CHEMICAL STUDIES ON TOBACCO SMOKE
XXIII. SYNTHESIS OF CARBON-14 LABELLED MYOSMINE, NORNICOTINE AND N'-NITROSONORNICOTINE.

M.W.Hu, W.E.Bondinell and D.Hoffmann.
Division of Environmental Carcinogenesis, Naylor Dana Institute for Disease Prevention, American Health Foundation, New York, NY 10021.
Received on October 16, 1973.

SUMMARY

dl-Nornicotine-2'-^{14}C was synthesized in four steps. Nicotinic acid (carboxyl-^{14}C) was first esterified with diazomethane to yield methyl nicotinate (carboxyl-^{14}C). The ester was condensed with 3-lithio-N-trimethylsilyl-2-pyrrolidone to give 3-nicotinoyl-N-trimethylsilyl-2-pyrrolidone (ketone carbonyl-^{14}C), which was hydrolyzed and decarboxylated in concentrated hydrochloric acid to give myosmine-2'-^{14}C. Reduction of myosmine-2'^{14}C gave nornicotine-2'-^{14}C. Reaction of nornicotine-2'-^{14}C in hydrochloric acid with sodium nitrite gave N'-nitrosonornicotine-2'-^{14}C. Purification of labelled myosmine, nornicotine, and N'-nitrosonornicotine was accomplished by preparative thin layer chromatography.

INTRODUCTION

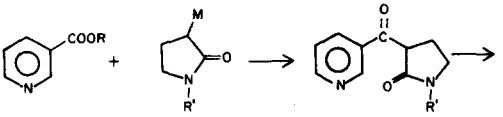
High concentrations of nornicotine (V) are present in tobacco smoke[1] where this compound may serve as a precursor of N'-nitrosonornicotine (VI), a potent carcinogen. We required labelled V and VI to facilitate chemical and analytical studies designed to evaluate the importance of these compounds as health hazards to smokers.[1]

Nornicotine (V) had been synthesized by several methods,[2-6] but the synthesis of labelled nornicotine had not been reported. We have developed a method for the preparation of ^{14}C-labelled nornicotine (V) with high specific radioactivity. We modified Korte and Schulze-Steiner's[7] method for the preparation of unlabelled nornicotine and prepared unlabelled myosmine (IV) by hydrolyzing the condensation product (IIIa) of ethyl nicotinate (Ia) and N-nicotinoyl-2-pyrrolidone (IIa). Our synthesis of ^{14}C-labelled nornicotine was also closely related to the synthesis of nicotine from ethyl nicotinate (Ia) and 3-sodio-N-methyl-2-pyrrolidone (IIc).[8] Our method utilized the condensation of methyl nicotinate (carboxyl-^{14}C) (Ib) with 3-lithio-N-trimethylsilyl-2-pyrrolidone (IIb). The resulting product, 3-nicotinoyl-N'-trimethylsilyl-2-pyrrolidone (ketone carbonyl-^{14}C) (IIIb), was hydrolyzed, decarboxylated and recyclized in concentrated hydrochloric acid to give myosmine-2'-^{14}C (IV). Reduction of myosmine-2'-^{14}C with sodium borohydride yielded nornicotine-2'-^{14}C (V). N'-nitrosonornicotine-2'-^{14}C (VI) was prepared by treating nornicotine-2'-^{14}C (V) with sodium nitrite in hydrochloric acid solution.

