SYNTHESIS OF 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE, 4-(CARBETHOXYNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE, AND N'-NITROSONORNICOTINE LABELLED WITH TRITIUM IN THE PYRIDINE RING.

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SUMMARY

The carcinogenic tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN), as well as the model compound 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone, were synthesized with tritium at the 5-position of the pyridine ring. In each case, the tritium labelled compound was prepared by catalytic tritium replacement of the corresponding brominated precursor.

Key Words: Tritium labeled tobacco-specific nitrosamines; NNK; NNN

INTRODUCTION

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) are carcinogenic nitrosamines that occur in tobacco and tobacco smoke (1). They form during the processing of tobacco or during smoking by nitrosation of the major tobacco alkaloid nicotine. These tobacco-specific nitrosamines cause tumors in the respiratory and digestive tracts of mice, rats, and hamsters (1). Their tumorigenic properties are believed to be due at least in part to reactions with DNA of electrophilic diazohydroxide metabolites that are formed by enzymatic hydroxylation of the carbons adjacent to the N-nitroso group. This process is illustrated in Scheme 1 for methyl hydroxylation of NNK and 2'-hydroxylation of NNN; the common intermediate formed is 4-(3-pyridyl)-4-oxobutyldiazohydroxide, 4.

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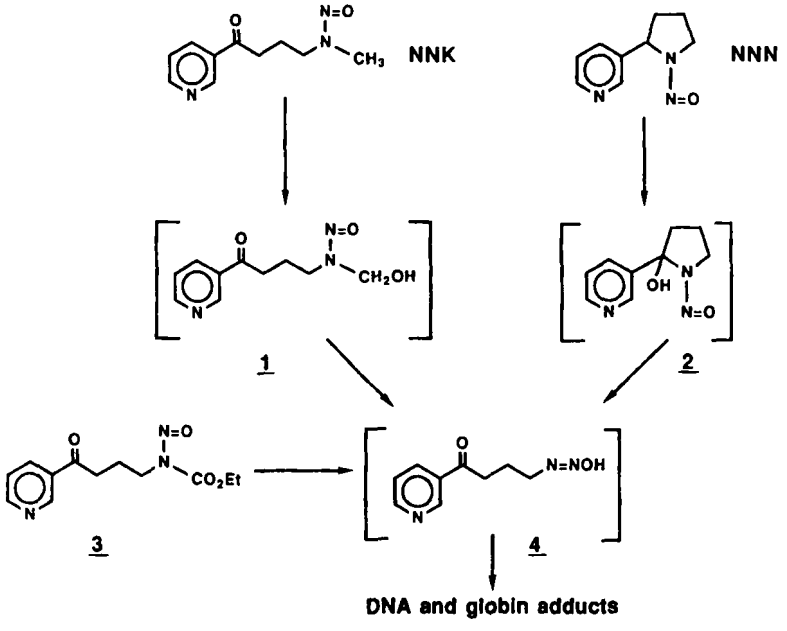
This intermediate as well as the α-hydroxy compounds 1 and 2 are unstable and have not been synthesized. However, 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone, 3, is a stable precursor to 4 (2,3). In order to investigate the interactions of NNK, NNN, and 3 with DNA and protein, the tritium labelled compounds were required. Metabolic studies of NNK and NNN indicate that hydroxylation of the pyridine ring is not a major process (4). Thus, to avoid exchange in vivo, tritium was specifically incorporated at the 5-position of the pyridine ring.

