THE SYNTHESES OF DEUTERIUM LABELLED TOBACCO ALKALOIDS: NICOTINE, NORNICOTINE AND COTININE

Trong-Lang Nguyen and Neal Castagnoli, Jr *
Department of Pharmaceutical Chemistry,
School of Pharmacy, University of California,
San Francisco, California 94143
Received August 26, 1977

SUMMARY

A variety of deuterium labelled tobacco alkaloids has been prepared for metabolic studies. Reduction of myosmine with sodium borodeuteride provided nornicotine-2'-d_1. Myosmine also underwent smooth base catalyzed exchange with deuterium oxide to myosmine-3',3'-d_2 which could be reduced to nornicotine-3',3'-d_2 and nornicotine-2',3',3'-d_3. Thus avenues to nicotine alkaloids labelled at C_2', and/or C_3', have been established. Conversion of nornicotine to N-ethoxycarbonyl-nornicotine followed by lithium aluminum deuteride reduction gave nicotine-N-methyl-d_3. Nicotine-2',5',5'-d_3 was prepared by oxidation of nicotine-2'-d_1 to cotinine-5-d_1 followed by lithium aluminium deuteride reduction. In each case we observed the partial exchange of pyridyl protons with deuterium derived from the lithium aluminium deuteride. In combination with existing methods, these

---

* To whom correspondence should be addressed.

0362-4803/78/0614-0919$01.00

reactions will be useful in the synthesis of a variety of deuterium labelled nicotine alkaloids

Key Words: Nicotine, Nornicotine, Cotinine, Deuterium

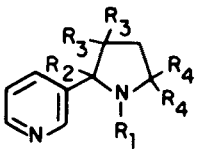
INTRODUCTION

The oxidative metabolism of nicotine (1) has been shown to involve the C_2', C_4' and C_5' positions of the five membered pyrrolidine ring (1). In order to facilitate our studies on nicotine metabolism, we required synthetic methods for the preparation of 1 and its principal metabolites nornicotine (2) and cotinine (3) containing specific deuterium labels in the five membered ring.

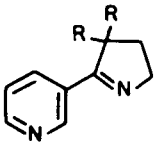
The synthesis of nornicotine-2'-d_1 (2a) has been achieved previously by catalytic reduction of myosmine (4) with deuterium gas over palladium on charcoal (2). Subsequent methylation of 2a provided nicotine-2'-d_1 (1a). However this procedure provided 1a and 2a with low deuterium incorporation. The same workers prepared nicotine-N-methyl-d_3 (1c) in low yield by methylation of 2 with methyl iodide-d_3 and nicotine-5',5'-d_2 (1b) by the lithium aluminium deuteride reduction of cotinine (3). The preparation of cotinine-3,3-d_3 (3a) by exchange with deuterium oxide has been shown to proceed in good yield with a high incorporation of label (3).

DISCUSSION

The reactions for the preparation of the various deuterium labelled tobacco alkaloids are summarized in Scheme I. Myosmine (4) is the key intermediate in this synthetic sequence. The imino functionality provides an opportunity to introduce deuterium atoms at C_2' (by reduction) and C_3' (by exchange) of the five membered ring.

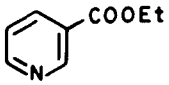


	R_1	R_2	R_3	R_4
1 :	CH_3	H	H	H
1a :	CH_3	D	H	H
1b :	CH_3	H	H	D
1c :	CD_3	H	H	H
1d :	CH_3	D	H	D
2 :	H	H	H	H
2a :	H	D	H	H
2b :	H	H	D	H
2c :	H	D	D	H
9 :	COOEt	H	H	H
9a :	COOEt	D	H	H