EXPERIMENTAL

1. Preparation of N-Trimethylsilyl-2-pyrrolidone

Trimethylsilylchloride (50 ml) was added slowly to a solution of 2-pyrrolidone (30.6 g; 0.36 mol) and triethylamine (40.2 g, 0.40 mol) in dry benzene (100 ml) stirred in a nitrogen atmosphere. The reaction mixture was allowed to



Ia, R=Et

Ib, R=Me

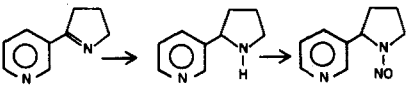
IIa, R'=  M = H

IIb, R' = $SiMe_3$ M = Li

IIc, R' = CH_3 M = Na

IIIa, R' = 

IIIb, R' = $SiMe_3$



IV V VI

stand overnight at room temperature. The solution was filtered, and the solvent was evaporated *in vacuo*. Distillation of the residual oil gave 37.6 g (66.5% yield) of N-trimethylsilyl-2-pyrrolidone, bp 65° (4.5 mm), (lit. bp 77-81°; 6 mm).[9]

2. Preparation of Methyl Nicotinate (carboxyl-^{14}C)

Nicotinic acid (carboxyl-^{14}C) (Amersham/Searle Corp.; 3.12 mg; 25 μmol; 1.73 mCi) in 10 ml methanol was mixed with unlabelled nicotinic acid (11.88 mg; 97 μmol; purified by sublimation). The solvent was evaporated to dryness (40°, 30-150 mm) and the nicotinic acid (carboxyl-^{14}C) (spec. act. 14.1 mCi/m mol) was stirred in 0.5 ml methanol and esterified with excess ethereal diazomethane (5 ml). The reaction mixture was allowed to stand at room temperature for 1 hr and

the solvent was carefully evaporated at 30° using a fractional distillation apparatus attached to a cold receiving flask (-78°) (methyl nicotinate is quite volatile and care must be exercised to prevent large losses during removal of solvent). The crude methyl nicotinate (carboxyl-^{14}C) was used directly for condensation with 3-lithio-N-trimethylsilyl-2-pyrrolidone.

3. Preparation of Myosmine-2'-^{14}C

n-Butyllithium (1 ml, 1.9 M in hexane) was added to a cold solution (-70°, Dry Ice/acetone) of diisopropylamine (202 mg; 2.0 mmol) in anhyd. ether (2ml) stirred in a nitrogen atmosphere. N-Trimethylsilyl-2-pyrrolidone (314 mg; 2.0 mmol) was then added and the solution was stirred for 15 min. at -70° and transferred quickly with a syringe to a flask containing methyl nicotinate (carboxyl-^{14}C). The mixture was stirred overnight at room temperature in a nitrogen atmosphere. Concentrated hydrochloric acid (4 ml) was added slowly to the crude 3-nicotinoyl-N-trimethylsilyl-2-pyrrolidone (ketone carbonyl-^{14}C). A slow stream of nitrogen was passed through the solution until the ether was evaporated and the solution was refluxed overnight. The reaction mixture was cooled and made alkaline with 9 ml of 20% sodium hydroxide solution. The solvent was evaporated at 40° (30-150 mm) using a fractional distillation apparatus which was attached to a cooled (-78°) receiving flask. The crude myosmine was purified by preparative thin layer chromatography (silica gel G, 0.25 mm thick, 20 x 20 cm, solvent 9:1 chloroform/methanol). The zone (R_f 0.56) on extraction with 10 ml methanol afforded myosmine-2'-^{14}C. The solution was trans-

ferred to a volumetric flask and diluted to 25 ml. The yield of myosmine-2'-^{14}C (4.35 mg; 29.8 μmol; 420 μCi; spec. activity 14.1 mCi/mmol) 24.4% yield from nicotinic acid (carboxyl-^{14}C) was determined by uv. The purity of myosmine-2'-^{14}C was determined by glc (5% UC-98 on Chromosorb W, 80/100 mesh, 160°, flow rate 40 ml/min. of helium; retention time 5.2 min.) and was found to be >99.9%.

