SYNTHESIS OF NORNICOTINE-2,4,5,6-d_4 AND ITS N'-NITROSO DERIVATIVE

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SUMMARY

Nornicotine-2,4,5,6-d_4 and its N'-nitroso derivative were synthesized from pyridine-d_5. Initially, pyridine-d_5 was brominated in fuming D_2SO_4 to give 3-bromopyridine-d_4. Treatment of this material with n-butyllithium and dry ice followed by acidification gave nicotinic acid-2,4,5,6-d_4. Conversion of this substance to the acid chloride hydrochloride (via thionyl chloride) followed by treatment with absolute ethanol gave ethyl nicotinate-2,4,5,6-d_4. The ester was condensed with N-trimethylsilyl-2-pyrrolidinone in the presence of n-butyllithium and diisopropylamine to give, after hydrolysis and decarboxylation, myosmine-2,4,5,6-d_4. Reduction of this material with sodium borohydride gave nornicotine-2,4,5,6-d_4. Conversion to the N'-nitroso derivative was achieved by treatment with nitrous acid.

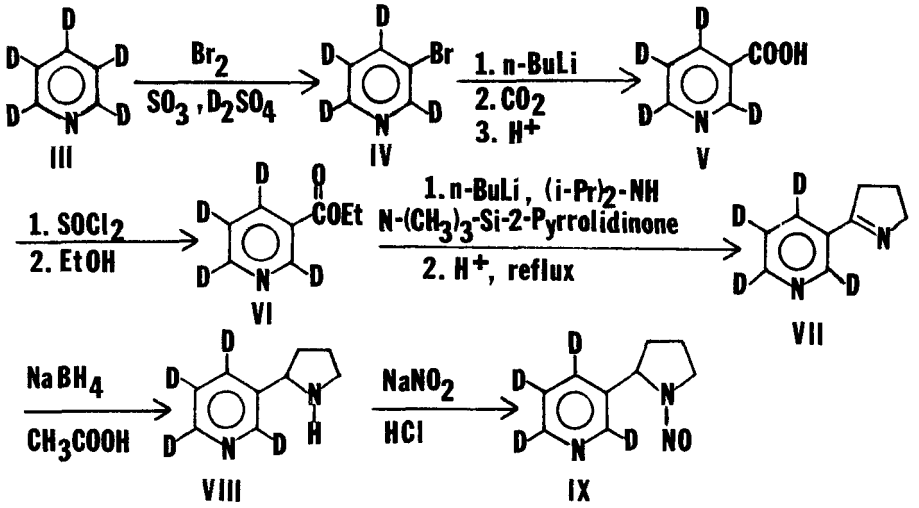
Key Words: Synthesis, 3-bromopyridine-d_5, nicotinic acid-2,4,5,6-d_4, myosmine 2,4,5,6-d_4, nornicotine-2,4,5,6-d_4, N'-nitroso-nornicotine-2,4,5,6-d_4.

INTRODUCTION

Nornicotine (I) is present in tobacco leaf (1) and may serve as a precursor for the carcinogenic N'-nitrosonornicotine (II). In order to evaluate tobacco products and tobacco smoke condensates for II it is necessary to develop a sensitive and selective analytical method for this compound. For our studies in this area we have chosen gas chromatography/mass spectroscopy (GC/MS) in the chemical ionization mode. By monitoring the signals corresponding to the parent ion (MH^+)

of II and a suitably labelled internal standard it is possible to accurately measure II. Furthermore, the use of a labelled internal standard allows us to compensate for incomplete recovery of II during the extraction procedures.