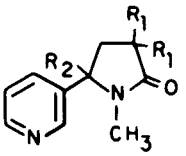


4 : R = H

4a : R = D



5

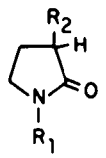


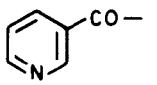
3 : R_1 = R_2 = H

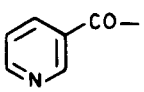
3a : R_1 = H ; R_2 = D

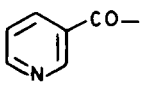
3b : R_1 = D ; R_2 = H

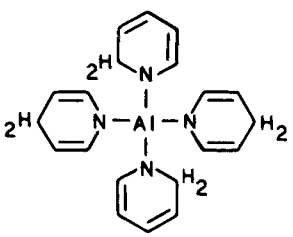
10 : R_1 = Br ; R_2 = H



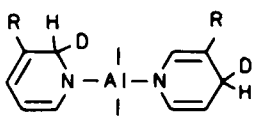
6: R_1 =  ; R_2 = H

7: R_1 = R_2 = 

8: R_1 = H ; R_2 = 

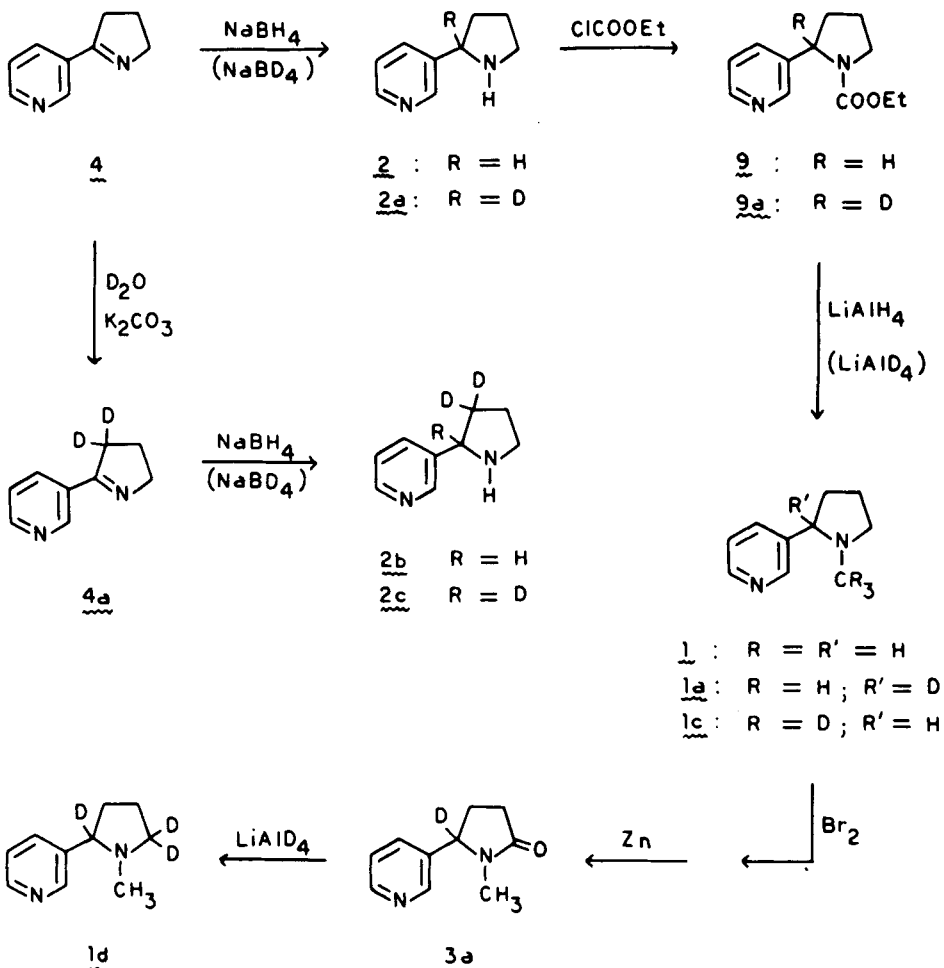


11a



11b

Several procedures leading to 4 have appeared in the literature (4). The procedure followed in this program is a modification of that reported by Korte and Schulze-Steinen (4a). This approach involves the condensation of ethyl nicotinate (5) with N-nicotinoyl-2-pyrrolidinone (6) followed by acid catalyzed hydrolysis and decarboxylation of the condensation product to myosmine. These authors suggested that the base induced condensation of 5 and 6 gives 1,3-dinicotinoyl-2-pyrrolidinone (7) which upon heating at reflux in hydrochloric acid is converted to myosmine via the intermediate 3-nicotinoyl-2-pyrrolidinone (8). However no characterization