4. Preparation of Nornicotine-2'-^{14}C

A solution of myosmine-2'-^{14}C (3.48 mg; 23.8 μmol; 335 μCi) in methanol (20 ml) was concentrated at 40° (30-150 mm) to 4 ml using a fractional distillation apparatus as described above and then mixed with 0.15 ml of glacial acetic acid. Sodium borohydride (300 mg; 12 mmol) was added slowly at 0 - 5° during a period of 30 min. to the solution stirred in a nitrogen atmosphere. The mixture was stirred at room temperature for 2 hrs and at 50° for 1 hr. The reaction mixture was allowed to stand at room temperature overnight. The excess sodium borohydride was decomposed by adding 2 ml of water and the mixture was concentrated to 2 ml. The aqueous solution was extracted with 4 x 8 ml of chloroform. The chloroform extracts were combined and evaporated (40°; 30 - 150 mm) to 1 ml using a fractional distillation apparatus. The crude nornicotine was chromatographed on a preparative thin layer plate (silica gel G, solvent $CHCl_3$/MeOH/conc. NHa_4OH, 85:15:2). The zone (R_f 0.48) was extracted with 3 ml methanol and diluted to 5.0 ml in a volumetric flask. The yield (2.62 mg; 17.7 μmol; 246 μCi; 74% from myosmine; spec. activity 14.0 mCi/mmol) was determined by

uv. The purity of nornicotine-2'-^{14}C was determined by glc (5% UC-98 on Chromosorb W, 80/100 mesh, 150°, helium flow rate 40 ml.min., retention time 8.75 min.) and was found to be >99.8%.

5. Preparation of N'-nitrosonornicotine-2'-^{14}C

A solution of sodium nitrite (400 mg; 5.8 mmol) in 0.5 ml water was added dropwise over 30 min. to a stirred solution of nornicotine-2'-^{14}C (0.52 mg; 3.5 μmol; 48.9 μCi) and hydrochloric acid (10%, 3 ml). The reaction mixture was allowed to stand overnight at room temperature and the solution was made alkaline with dilute sodium hydroxide and extracted with 2 x 10 ml of chloroform. The chloroform extracts were concentrated to 1 ml at 40° (30 - 150 mm) using a fractional distillation apparatus. The crude N'-nitrosonornicotine-2'-^{14}C was chromatographed on preparative thin layer plates (silica gel G, 0.25 mm thick, 20 x 20 cm, eluted with 9:1 chloroform/methanol). The zone (R_f 0.60) was extracted with 3 ml methanol and diluted to 4 ml in a volumetric flask. The yield (0.59 mg; 3.3 μmol; 46.7 μCi; 95% yield from nornicotine-2'-^{14}C; spec. activity 14.1 mCi/mmol) was determined by uv. The purity of N'-nitrosonornicotine-2'-^{14}C was determined by glc (5% UC-98 on Chromosorb W, 80/100 mesh, at 160°, helium flow rate 40 ml/min., retention time 13.4 min.) was found to be >99%.

6. Preparation of 3-Nicotinoyl-2-pyrrolidone

n-Butyllithium (79 ml; 1.9 M in hexane) was added to a cold solution (-70°, Dry Ice/acetone) of diisopropylamine

(20.2 g; 0.2 mol) in anhydrous ether (200 ml) stirred in a nitrogen atmosphere. N-Trimethylsilyl-2-pyrrolidone (25 g; 0.16 mol) was added and the solution was stirred for 15 min. at -70°. The ethyl nicotinate (15.1 g; 0.1 mol) was added. The reaction was stirred at room temperature overnight for 18 hrs, water (20 ml) was added, and the ether layer was removed and discarded. The aqueous solution was neutralized to pH 7 with 10% hydrochloric acid and was extracted with chloroform. Removal of solvent gave a gummy solid (21.67 g) which was used directly to prepare myosmine without further purification. A small amount of solid (2 g) was recrystallized from ether-ethanol to give colorless crystals (mp 102-106°); the infrared spectrum showed absorptions at 1679 cm^{-1} (amide, C=O), and 1723 cm^{-1} (C=O, ketone); tlc, R_f 0.36 (silica gel G, $CHCl_3$/MeOH/NH_4OH, 9.5:0.5:0.1, uv detection), nmr ($CDCl_3$: 2.0 -3.7 ppm (m, 4H), 4.4 ppm (q, 1H), 7.4 ppm (m, 2H), 8.4 ppm (d, 1H), 8.8 ppm (d, 1H), and 9.3 ppm (s, 1H), mass spectrum (70 eV) m/e (rel. intensity):190 (34), 162 (20), 106 (100), 84 (37.5), and 78 (86).