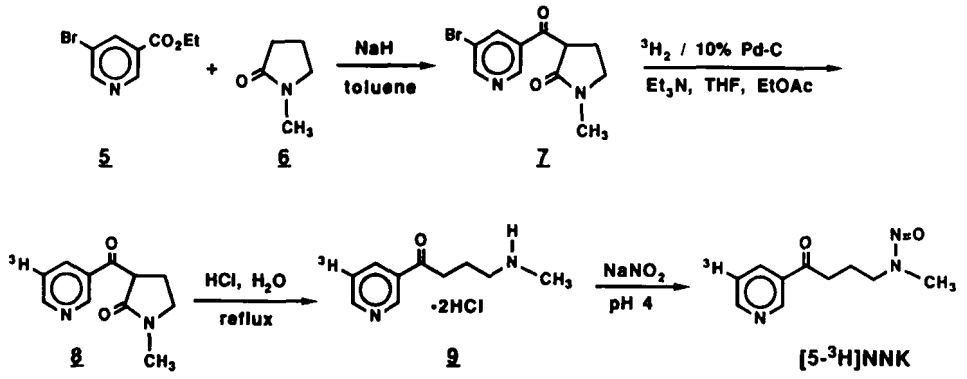


Scheme 1

RESULTS AND DISCUSSION

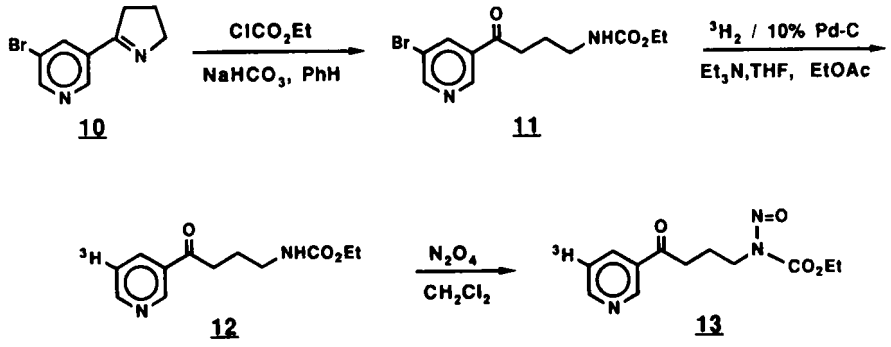
The synthesis of [5-^{3}H]NNK is illustrated in Scheme 2. The method is based on that used for unlabelled NNK (5,6). Ethyl 5-bromonicotinate, 5, was reacted with N-methylpyrrolidinone, 6, to give the intermediate condensation product 7. Tritiation of 7 by

catalytic tritium-bromine replacement, using a Toepler pump for tritium transfer, provided 8 in 90% yield. This material was hydrolyzed under acidic conditions to give the labelled dihydrochloride 9 in 96% yield. The tritium-labelled dihydrochloride, 9, was nitrosated to provide [5-^{3}H]NNK in 37% yield. Careful control of pH was necessary to avoid formation of an oxime α- to the carbonyl group.



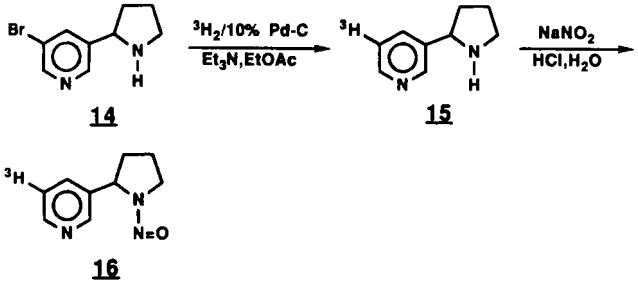
Scheme 2

The synthesis of 4-(carbethoxynitrosamino)-1-([5-^{3}H]3-pyridyl)-1-butanone, 13, is outlined in Scheme 3. 5-Bromomyosmine, 10, was converted to the bromocarbamate 11 under conditions similar to those used for preparation of the unbrominated carbamate (2). The bromocarbamate 11 was tritiated by catalytic replacement of bromine with tritium to give 12 in 66% radiochemical yield. The tritiated carbamate 12 was nitrosated using nitrogen tetroxide to provide 4-(carbethoxynitrosamino)-1-([5-^{3}H]3-pyridyl)-1-butanone, 13, in 39% radiochemical yield. This material proved to be unstable at a specific activity of 3 Ci/mmol and it was therefore diluted to a specific activity of 821 mCi/mmol. After storage at -20°C for 2 months, the radiochemical purity had decreased to 60%.



Scheme 3

The starting material for the synthesis of [5-^{3}H]NNN was 5-bromonornicotine, prepared as described previously (7,8). It was converted to [5-^{3}H]nornicotine, 15, by tritium-bromine replacement, in a manner analogous to that employed for the synthesis of [5-^{3}H]NNK. Nitrosation as described (9) gave [5-^{3}H]NNN, 16, in an overall radiochemical yield of 12%. The synthesis route is shown in Scheme 4.



Scheme 4

EXPERIMENTAL SECTION

NMR spectra were recorded on a JEOL Model FX90Q spectrometer. Thin-layer chromatography (TLC) was carried out on silica gel 60 LK 6F plates (Whatman). Radiochemical purity was determined with a Bioscan BID 100 System. UV-VIS spectra were recorded on a IBM 9430 UV-VIS Spectrophotometer. Scintillation counting was performed with a Beckman LS 3801. Tritium gas was purchased from Amersham International (England).