Scheme I. Synthetic sequence for the syntheses of deuterated tobacco alkaloids.

of 7 or 8 was reported. We have now isolated this condensation product and established its structure as 3-nicotinoyl-2-pyrrolidinone (8) by analytical and spectral data (see experimental). Compound 8 presumably is formed during work-up from cleavage of the amide linkage of 7. Hu, et al. (4e) recently reported the synthesis of 8 by an independent route.

Treatment of myosmine with potassium carbonate in deuterium oxide led to the incorporation of two deuterium atoms. The mass spectrum of the product shows a shift of two mass units in the molecular ion (from 146 to 148) but no shift in the base peak at m/e 118 ($M^+ - C_2H_2D_2$). This established that the label is located in the pyrroline and not the pyridyl ring. The nmr signals of the pyrroline protons of myosmine-d_0 include two triplet of triplets centered at δ 2.9 and 4.15 ppm for the C_3' and C_5' protons. The signal at δ 2.9 ppm, which is absent in the myosmine-d_2 spectrum, must be due to the C_3' protons since the signal for the C_5' methyleneamino protons will occur downfield relative to the signal for the C_3' allylic protons (5). Therefore we have assigned the structure of this product as myosmine-3',3'-d_2 (4a).

Reduction of 4a with sodium borohydride gave nornicotine-3',3'-d_2 (2b) without any significant exchange of the label with the medium. Similarly sodium borodeuteride reduction of 4 and 4a gave nornicotine-2'-d_1 (2a) and nornicotine-2',3',3'-d_3 (2c), respectively, again without detectable exchange of the desired label. The mass and nmr spectra of these products are completely in accord with the assigned structure (see experimental for details). Exchange between borohydride (or borodeuteride) and the medium is known to occur at low pH (6) while, as we have shown, exchange between myosmine and the medium occurs at high pH. Fortunately at the pH of an aqueous alcoholic solution of borohydride neither of these processes presents a problem.

Trideuteriomethylation of nornicotine (2) to form nicotine-N-methyl-d_3 (1c) was accomplished by reduction of N-ethoxycarbonylnornicotine (9) with lithium aluminium deuteride. Both the N-ethoxycarbonylation and the deuteride reduction proceed in acceptable yields (80 and 73%, respectively). Similarly nornicotine-2'-d_1 (2a) was converted to nicotine-2'-d_1 (1a).

The synthesis of nicotine-5',5'-d_2 was achieved as described in the literature (2) by lithium aluminium deuteride reduction of cotinine (3). In order to establish the versatility of this approach to labelled nicotine alkaloids, we examined the oxidation of nicotine to 3,3-dibromocotinine (10) and the conversion of 10 to cotinine in deuterated solvents. The product isolated was exclusively cotinine-3-3-d_2 (3a) showing that this reaction sequence can be carried out in protio solvents on nicotine bearing deuterium at 2',3' and the N-methyl group without loss of label. Nicotine-2',5',5'-d_3 (1d) was successfully prepared in this way. Consequently by using the appropriate combination of the above synthetic steps nicotine and related alkaloids with a variety of patterns of labelling in the five membered ring can be prepared.

