the two natural isotopes of bromine and by abundant M-1 ions.

(±)-5-Bromonornicotine-3',3'-d_2 (3). Crude 5-bromomyosmine-3',3'-d_2 (10 g, 44 mmol) was added to 150 mL of 80:20 methanol-acetic acid which had been cooled to ca -78°C with a dry ice-acetone bath. Sodium borohydride (10 g, 260 mmol) was added portionwise with vigorous stirring over 30 minutes, which resulted in vigorous evolution of hydrogen. The mixture was allowed to warm to room temperature, worked up by pouring into 800 mL of water, and made basic with ammonia. Extraction with methylene chloride (2 x 100 mL), evaporation of the combined extracts using a rotary evaporator, and a bulb-to-bulb distillation (Kugelrohr oven, 100-120°, 0.1 mmHg) provided 8.5 g of a light yellow liquid. The isotopic purity was determined by GC-MS to be >99% by the lack of observable ion current (<1% relative abundance) at m/z 71, which is one mass unit less than the base peak m/z 72 resulting from loss of the pyridine ring.

(S)-5-Bromonornicotine-3',3'-d_2 (+)-MTPA Salt. A solution of 12.8 g (56 mmol) of (±)-5-bromonornicotine-3',3'-d_2 in 100 mL ethyl acetate was added to a solution of 7.4 g (32 mmol) of (+)-α-methoxy-α-trifluoromethylphenylacetic acid ((+)-MTPA, Aldrich). This resulted in formation of a white precipitate, which was collected by filtration and air-dried to give 8.8 g of a white solid. This was recrystallized four times from boiling acetonitrile (~ 10 mL/g) to give 6.0 g of white needles, mp 180-181°C. The melting point of the corresponding unlabelled compound was 178-179°C [8].

Determination of 5-Bromonornicotine Optical Purity. 5-Bromonornicotine-3',3'-d_2 (+)-MTPA salt (2 mg) was added to a 0.1 M solution of (-)-camphanic acid chloride (Aldrich) in methylene chloride, followed by addition of 5 drops of triethylamine. The solvent was evaporated using a current of nitrogen, 0.5 mL of 50% aqueous potassium carbonate was added, and the mixture was extracted with 1 mL of toluene. The toluene extract was diluted 20:1 and analyzed by gas chromatography on a 25 m x 0.31 mm fused silica capillary column coated with 5% phenylmethylsilicone, 0.33 μ film thickness. The injection was made in the splitless mode and column oven was temperature programmed from 90-275°C, then held at the upper limit until the peaks of interest eluted. The amide derivative of (R)-5-bromonornicotine-d_2 eluted at 15.52 min, and the amide of (S)-5-bromonornicotine-d_2 eluted at 15.77 min, with nearly baseline separation of the diasteriomeric amines. By integration of the peaks, the optical purity was determined to be 99.7%. That the peak with the longer retention time was the (S)-isomer was determined by carrying out the analysis of unlabelled (R)- and (S)-5-bromonornicotine of established configuration [8].

(S)-Nornicotine-3',3'-d_2 (4). (S)-5-Bromonornicotine (+)-MTPA salt (3.0 g, 6.5 mmol) was added to 50 mL of 1 M KOH, and the mixture was extracted twice with 50 mL portions of methylene chloride. The combined extract was washed with a small quantity of aqueous KOH, then evaporated to a colorless oil. This was dissolved in 50 mL of anhydrous ethanol, to which was added 2 mL of triethylamine and 0.5 g of 5% palladium on charcoal. The mixture was hydrogenated at ca. 1 atmosphere using a Brown automatic gasimeter (Ace Glass Co.) for 1 hour. Analysis by TLC (ethyl acetate-methanol-conc ammonia 85:10:1) on silica gel indicated that hydrogenolysis was complete. The mixture was filtered through Celite and the filter cake was washed with 50 mL of methanol containing 3 mL of conc. ammonia. The filtrate was evaporated on a rotary evaporator to an almost colorless oil. A small portion was converted to the picrate salt, which after recrystallization from 90% aqueous ethanol melted at 189-190°C. Unlabelled (S)-nornicotine picrate melted at 188.5-189.5°C [8]. EIMS m/z (%) M^+ 150 (16), 149 (26), 120 (29), 119 (100), 72 (75).

(S)-Nicotine-3',3'-d_2 (5) bis-tartrate dihydrate. The crude (S)-nornicotine-d_2 base obtained in the previous reaction was dissolved in 40 mL of methanol containing 10 mL of acetic acid, and then cooled with an ice bath. Formaldehyde (10 mL of 30% aqueous) was added,

followed by the portionwise addition of sodium borohydride (2 g) with vigorous stirring over 20 min. TLC analysis indicated complete reaction. The solution was warmed to room temperature, most of the solvent was removed using a rotary evaporator, and enough aqueous ammonia was added to make the mixture basic. The resulting solution was extracted twice with 100 mL portions of methylene chloride, which were combined and evaporated on a rotary evaporator. The residue was dissolved in 50 mL of 1 M sulfuric acid, washed with methylene chloride (2 x 50 mL), and then made basic with potassium hydroxide. This was extracted twice with 50 mL portions of methylene chloride, which were combined, evaporated on a rotary evaporator and distilled bulb-to-bulb using a Kugelrohr oven (130-135°C, 25 mmHg) to give 0.95 g (5.8 mmol, 89% yield based on 5-bromonornicotine-3',3'-d_2) of colorless liquid.