3-(5-Bromo-3-pyridoyl)-N-methylpyrrolidin-2-one 7

A solution of ethyl-5-bromonicotinate (5, 2.0 g, 8.69 mmol) (7,8) and N-methylpyrrolidinone (6, 0.86 g, 8.70 mmol) in 11 ml of toluene was added dropwise to a stirred suspension of NaH (315 mg, 13.1 mmol) in 4.5 ml of dry toluene. The mixture was heated under reflux for 6 h and then cooled and acidified with 15 ml of 2N HCl. After the pH was adjusted to 4.3 with 10% NaOH, the organic layer was separated and the aqueous phase was extracted with $CHCl_3$ (6x50 ml). The organic layers were combined and dried (Na_2SO_4). Evaporation provided 2.3 g of a light brown solid. This solid was purified by chromatography on a 2.5 x 5.5 cm column of silica gel 60 eluted with CH_2Cl_2/CH_3CN (7:1) to give 7, 1.4 g, 4.94 mmol, 57%, mp 94-97° , TLC, silica gel 60, benzene/dioxane/acetic acid (90:25:4), Rf=0.30 (one spot).

3-(3-[5-^{3}H]Pyridoyl)-N-methylpyrrolidin-2-one 8

A mixture of the bromo derivative 7 (43 mg, 0.15 mmol), 0.5 ml of tetrahydrofuran, 0.5 ml of ethyl acetate, 0.065 ml of triethylamine, 5 mg of 10% Pd/C and tritium gas (10 Ci, 0.175 mmol) was stirred at room temperature overnight. The resulting mixture was then stirred under 1 atm of hydrogen gas for another 4 h. The mixture was filtered, diluted with 710 mg of 3-(3-pyridoyl)-N-methylpyrrolidin-2-one, and purified by chromatography, on a 2.5 x 28 cm silica gel column with elution by CH_2Cl_2 /methanol (15:1) to provide 8, 649 mg, 3.18 mmol, 3.7 Ci, as a yellow oil. TLC, silica gel 60, CH_2Cl_2/methanol (15:1), one spot, Rf=0.40.

4-(Methylamino)-1-(3-[5-^{3}H]pyridyl)-1-butanone dihydrochloride 9

Concentrated HCl (5.0 ml, 60 mmol) was added to 3-(3-[5-^{3}H]-pyridoyl)-N-methylpyrrolidin-2-one (8, 649 mg, 3.7 Ci, 3.18 mmol) and the resulting solution was stirred for 120 h at 95° . The solution was then cooled in an ice-bath, adjusted to pH 12 with a 20% NaOH solution and quickly extracted with CH_2Cl_2 (4x20

ml). The CH_2Cl_2 extracts were immediately washed with 2x20 ml portions of 2N HCl. The aqueous layers were combined and evaporated under vacuum to give 9, 767 mg, 3.05 mmol, 96%, 2.5 Ci, mp 196-198°, lit[10] mp 196-198° , TLC, silica gel 60, ethyl acetate/methanol/ammonium hydroxide (65:35:11), Rf=0.70. Purity was 98% by radio-TLC.

4-(Methylnitrosamino)-1-(3-[5-^{3}H]pyridyl)-1-butanone ([5-^{3}H]NNK)

A solution of 383 mg, 1.52 mmol, 1.25 Ci of 9 in 4 ml of H_2O at 0° was adjusted to pH 4 with 10% NaOH solution. To this solution was added dropwise a solution of 190.2 mg, 2.8 mmol, of $NaNO_2$ in 1.0 ml of H_2O, while maintaining a pH of 4. The mixture was stirred at room temperature for 17 h and then extracted with 4x50 ml portions of CH_2Cl_2. The organic layers were combined and washed with 10% NaOH (2x20 ml) and H_2O (2x20 ml), dried over Na_2SO_4 and concentrated to give 181 mg (700 mCi) of a crude brown oil. The crude product was purified by chromatography on a 2.5x22 cm silica gel column, eluted with CH_2Cl_2/methanol (15:1). The eluate was evaporated to give 143 mg of an oil which was crystallized from CH_2Cl_2/Et_2O to give 118 mg, 0.57 mmol, 38%, 667 mCi (specific activity 1190 mCi/mmol) of [5-^{3}H]NNK as white crystals, mp 65-66° , lit[5] mp 63-65° , TLC, silica gel 60, CH_2Cl_2/methanol (15:1), Rf=0.40, CH_2Cl_2/acetonitrile (5:2), Rf=0.28, acetonitrile, Rf=0.40. The [5-^{3}H]NNK was >98% pure by chemical and radio-TLC and by UV in comparison with the unlabelled standard.