Anal. Calcd. for $C_{10}H_{10}N_2O_2$: C, 63.16; H, 5.26; N, 14.74; Found: C, 63.05; H, 5.48; N, 14.65

7. Preparation of Myosmine

Crude 3-nicotinoyl-2-pyrrolidone (18.06 g) was heated under reflux in 100 ml of conc. hydrochloric acid overnight. The crude product, isolated in a manner similar to that described for myosmine-2'-^{14}C, was distilled to give pure myosmine (9.75 g; 80.1% yield from ethyl nicotinate, bp 84°; 0.25 mm, mp 41.5 - 43°; reported mp 44° [7]); the infrared

spectrum (neat liquid) showed absorption at 1620 cm^{-1} (imine); tlc, R_f 0.56 (silica gel G, $CHCl_3$/MeOH, 9:1); glc, rt 5.2 min (5% UC-98 on Chromosorb W, 80/100 mesh, 160°, helium flow rate 40 ml/min.); uv λ max. (ε): 233 (10,599), 266 (3,580) in MeOH [reported λ max. (ε): 234 (11,360), 266 (3,890), in 95% ethanol [10]]; nmr ($CDCl_3$): 2.05 ppm (m, 2H), 2.95 ppm (m, 2H), 4.08 ppm (m, 2H), 7.32 ppm (q. 1H), 8.18 ppm (d, 1H), 8.68 ppm (d, splitting 1H), and 9.00 ppm (s, splitting 1H); mass spectrum (70 eV) m/e (rel. intensity): 146 (70), 145 (56), 118 (100), 105 (25), 78 (22).

Anal. Calcd. for $C_9H_{10}N_2$: C, 73.97; H, 6.85; N, 19.18; Found: C, 73.99; H, 6.94; N, 19.16

The picrate, recrystallized from 50% aqueous ethanol gave yellow crystals, mp 184 - 187° (reported mp 184 - 185 [7]).

8. Preparation of Nornicotine

Sodium borohydride (3.79 g; 0.1 mol) was added to a stirred solution of myosmine (34.9 mmol; 5.1 g) in methanol (35 ml) and glacial acetic acid (2.0 ml) at 0 - 5° over a period of 30 min. in a nitrogen atmosphere. The mixture was stirred at room temperature for 2 hrs and at 50° for 1 hr. After work up, as described for nornicotine-2'-^{14}C, the crude oil was distilled to give nornicotine (4.38 g; 85.7% yield; bp 106 - 107°; 0.25 mm): tlc, R_f 0.48 (silica gel G, $CHCl_3$/MeOH/conc. NH_4OH, 85:15:2), glc, rt 8.75 min (5% UV-98 on Chromosorb W, 80/100 mesh, 150°, flow rate of helium 40 ml/min.); uv, λ max. (ε), 262 (3,100), MeOH, [reported [10]

λ max. (ε), 262 (2,900), 95% ethanol]. The IR, NMR, and mass spectra were identical to published data. The picrate (mp 182 - 186°, reported mp 186° [3]) recrystallized from ethanol gave satisfactory elemental analysis.