One complication was noted in these studies. Reduction of cotinine (3) and N-ethoxycarbonylnornicotine (9) with lithium aluminium deuteride led to an excess incorporation of deuterium in the final products. The mass spectrum of the deuterium labelled nicotines displayed the excess label in the parent ion but not in the base peak (M^+-pyridine) indicating that partial incorporation of deuterium into the pyridine ring was occuring. When lithium aluminium hydride is dissolved in pyridine at room temperature the solution immediately turns orange. It has been established by nmr and ir studies that under these conditions the tetrakis (N-1,2- and/or N-1,4-dihydropyridyl) aluminate (11a) is formed (7). It seems likely

that the pyridine moiety of 3 and 9 also can react with lithium aluminium deuteride to form a complex (11b) similar to that formed by pyridine itself. Apparently this complex is subsequently air oxidized to regenerate the original pyridine compound. The net result is a partial incorporation of deuterium into the pyridine moiety.

When nicotine-d_0 was allowed to react with lithium aluminium deuteride in THF a similar partial incorporation of deuterium was observed. Figure I shows the nmr spectra of the pyridyl protons of nicotine-d_0 (bottom) and the corresponding set of signals from the nicotine recovered from the lithium aluminium deuteride reaction. The spectrum of the partially deuterated sample shows that the signal for proton D has collapsed into two doublets indicating that the major coupling with proton C (J_{CD} = 8 Hz) is unchanged and that proton A or B has been replaced by a deuterium atom. Since the major coupling of proton C with proton B (J_{BC} = 4.8 Hz) is unchanged, we have concluded that proton A and not B is replaced. The integration confirms this. There is an obvious decrease in the intensity of the signal for proton A. The relative intensities of the signals for A + B, D, C and the pyrrolidine protons are 1.2, 0.7, 0.8 and 10.0. It appears therefore that there is a slight replacement of deuterium for hydrogen at other positions of the pyridyl ring as well. The excess deuterium incorporation in the synthetic compounds obtained in this way amounted to as much as 20%. Fortunately, this does not interfere with the mass spectral analyses of these compounds since we were able to rely on the fragment ions arising from the loss of the pyridine moiety.

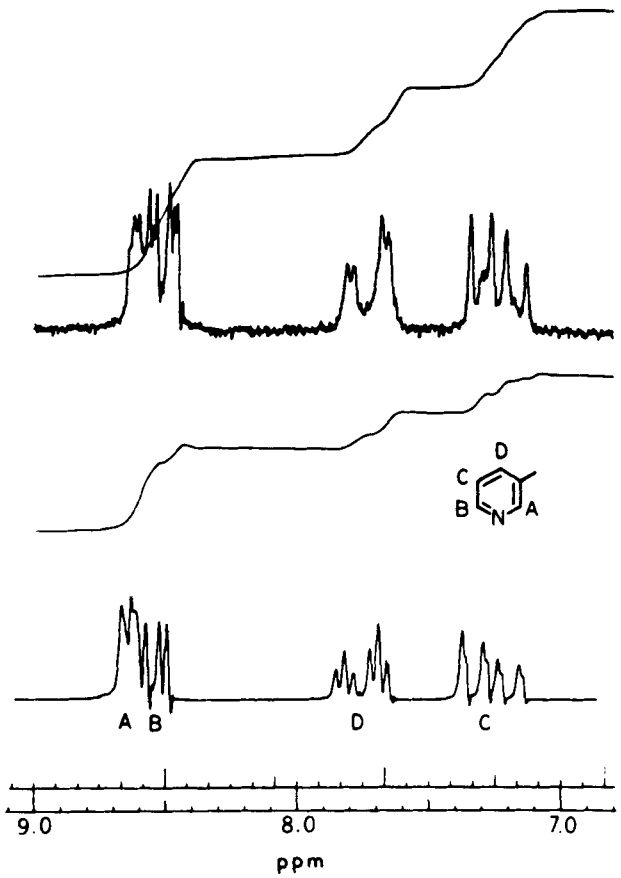


Figure 1. The nmr spectra (60 MHz, $CDCl_3$) TMS) of the pyridyl protons of nicotine-d_0 (bottom) and of the corresponding set of nicotine recovered from the reaction of nicotine-d_0 with $LiAlD_4$ in THF (top).