We have synthesized N'-nitrosonornicotine labelled with deuterium atoms in the 2,4,5 and 6 positions of the pyridine ring. This compound is being used as the internal standard for the mass fragmentography analysis of II in tobacco smoke and tobacco smoke condensates. The synthesis reported here involves the conversion of pyridine-d_5 (III) to 3-bromopyridine-d_4 (IV) according to the method of den Hertog et al. (2), conversion of the 3-bromopyridine-d_4 to nicotinic acid-2,4,5,6-d_4 (V) by a modification of the method of Gilman and Spatz (3), formation of the ethyl ester (VI) of V, condensation of N-trimethylsilyl-2-pyrrolidinone, to form myosmine-2,4,5,6-d_4 (VII) by the method of Hoffman, et al. (4), and reduction of the labelled myosmine to nornicotine-2,4,5,6-d_4 (VIII). The N'-nitrosonornicotine-2,4,5,6-d_4 (IX) was prepared by nitrosation of VIII with nitrous acid.



EXPERIMENTAL

1. Preparation of 3-Bromopyridine-d_4

Sulfuric acid-d_2 (14 ml) was dissolved in sulfur trioxide (γ -form) (26 ml) with cooling in an ice-water bath. Cooling was continued while pyridine-d_5

(8.2 ml, 8.05 g) was added slowly. This mixture was heated in a sealed glass tube with 1.4 ml (4.37 g) of bromine for 8 hours at 130°. The contents were poured carefully onto 125 g of cracked ice. The pH of the resulting solution was adjusted to 8.5 by dropwise addition of concentrated sodium hydroxide solution. The mixture was extracted with 6 x 200 ml of ether, the combined ether layers dried over anhydrous Na_2SO_4, and the solvent removed under a nitrogen flow at room temperature. The residue was distilled at atmospheric pressure to give 5.53 g of material boiling at 168-178° (lit. value for unlabelled 3-bromopyridine 174° at 758 mm (5). The mass spectrum showed parent ions at 161 and 163 m/e of almost the same intensity. The infrared spectrum (neat liquid on NaCl plates) showed major absorptions at 2300 cm^{-1} (C-D stretch) and at 1540, 1365, 1350, 1055, 1040, 1030, 1015, 910, 860 and 705 cm^{-1}. A 35.6% conversion of pyridine-d_5 to 3-bromopyridine-d_4 was achieved even though pyridine-d_5 was in excess. (Pyridine-d_5 was kept in excess to reduce the production of di-brominated products.)

2. Preparation of Nicotinic Acid-2,4,5,6-d_4

A solution of 1.6N n-butyllithium in hexane (35 ml) and dry ether (80 ml) was placed in a three-necked flask fitted with a mechanical stirrer, nitrogen bubbler and rubber septum. This solution was cooled to -70° (isopropyl alcohol-dry ice). A solution of 3-bromopyridine-d_4 (5.525 g in 25-30 ml of dry ether) was added *via* a syringe through the rubber septum. The solution was stirred for an additional minute, then an excess of dry ice in the form of large lumps was added directly to the reaction mixture during a five minute interval. The reaction mixture was allowed to stand at room temperature overnight while stirring was continued. The mixture was then filtered, and the precipitate was washed with ether and dissolved in water. The aqueous solution was filtered and its pH was adjusted to 3.1 with 1N hydrochloric acid. The water was then evaporated and the residue was recrystallized from methanol to give 2.565 g of nicotinic acid-2,4,5,6-d_4 (59% yield). An analytical sample recrystallized three times from absolute ethanol had m.p. 235-236° (lit. value [6] for unlabelled nicotinic acid 236-237°). An infrared spectrum (KBr wafer) showed absorptions at

2440 cm^{-1} (broad, N-H stretch), 1720 cm^{-1} (C = O stretch), 1560 and 1550 cm^{-1} (pyridine ring) and peaks at 1380, 1330, 1290, 1100, 1000, 870, 780, 765, 680, 635, 560, 500 and 390 cm^{-1}. The mass spectrum showed a parent ion at m/e 127 and a fragmentation pattern corresponding to that reported by Neeter and Nibbering for nicotinic acid-2,6-d_2 (7).