The tartrate salt was prepared by combining the free base with two equivalents of (+)-tartaric acid (1.74 g) in 20 mL of methanol. The solvent was removed with a rotary evaporator to give a viscous liquid, which was triturated with a few mL of 80% aqueous ethanol. Stirring and scratching with a glass rod led to crystallization of the product, which was collected by filtration, and then recrystallized three times from 10 mL portions of boiling 80% aqueous ethanol. Following the final recrystallization, the product was vacuum dried at 0.1 mmHg to give 1.74 g of white crystalline powder. The melting behavior was identical to that of a sample of unlabelled nicotine bitartrate dihydrate: at 99-101° C both samples went to a viscous melt, with bubbling, presumably evolution of H_2O. Our previous experience has been that the melting point of nicotine tartrate is variable, depending upon the rate of heating. A microanalysis confirmed the composition of (S)-nicotine-3',3'-d_2 bis-tartrate dihydrate: Anal. Calcd for $C_{18}H_{28}D_2N_2O_{14}$: C, 43.20; H + D, 6.44; N, 5.60. Found: C, 43.31; H + D, 6.49; N, 5.25. EIMS m/z (%) M^+ 164 (14), 163 (12), 133 (23), 86 (100); deuterium enrichment >99% d_2 (Fig. 2).

Determination of Nicotine-3',3'-d_2 Optical Purity.

Ten mg of (S)-nicotine-d_2 tartrate was added to 1 mL of 50% potassium carbonate, which was extracted with 2 mL of chloroform. The extract was added to 5 mg of m-chloroperbenzoic acid (85% purity) and boiled 30 sec on a steam bath. The solution was cooled, and extracted with 1 mL of water. The aqueous layer (containing nicotine-d_2 N-oxide) was separated, treated with 100 mg of ferrous sulfate, and heated on a steam bath for 5 min. The mixture was cooled, made basic by adding 1 mL of 20% potassium carbonate, and extracted with 2 mL of ethyl acetate. To the extract was added 5 mg of (-)-camphanic acid chloride, and after standing 10 min the solution was washed with 1 mL of 50% potassium carbonate, evaporated with a current of nitrogen, and reconstituted with 1 mL of 90:10 toluene-butanol. This was then diluted 1:10, and 1 µl analyzed by GC-MS. The separation was carried out on a 12 m x 0.2 mm fused silica capillary column, coated with a 0.33 µ film of cross-linked methylsilicone. The column oven was temperature programmed from 70° to 225°C and held at the upper limit until the peaks of interest eluted. The mass spectrometer was tuned to monitor m/z 330, the molecular ion of the

(-)-camphanic acid amide derivative of nornicotine-d_2. The m/z 330 ion chromatogram (Fig. 3, upper panel) displayed a single peak, retention time 16.30 min, corresponding to the (S)-isomer. Racemic nornicotine-d_0 was converted to the diasteriomeric (-)-camphanic acid amides, and analyzed using the same chromatographic conditions. Two well resolved peaks with retention times of 16.09 and 16.30 min in the m/z 228 ion chromatogram correspond to the (R)- and (S)-isomers, respectively, of nornicotine. That the peak with the longer retention time is the (S)-isomer was shown by analysis of nornicotine enantiomers with known configuration [8].

(S)-Cotinine-4',4,'-d_2 (6) perchlorate. To 0.35 g (2.1 mmol) of (S)-nicotine-3',3'-d_2 in 25 mL of 80% aqueous acetic acid was added 3 mL (8.7 g, 54 mmol) of bromine, and the resulting deep red solution was heated on a steam bath for 4 hr. The solution was cooled with an ice bath, and with vigorous stirring 15 g of zinc dust was added portionwise over 5 min (exothermic). Then 20 mL of 5% aqueous HCl was added in small portions, which resulted in vigorous gas evolution and foaming. After stirring for 10 min at room temperature, the mixture was filtered through Celite. The filtrate was made basic with ammonia, and then extracted twice with 100 mL portions of methylene chloride. The combined extracts were back-extracted into 50 mL of 1 M sulfuric acid, which was then made basic with ammonia and extracted with two 100 mL portions of methylene chloride. These final extracts were combined, evaporated on a rotary evaporator, and distilled bulb-to-bulb (Kugelrohr oven, 100-110°C, 0.1 mmHg) to give a light yellow oil. This was taken up in 5 mL of isopropyl alcohol, which was then added to 0.5 mL of 60% perchloric acid in 5 mL of isopropyl alcohol. The solution went cloudy, and then precipitated a white solid. After standing 15 min at room temperature, the product was collected by filtration, washed successively with small portions of isopropyl alcohol and methyl tert-butyl ether, and air dried. There was obtained 0.17 g (0.61 mmol, 30% yield) of fluffy white crystals, mp 216.5-217.5°C (dec). A sample of unlabelled cotinine perchlorate melted at 217.5-218.5°C (dec), lit. (15) mp 218-219°C.