EXPERIMENTAL

All reactions were carried out under a nitrogen atmosphere unless otherwise stated. Melting points and boiling points are uncorrected. All chemicals used were reagent grade unless otherwise specified. Infrared (ir) spectra were taken on a Perkin Elmer 337 grating spectrophotometer. Intensities of ir absorption bands are given as (sh) sharp, (s) strong, (m) medium, (w) weak, and (b) broad. A Varian A 60 instrument was used to obtain nuclear magnetic resonance (nmr) spectra. Chemical shifts are given in parts per million (ppm) downfield from internal tetramethylsilane (TMS) in all solvents except D_2O where sodium 2,2-dimethyl-2-silapentane-5-sulfonate (SDSS) was used. Spin multiplicity is given as (s) singlet, (d) doublet, (t) triplet, (q) quartet, (p) pentet or (m) multiplet. Ultraviolet (uv) spectra were recorded on a Cary 15 Spectrophotometer. Electron impact mass spectra (eims) were obtained either by direct insertion or by gas chromatography mass spectrometry (gcms) on an AEI MS-12 mass spectrometer which is interfaced to a PDP 8/I computer using the DS-30 software. An Infotronics 2400 gas chromatograph using helium as the carrier gas is interfaced to the mass spectrometer via a Biemann-Watson molecular separator. Unless otherwise stated, mass spectra were taken with an 8 kV accelerating voltage, a trap current of 500 μA, an electron beam energy of 70 ev, a source temperature of 200° C and a resolving power of 1,200. Chemical ionization mass spectra (cims) 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 and the probe temperature 180-200°.

N-Nicotinoyl-2-pyrrolidinone (6) -- The following modified literature (4a) procedure provided reasonable yields of compound 6. A mixture of

nicotinic acid (10 g, 8.13 mmol) and thionyl chloride (30 g, 250 mmol) was heated with vigoroug stirring under reflux for 2.5 hr and the residue obtained after removing the excess thionyl chloride (using co-distillation with benzene for last traces) was treated with pyridine (10 g, 127 mmol) and anhydrous dichloromethane (40 ml) followed by the dropwise addition of 2-pyrrolidinone (30 g, 310 mmol) in dichloromethane (60 ml) over a period of 30 min. The reaction mixture was then heated briefly to 50-60^{o} to help break up large chunks of solid and stirring was continued at room temperature for 15 hr. Water (100 ml) was added and the pH of the aqueous layer adjusted to 4-5 with dilute hydrochloric acid. Extraction with dichloromethane (7 x 350 ml) gave after drying (anhydrous sodium sulfate) and removing the solvent a brown residue which by programed (75^{o} for 1 min then increasing 10^{o}/min) gcms on 3% OV17 was found to contain the desired compound 6, pyridine and 2-pyrrolidinone. After solidifying, the oily impurities were decanted and the residue recrystallized alternately from ethyl acetate and benzene to obtain colorless needles (8.45 g, 50%): mp 103-105^{o} (lit.[4b] mp 103-105^{o}); nmr ($CDCl_3$) δ ppm 1.8-2.3 (distorted p, 2H, C_4), 2.2-2.9 (q, 2H, C_3), 3.9 (t, J = 6.5 Hz, 2H, C_5), 7.0-9.0 (3m, 4H, the typical 3-substituted pyridine pattern[8] observed for all pyridyl systems studied; eims m/e (%) 190 (35), 162 (25), 106 (100), 78 (74).

3. Nicotinoyl-2-pyrrolidinone (8) -- The procedure followed was that which Korte and Schulze-Steinen (4a) reported for the preparation of 1,3-dinicotinoyl-2-pyrrolidinone (7). The crude product obtained solidified upon standing at room temperature for two days. Recrystallization from toluene followed by sublimation (90^{o} C, 0.1 mm) provided an analytically pure product in 69% yield: mp 100-101^{o} (lit.[4e] mp 102-106^{o}); nmr ($CDCl_3$) δ 2.0-3.7 ppm (2m, 4H, C_4 and C_5), 4.45 (2d, 1H, C_3), 7.68 (broad s, 1H, NH);

ir ($CHCl_3$) ν 3440 cm^{-1} (sh, m), 3325 (b, m), 1720 (s), 1700 (s); uv (EtOH) λ_{max} 300 nm (ε 1100), 267 (ε 3750), 225 (ε 9800); cims MH^+ 191; eims m/e (%) 190 (30), 162 (16), 106 (100), 84 (52), 78 (90).

Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.16; H, 5.29; N, 14.73. Found: C, 63.33; H, 5.30; N, 14.84.

Myosmine (4) -- Myosmine-d_0 (4) was prepared from 8 according to the published procedure (4a). Recrystallization from diethyl ether gave a 63% yield of yellowish white crystals: mp 42-44° (lit.[4a] mp 44° C); nmr ($CDCl_3$) δ 2.1 ppm (distorted p, 2H, C_4'), 3.0 (distorted t of t, 2H, C_3'), 4.15 (t of t, 2H, C_5'); ir ($CHCl_3$) ν 1621 cm^{-1} (sh, s, C=N) (lit.[9] 1621 cm^{-1}); eims, m/e (%) M^+ 146 (83), 145 (43.8), 118 (100), 91 (7.7) (similar to lit.[2] spectrum).

Myosmine-3',3'-d_2 (4a) -- All glassware was baked overnight in the oven. Potassium carbonate was placed in a test tube and heated by a free flame to expel absorbed moisture and was then baked in the oven overnight before use.

A solution of myosmine (730 mg, 5 mmol) and potassium carbonate (305 mg, 2.2 mmol) in D_2O (5 ml, 99.8% D, 275 mmoles) was heated with stirring at 80-90° for five days and then extracted with dry chloroform (4 x 10 ml). The extracts were combined, dried (anhydrous sodium sulfate) and the solvent was removed to yield essentially pure myosmine-3',3'-d_2: nmr (D_2O) δ 2.1 ppm (t, J = 8 Hz, 2H, C_4'), 4.1 (t, J = 8 Hz, C_5'); no signal was observed at 2.9 ppm. The eims of myosmine-3',3'-d_2: m/e (%) M^+ 148 (64.6), 147 (43), 118 (100). The isotopic composition of myosmine-3',3'-d_2 could not be determined directly due to the interference of the $(M-1)^+$ and $(M-2)^+$ peaks in the molecular region. The minimum deuterium incorporation

as determined indirectly by reduction of myosmine-3',3'-d_2 to nornicotine-3',3'-d_2 (see below) was 96% d_2 and 4% d_1.

Nornicotine-d_0 (2) and deuterium labelled nornicotine 2a, 2b and 2c -- The following procedure for nornicitine-d_0 (2) also was followed for the deuterium labelled compounds. Myosmine-d_0 (4, 1.46 g, 10 mmol) and sodium borohydride (0.76 g, 20 mmol) were stirred for 5 days at room temperature in a 1:3 ethanol:water mixture (120 ml). The combined chloroform (5 x 140 ml) extract was dried (anhydrous sodium sulfate) and removed to yield a yellow residue (1.37 g) which on short path distillation (94°/0.5 mm) gave pure nornicotine-d_0 (1.30 g, 90%): nmr ($CDCl_3$) δ 1.2-2.4 ppm (m, 4H, C_3', and C_4'), 2.65 (s, 1H, NH), 3.1 (m, 2H, C_5'), 4.15 (t, 1H, C_2'); eims m/e (%) M^+ 148 (23), 147 (32), 120 (34), 119 (100), 118 (34), 70 (90), (similar to lit.[2] spectrum) uv and ir spectra agreed with lit.[10] The following characteristics were obtained for the deuterium labelled compounds. Nornicotine-2'-d_1 (2a): nmr ($CDCl_3$) δ 1.2-2.4 (m, 4H, C_3' and C_4'), 2.6 (s, 1H, NH), 2.8-3.4 (m, 2H, C_5'); the signal at 4.15 ppm for C_2' was absent; eims m/e (%) M^+ 149 (30), 120 (100), 71 (99), 70 (7.7). The ions at m/e 71 and 70 (the pyrrolinium species)[2] established the deuterium enrichment to be 96% d_1. Nornicotine-3',3'-d_2 (2b): eims m/e (%) M^+ 150 (28), 119 (100), 72 (91), 71 (3.9), deuterium enrichment 96% d_2 and 4% d_1. Nornicotine-2',3',3'-d_3 (2c): nmr ($CDCl_3$) δ 1.8 ppm (broad t, 2H, C_4'), 2.65 (s, 1H, NH), 2.8-3.4 (m, 2H, C_5'); a weak signal (8% of 1H) appeared at 4.1 ppm for C_2'); eims m/e (%) M^+ 151 (35), 120 (100), 73 (99), 72 (11.6); deuterium enrichment 94% d_3 and 6% d_2.