3. Preparation of Ethyl Nicotinate-2,4,5,6-d_4

Nicotinic acid-2,4,5,6-d_4 (2.13 g) in a 25 ml round bottom flask was cooled in ice water while freshly redistilled thionyl chloride (6 ml) was added dropwise over 30 minutes. The mixture was refluxed for two hours, and the excess thionyl chloride was distilled off at reduced pressure leaving an off-white solid. Dry benzene (7.0 ml) was added and the mixture was stirred for several minutes. Absolute ethanol (3.0 ml) was added dropwise over a 30 minute period. The resulting mixture was refluxed for two hours to ensure complete reaction. At the end of this time 20 ml of 20% Na_2CO_3 aqueous solution was added. The addition was carried out very slowly to avoid serious frothing. The resulting mixture was stirred for 15 minutes. The benzene layer was then separated and the aqueous portion was extracted twice with 25 ml portions of ether. The benzene layer and the ether extracts were combined and dried over anhydrous $MgSO_4$ overnight. The solvents were evaporated and the residue was distilled at 14-15 mm to give 1.879 g of ethyl nicotinate-2,4,5,6-d_4, b.p. 106-109°, in 72% yield from the free acid. The infrared spectrum (neat liquid on AgCl discs) showed the following peaks: 3000, 2955, 2920 and 2890 cm^{-1} (ethyl C-H stretch); 2300 and 2280 cm^{-1} (pyridyl C-D stretch); very intense peak near 1730 cm^{-1} (C = O stretch); 1570 (intense) and 1550 cm^{-1} (pyridyl ring stretching frequencies); other major peaks at 1470, 1450, 1400, 1390 (intense), 1345 (intense and very sharp) 1330 (very sharp), 1265 and 1240 (very intense), 1100 (very intense), 1040 (very sharp), 1015, 900, 825, 765 and 618 (very sharp), 572 cm^{-1} (intense).

4. Preparation of N-Trimethylsilyl-2-pyrrolidinone

This material, a colorless liquid with a mint-like odor, was prepared by the method of Hoffman (4). It was purified by distillation, b.p. 75-85° (5.5-6.5 mm)

(lit [8] b.p. 77-81° at 6 mm). The infrared spectrum (neat liquid on AgCl discs) was characterized by the following peaks: 2975 and 2900 (C-H stretch); 1690 cm^{-1} (very intense, C = O stretch); strong bands at 1390, 1260, 1140, 970, 860 and 775 cm^{-1}; weaker bands at 1495, 1465, 1180, 1025, 725, 645, 560, 480 and 400 cm^{-1}. The proton NMR spectrum (neat liquid plus TMS) showed a sharp singlet at δ = 0.22 (trimethylsilyl protons) and a multiplet at δ = 2.1 and a triplet at δ = 3.32 (2-pyrrolidinone ring protons); the integration of these peaks is consistent with the assigned structure.

Although apparently not previously reported, our experience with N-trimethylsilyl-2-pyrrolidinone seems to indicate that it is partially decomposed by allowing it to stand at room temperature for a few days and that it is somewhat moisture sensitive. However, it may be stored in an air-tight container below 0° for several weeks without undergoing noticeable decomposition.

5. Preparation of Myosmine-2,4,5,6-d_4

n-Butyllithium (11 ml, 1.6 N in hexane) was added to a stirred solution at -70° (dry ice-isopropyl alcohol) of diisopropylamine (3.5 ml) in anhydrous ether (30 ml) under a nitrogen atmosphere. N-trimethylsilyl-2-pyrrolidinone (3.096 g) in 10-20 ml of dry ether was added, and the reaction mixture became pale yellow. After 15 minutes of stirring at -70°, ethyl nicotinate-2,4,5,6-d_4 (1.87 g in 10-20 ml of dry ether) was added, and the solution turned bright yellow. The mixture was stirred at room temperature overnight, during which time a precipitate developed. The mixture was treated with 25 ml of water and stirred for 30 minutes. The water layer was separated, and the ether layer was extracted again with 25 ml of water. The combined aqueous layers were adjusted to pH 7 with 1N HCl. The aqueous phase was then extracted three times with chloroform, and the combined chloroform layers were dried over $MgSO_4$. The solvent was removed under a stream of nitrogen leaving a residue (3.9 g) of clear brown oil that crystallized slowly on standing. Concentrated hydrochloric acid (15 ml) was added, and the solution was refluxed overnight. After allowing the solution to cool to room temperature, it was made alkaline (pH 11) with 20% Na_2CO_3 and extracted with 5 x 40 ml portions of ether. The combined ethereal extracts were dried over anhydrous $MgSO_4$ overnight. Following filtration, the ether was