Anal. Calcd. for $C_{12}H_{18}N_8O_{14}$, C, 41.58; H, 2.97; N, 18.48; Found: C, 41.71; H, 3.00; N, 18.33

9. Preparation of N'-Nitrosonornicotine

A mixture of sodium nitrite (7.7 g; 0.112 mol) in 7 ml of water was added dropwise over 30 min. to a stirred solution of nornicotine (4.0 g; 9.927 mol) and hydrochloric acid (57.7 ml; 10%). The reaction mixture was allowed to stand at room temperature overnight, and the product was isolated as described for N'-nitrosonornicotine-2'-^{14}C. After work up, the crude oil was distilled to give pure N'-nitrosonornicotine (yellow oil, bp 154°, 0.2 mm, 4.46 g, 93% yield): the infrared spectrum contained no bands in the OH and NH region; tlc, R_f 0.60 (silica gel G, 9:1 $CHCl_3$/MeOH); glc, rt 13.4 min. (5% UC-98 on Chromosorb W, 80/100 mesh, 160°, helium flow rate 40 ml/min.); uv λ max. (ε) 237 (7,580), 357 (80), 261 (sh) (4,431), and 269 (sh) (2,918) in MeOH; nmr ($CDCl_3$): 2.10 ppm (m, 4H), 3.76 ppm (5' protons, E form, m, 1.24H), 4.55 ppm (5' protons, E form, m, 0.76H), 5.25 ppm (2' protons, E form, 0.31H), 5.69 ppm (2' protons, Z form, 0.68),[11,12] 7.10 - 7.66 ppm (m, 2H), 7.90 - 8.25 ppm (m, 2H); mass spectrum (70 eV) m/e (rel. intensity), 177 (100), 147 (58), 118 (46), 105 (82), 92 (32), and 78 (44).

Anal. Calcd. for $C_9H_{11}N_2O$: C, 61.02; H, 6.21; N, 23.73; Found: C, 60.78; H, 6.37; N, 23.67.

N'-Nitrosonornicotine methiodide, prepared by refluxing nitrosonornicotine (500 mg) and methyl iodide (0.5 ml) in methanol (0.5 ml) for two hrs, was recrystallized from ethanol to give yellow crystals, mp 140 - 144° (reported mp 144°)[13].

Anal. Calcd. for $C_{10}H_{14}N_3OI$: C, 37.63; H. 4.39; N, 13.17; Found: C, 37.78; H, 4.43; N, 13.11.

REFERENCES

1. Wynder, E.L. and Hoffmann, D. - "Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis" Academic Press, Inc., New York, 1968, p. 377

2. Craig, L.C. - J. Am. Chem. Soc., 55 : 2854 (1933)

3. Zymalkowski, F. and Trenktroy, B. - Arch. Pharm., 292 : 9 (1959)

4. Hellmann, H. and Dietrich, D. - Liebigs Ann. Chem., 672 : 98 (1962)

5. Mizoguchi, T., - Chem. Pharm. Bull., 9 : 818 (1961)

6. Leete, E., Chedekel. M.R., and Boden, G.B. - J. Org. Chem., 37 : 4465 (1972)

7. Korte, F. and Schulze-Steiner, H.J. - Chem. Ber., 95 : 2444 (1962)

8. Decker, K. and Sammeek, R., - Biochem. Zeitschrift, 340 : 326 (1964)

9. DeBenneville, P.L. and Hurwitz, M.J. - Chem. Abstr., 53 : 12321 (1959)

10. Swain, M.L., Eisner, A., Woodward, C.F. and Bruce, B.A. - J. Am. Chem. Soc., 71 : 1341 (1949)

11. Liberek, B., Ciarkowski, J., Steporowska, K., Stachowiak, K. and Jereczek, E., - Roczniki Chemi; Ann. Soc. Chim. Polonium, 46 : 1457 and 1895 (1972)

12. Lijinski, W., Keefer, L. and Leo, J. - Tetrahedron, 26 : 5137 (1970)

13. Braun, J.v., and Weissbach, K. - Ber. 63B : 2018 (1930) [C.A. 25, 300 (1931)]