N-Ethoxycarbonylnornicotine (9) -- To a well stirred solution of nornicotine (222 mg, 1.5 mmoles) and triethylamine (25. mg, 2.5 mmoles) in 10 ml of freshly distilled ether was added dropwise ethyl chloroformate (267 mg,

2.5 mmoles) in 3 ml ether. After 5 minutes the mixture was filtered and the solvent was removed to give the crude product which was distilled at 133°/65 μ in a Kugelrohr oven to yield a colorless liquid (256 mg, 80%): nmr ($CDCl_3$) δ 1.1 (t, 3H, J = 6.5 Hz, OCH_2CH_3), 1.6-2.5 (m, 4H, C_3' and C_4'), 3.6 (t, 2H, C_5'), 4.0 (q, 2H, J = 6.5 Hz, OCH_2CH_3), 4.9 (2d, 1H, C_2'); ir ($CHCl_3$) ν 1710 cm^{-1} (s, C=O); eims m/e (%) M^+ 220 (34.8), 191 (100), 147 (60), 142 (51.5).

Anal. Calcd for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.71. Found: C, 65.24; H, 7.27; N, 12.64.

Nicotine-d_0 (1) -- Crude N-ethoxycarbonylnornicotine (9, 210 mg, 0.95 mmol), prepared according to the procedure described above, in freshly distilled tetrahydrofuran (10 ml) was added dropwise to a well stirred suspension of lithium aluminium hydride (76 mg, 2 mmol) in freshly distilled tetrahydrofuran (5 ml). The yellow reaction mixture was stirred at room temperature for 1.5 days and after cooling, water (1/4 ml) followed by 15% NaOH (1/4 ml) and finally additional water (1.2 ml) were added carefully. After filtering the liquid was dried over anhydrous sodium sulfate and the solvent was removed. A short path distillation (84° C 0.6 mm) gave 118 mg (73%) of a colorless liquid. All the spectral data (ir,[10] nmr,[8] eims[2]) were identical to those reported for nicotine.

(S)-Cotinine (3) -- This compound was synthesized according to the method of Bowman and McKennis (11). A 70% yield of a colorless liquid was obtained which solidified upon storage at 0° C: nmr ($CDCl_3$) δ 1.8-2.8 ppm (complex pattern, 4H, C_3 and C_4), 2.75 (s, 3H, NCH_3), 4.75 (distorted t, 1H, C_5); eims m/e (%) M^+ 176 (40), 175 (12), 98 (100).

Nicotine-2',5',5'-d_3 (1d) -- Nicotine-2'-d_1 was prepared from nornicotine-2'-d_1 (2a) via 9a according to the procedure described above for the synthesis

of nicotine-d_0. Crude 1a was converted directly to cotinine-5-d_1 (3a) according to the method of Bowmaan and McKennis (11): eims m/e (%) M^+ 177 (50.3), 99 (100), 98 (8.9); deuterium enrichment 93% d_1. Reduction of cotinine-5-d_1 with lithium aluminium deuteride gave nicotine-2',5',5'-d_3 (1d): eims m/3 (%) M^+ 165 (20.7), 136 (20), 87 (100), 86 (13.8), 85 (2.7), 84 (3.0); deuterium enrichment 86% d_3 and 13% d_2.