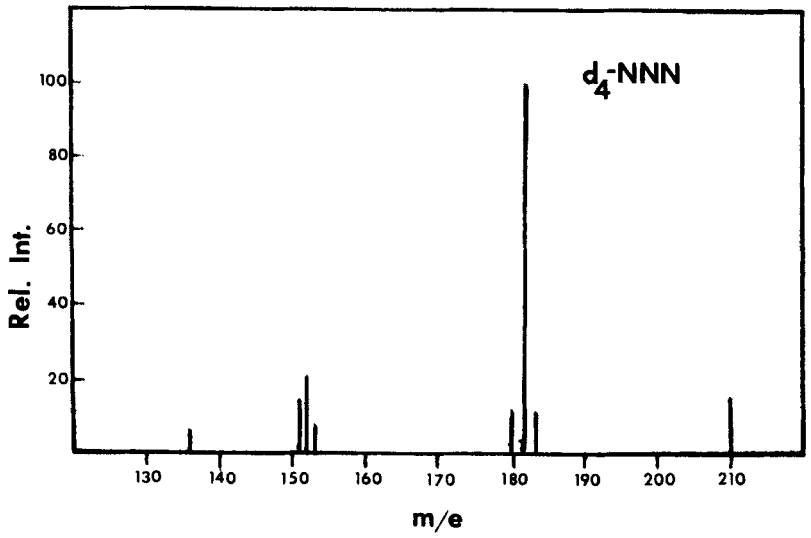
evaporated under a steam of nitrogen. The residue obtained was a low-melting solid that weighed 1.178 g (60% yield from labelled ethyl nicotinate). Unlabelled material prepared in an analogous manner was identified as myosmine by its melting point (9) and infrared spectrum (10). The crude labelled myosmine was not purified but was used directly in preparing labelled nornicotine.

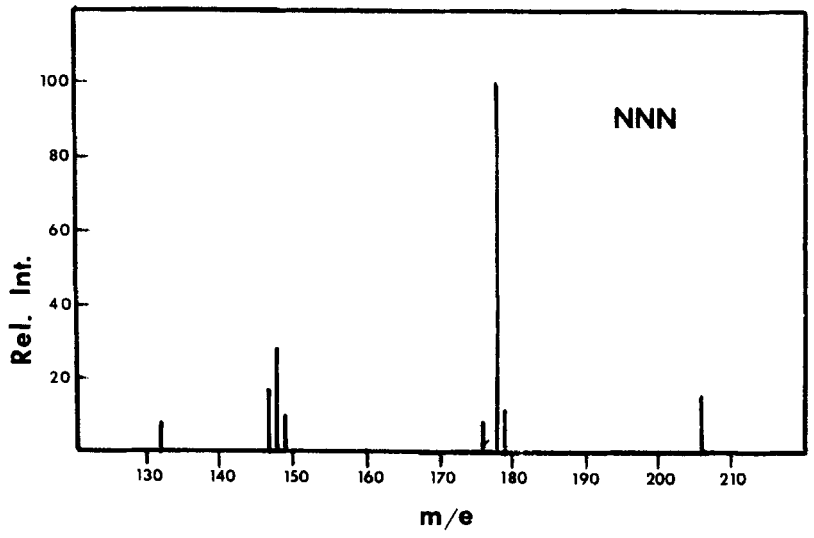
6. Preparation of d,l-Nornicotine-2,4,5,6-d_4