Anal. Calcd for $C_{10}H_{11}D_2N_2O_5Cl$: C, 43.09; H + D, 5.43; N, 10.05. Found: C, 43.08; H + D, 5.19; N, 9.93. EIMS m/z (%) M^+ 178 (31), 119 (14), 100 (100); deuterium enrichment >99% d_2 (Fig. 4).

(±)-N'-Formylnornicotine-3',3'-d_2 (8). Racemic nornicotine-3',3'-d_2 (7) was prepared by reduction of myosmine-3',3'-d_2 [9] according to the procedure described above for reduction of 5-bromomyosmine-3',3'-d_2. A solution of 1 g (6.7 mmol) of 7 in 15 mL of n-butyl formate was heated on a steam bath overnight. The solution was cooled and extracted with 25 mL of 1 M sulfuric acid. The acid layer was separated, washed with 25 mL methylene chloride, made basic with potassium carbonate, and extracted with two 25 mL portions of methylene chloride. The combined extracts were evaporated and distilled bulb-to-bulb (Kugelrohr oven, 120-125°C, 0.1 mmHg) to give 1 g of a light yellow liquid. EIMS m/z (%) M^+ 178 (79), 149 (100), 119 (70), 92 (49), 72 (53).

(±)-Nicotine-3',3'-d_2-N-methyl-d_2 (9) Picrate. Lithium aluminum deuteride (Aldrich, 99% D, 0.4 g, 9.5 mmol) was added portionwise to 0.8 g (4.5 mmol) of N-formylnornicotine-3',3'-d_2 (8) in 50 mL anhydrous ether, with ice cooling over 5 min. After stirring for 15 min, the reaction was worked up by addition of 5 mL isopropyl alcohol (gas evolution) followed by 1 mL of 15% aqueous sodium hydroxide. The mixture was filtered, the filter cake was washed with 10 mL isopropyl alcohol, and then the filtrate was evaporated with a rotary evaporator. The residue was taken up in 50 mL 1 M HCl, washed with 50 mL methylene chloride, made basic with ammonia, and then extracted twice with 25 mL of methylene chloride. The combined extracts were evaporated with a rotary evaporator and distilled bulb-to-bulb (Kugelrohr oven, 110-120°, 20 mmHg) to give 0.5 g of a colorless liquid. A 0.16 g portion was added to 0.5 g of picric acid in 10 mL of boiling ethanol, which resulted in precipitation of a yellow solid. The mixture was cooled, the product was collected by filtration, washed with ether, and air-dried yielding 0.3 g of fine yellow crystals, mp 214-216°C (dec). EIMS m/z (%) M^+ 166 (13), 165 (12), 135 (24), 88 (100); deuterium enrichment 93% d_4, 7% d_3.

(±)-Cotinine-3',3'-d_2-N-Methyl-d_2 (10)-Perchlorate. The reaction of 0.34 g (2.0 mmol) of (±)-nicotine-3',3'-d_2-(N-methyl-d_2) (9) with 3 mL (8.7 g, 54 mmol) of bromine in 50 mL 80% aqueous acetic acid was carried out as described above for the synthesis of (S)-cotinine-3',3'-d_2. Bulb-to-bulb distillation of the crude product gave a yellow viscous liquid, which was converted to the perchlorate salt, 0.35 g (1.25 mmol, 63% yield) of a bright white crystalline powder, mp 234-236°C (dec). EIMS m/z (%) M^+ 180 (32), 121 (11), 102 (100); deuterium enrichment 88% d_4, 12% d_3.

(S)-Nicotine-3',3'-d_2-N-Methyl-d_3 (11) Picrate. The conversion of 4 to 11 was analogous to the method used by Seeman et al. [10] for synthesis of (±)-nicotine-N-methyl-d_3. EIMS m/z (%) M^+ 167 (13), 166 (13), 136 (26), 89 (100); deuterium enrichment 95% d_5, 5% d_4. The bis-picrate salt of 11, recrystallized from 75% aqueous ethanol, had mp 223-224°C (dec). It is interesting to note that the picrate of 11 had a significantly higher melting point than the picrate of 9, presumably due to the fact that 9 is a racemate, whereas 11 is the (S)-isomer. Likewise, racemic cotinine-3',3'-d_2-N-methyl-d_2 (10) perchlorate also had a melting point that differed considerably from the (S)-isomers of unlabelled cotinine perchlorate and cotinine-3',3'-d_2 perchlorate (see above).

ACKNOWLEDGEMENT

Financial support from U.S. Public Health Service grants DA02277, CA32389, and DA01696 is gratefully acknowledged. The authors wish to express their appreciation to Clarissa Ramstead for her help in preparation of the figures, and to Kaye Welch for administrative assistance and preparation of the manuscript.

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