Nicotine-N-methyl-d_3 (1c) -- Reduction of N-ethoxycarbonylnornicotine (9) with lithium aluminium deuteride instead (see synthesis of nicotine-d_0 above) gave 1c: nmr ($CDCl_3$) no signal at δ 2.17 ppm for the NCH_3 group; eims m/e (%) M^+ 165 (33.8), 135 (36), 87 (100), 86 (6.7), 85 (9.0), 84 (1.6); deuterium enrichment 96% d_3 and 4% d_2.

Incubation of nicotine with lithium aluminium deuteride -- To a well stirred suspension of lithium aluminium deuteride (168 mg, 4 mmol) in 10 ml of freshly distilled tetrahydrofuran was added slowly nicotine-d_0 (648 mg, 4 mmol). The yellow mixture was stirred at room temperature for 10 days and after cooling was worked up by adding water (0.2 ml) followed by NaOH (0.2 ml) and additional water (0.6 ml). After filtering, the tetrahydrofuran was removed and water (10 ml) was added to the wet residue and the nicotine extracted with chloroform (4 x 10 ml). After drying (anhydrous sodium sulfate) the solvent was removed and the residue distilled (81^o 0.2 mm) to give 358 mg (54%) of a colorless liquid. The nmr spectrum (between 7.0-9.0 ppm) of this material in $CDCl_3$ is shown in Figure 1.

ACKNOWLEDGEMENT

Financial support from GM 16496 is gratefully acknowledged. The authors also wish to express their appreciation to Professor John C. Craig for providing use of his MS-12 gcms system.

REFERENCES

1. Gorrod J.W. and Jenner P. - The Metabolism of Tobacco Alkaloids, in "Assays in Toxicology" Vol. 6, Academic Press, 1975
2. Duffield A.M., Budzikiewicz H. and Djerassi C. - J. Am. Chem. Soc. 87: 2926 (1965)
3. Dagne E., Gruenke L. and Castagnoli, Jr. N. - J. Med. Chem. 17:1330 (1974)
4. a. Korte F. and Schulze-Steinen H.J. - Chem. Ber. 95:2444 (1962)
   b. Mundy B.P., Larson B.R., McKenzie L.F. and Braden G. - J. Org. Chem. 37:1635 (1972)
   c. Leete E., Chedekel M. and Bodem G. - J. Org. Chem. 37:4465 (1972)
   d. Stein M.L. and Burger A. - J. Am. Chem. Soc. 79:154 (1957)
   e. Hu M.W., Bondinell W.E. and Hoffman D. - J. Labelled Cpds 10:79 (1974)
5. Cheng S.S., Piantadosi C. and Irvin J.L. - J. Pharm. Sci. 57:1910 (1968)
6. a. Gaylord N.C. in Reduction with Complex Metal Hydrides, Interscience Publishers, New York 1956
   b. Girardot P.R. and Parry R.W. - J. Am. Chem. Soc. 73:2368 (1951)
   c. Jolly W. and Mesmer R.E. - J. Am. Chem. Soc. 83:4470 (1961)
7. Lansburry P.T. and Peterson J.O. - Am. Soc. 83:3537 (1961), 85:2236 (1963)
8. Varian NMR Catalog, Vol. 1, 1962, Spectrum No. 269
9. Witkop B. - J. Am. Chem. Soc. 76:5597 (1954)
10. A. Swain M., Eisner A., Woodward C. and Brice B. - J. Am. Chem. Soc. 71:1341 (1949)
    b. Eddy C.R. and Eisner A. - Anal. Chem. 26:1428 (1954)
11. Bowman E. and McKennis, Jr. H. - Biochem. Prep. 10:36 (1963)