Sodium borohydride (0.876 g) was added to a stirred solution of myosmine-2,4,5,6-d_4 (1.1782 g) in methanol (10 ml) and glacial acetic acid (0.46 ml) at 0 to 5° over a period of two hours in a nitrogen atmosphere. (During the course of the addition much of the solvent evaporated away, and more methanol had to be added in order to maintain the volume of the solution at 10-15 ml.) Toward the end of the addition a foam had built up in the reaction mixture, and it had to be broken up with a stirring rod in order to facilitate the addition of the rest of the sodium borohydride. The mixture was stirred at room temperature for four hours, at 50° for one hour, and at room temperature overnight. It was then treated with 6 ml of water, and the total volume of the mixture was reduced to 5 ml; at this point the mixture separated into two layers. The mixture was extracted with 6 x 10 ml of chloroform, and the combined extracts were dried over anhydrous $MgSO_4$. The solvent was removed to give about 1 ml (0.90 g) of a cloudy light brown liquid. The resulting d,l-nornicotine-2,4,5,6-d_4 was purified by vacuum distillation, b.p. 131-136° at 11-12 mm (lit [11] for unlabelled nornicotine 131° at 11 mm). The yield after purification was 0.57 g (48% yield from myosmine-2,4,5,6-d_4). Two dimensional thin layer chromatography using benzene and methanol as the developing solvents indicated the product was chromatographically pure. The infrared spectrum (neat liquid on NaCl plates) showed peaks at 3290 cm^{-1} (broad, N-H stretch), 2965 and 2870 cm^{-1} (C-H stretch), 2250 cm^{-1} (C-D stretch) and peaks at 1620 (weak), 1545, 1460 (weak), 1360, 1290, 1095, 1080, 900 (weak), 885 and 840 cm^{-1}. A proton NMR spectrum (neat liquid plus TMS) gave the following information: multiplet at 1.2 - 2.2, sharp singlet at 2.50, multiplet at 2.65 - 3.15, triplet at 3.95; integration of these peaks is approximately 4:1:2:1, respectively. (No significant absorption was noted between 5 δ and 10 δ.) The pattern of peaks in the

0-5 δ region in an unlabelled sample of d,l-nornicotine in $CDCl_3$ and 1% TMS was nearly identical to that reported above. The mass spectrum showed an apparent parent peak at 152 m/e.

7. Preparation of d,l-N'-Nitrosonornicotine-2,4,5,6-d_4





A solution of sodium nitrite (0.39 g) in 2 ml of water was added dropwise over 30 minutes to a stirred solution of d,l-nornicotine (0.200 ml, 0.214 g) in 0.5 ml of concentrated hydrochloric acid and 4.5 ml of water. The resulting

mixture was allowed to stand overnight. The pH of the solution was then adjusted to 12 with an aqueous solution of 2N sodium hydroxide. The solution was then extracted with 3 x 10 ml of chloroform, and the combined extracts were dried over anhydrous Na_2SO_4. After removal of solvent the residual yellow oil was taken up in 3 ml of chloroform and chromatographed on a preparative thin layer chromatography plate (Silica Gel 60F-254, Cat. 5766 from EM Laboratories, Inc.) using chloroform/methanol, 9:1, as the developing solvent. The zone of R_f 0.40 was extracted with 2 x 10 ml of hot methanol, and the methanolic extracts were filtered and the solvent removed. Two dimensional thin layer chromatography using methanol and chloroform as the developing solvents indicated the yellow oily product was chromatographically pure. The infrared spectrum (neat liquid on NaCl plates showed the following peaks: 2980, 2950, 2890 cm^{-1} (C-H stretch); 2260 (C-D stretch); 1565 and 1550 cm^{-1} (pyridyl ring stretching frequencies); other peaks at 1450, 1420 (intense), 1300 (broad, very intense), 1200, 1030, 975, 890, 870, 840, 790, and 715 cm^{-1}.

Chemical ionization mass spectra (reagent gas-methane) of labelled and unlabelled nitrosonornicotine are shown in Figure 1. Very little fragmentation or overlap of spectra is observed.

ACKNOWLEDGEMENT

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