4-(Carbethoxyamino)-1-(5-bromo-3-pyridyl)-1-butanone 11

5-Bromomyosmine (10, 511 mg, 2.3 mmol, 7) was added to 5 ml of benzene and 5 ml of pH 5 buffer containing 0.5 g of $NaHCO_3$, at 0°. Ethyl chloroformate (0.5 ml) was added dropwise to this mixture at -10° . The mixture was stirred at 0° for 4 h, and another 0.5 ml portion of ethyl chloroformate was added. The pH was adjusted to 7 with 0.1 N HCl. After overnight stirring at

0° , the mixture was basified and extracted 4 times with CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried, and concentrated to a residue which showed several spots upon analysis by silica gel TLC. Silica gel chromatography with elution by CH_2Cl_2 and 0.5% CH_3OH in CH_2Cl_2 gave 85 mg, 0.32 mmol, 14%, of 4-(carbethoxyamino)-1-(5-bromo-3-pyridyl)-1-butanone, 11, mp 89-90° , R_f=0.69, silica gel, $CHCl_3$/methanol (15:1). NMR ($CDCl_3$) δ 1.20 (t, 3, CH_3), 2.0 (q, 2, $CH_2\underline{CH_2}CH_2$), 3.10 (t, 2, $\underline{CH_2}$-C=O), 3.30 (q, 2, $\underline{CH_2}$-NH), 4.10 (q, 2, $\underline{CH_2}CH_3$), 5.35 (bt, 1, NH), 8.35 (m, 1, pyr-4H), 8.81 (bs, 1, pyr-2H), 9.07 (bs, 1, pyr-6H); MS (m/e, rel. intensity) 316 (M^+, 0.6), 271 (3.4), 269 (3.5), 227 (48), 225 (50), 202 (98), 200 (100), 186 (48), 184 (48), 158 (62), 156 (60), 115 (60), 102 (32); High resolution MS, calcd for $C_{12}H_{15}N_2O_3Br$ 314.02655 found, 314.02660.

4-(Carbethoxyamino)-1-(3-[5-^{3}H]pyridyl)-1-butanone 12

A mixture of the bromo derivative 11 (54 mg, 0.17 mmol), 3.2 ml of tetrahydrofuran, 3.2 ml of ethyl acetate, 0.10 ml of triethylamine, 6 mg of 10% Pd/C and tritium gas (10 Ci, 0.175 mmol) was stirred at room temperature overnight. The resulting mixture was then stirred under 1 atm of hydrogen gas for an additional 4 h. Labile tritium was removed by washing the reaction mixture with 2x2 ml of absolute ethanol. The reaction mixture was filtered, and the filtrate was assayed for 12 by UV; it contained 0.16 mmol, 3.3 Ci, specific activity 20.6 Ci/mmol. Analysis by TLC and radio-TLC using silica gel 60 and CH_2Cl_2/methanol (15:1), gave Rf=0.48 and purity of 96%.

4-(Carbethoxynitrosamino)-1-(3-[5-^{3}H]pyridyl)-1-butanone 13

Sodium bicarbonate (35.3 mg) was added to a solution of 221 mCi, 2.6 mg, 0.011 mmol of 12 and 49.5 mg, 0.21 mmol of unlabeled 4-(carbethoxyamino)-1-(3-pyridyl)-1-butanone in 2.2 ml of CH_2Cl_2. The mixture, under argon, was cooled to -30° in a dry ice-acetone bath. A stock solution of N_2O_4 (10 mg/ml in CH_2Cl_2)

was added until the reaction mixture turned light green. The disappearance of starting material was monitored by TLC, silica gel, $CHCl_3$/acetonitrile (2:1). After 30 min the reaction mixture had turned light yellow. TLC showed that 25% of the starting material remained. More of the N_2O_4 stock solution was added until the reaction mixture again turned green. After stirring for 1 h at -30°, TLC showed that no starting material was present. The reaction mixture was quenched with 3.5 ml of 10% $NaHCO_3$ and stirred an additional 10 min. The organic and H_2O layers were separated and the H_2O layer was extracted with CH_2Cl_2. The CH_2Cl_2 layers were combined and dried over Na_2SO_4. TLC and radioassay showed that the CH_2Cl_2 layer contained 186 mCi of 86% pure 13.

A 96 mCi portion of crude 13 was purified by chromatography on a 3.5 x 46 cm column of silica gel 60 eluted with CH_2Cl_2/CH_3CN (2:1). This treatment provided 13, 14.3 mg, 0.054 mmol, 44 mCi, 39% radiochemical yield, specific activity 821 mCi/mmol (UV at 266 nm). It was 98% pure by TLC on silica gel 60, $CHCl_3$/acetonitrile (2:1), Rf=0.36; ethyl acetate/CH_2Cl_2 (2:1), Rf=0.30; CH_2Cl_2/methanol (15:1), Rf=0.57.

[5-^{3}H] Nornicotine 15

A mixture of 5-bromonornicotine (7,8) (18mg, 0.08 mmole), triethylamine (23 mg, 0.23 mmole) and 10% Pd/C (4 mg, Alfa) in 0.5 ml of ethyl acetate was stirred under 5 Ci (0.09 mmole) of tritium gas. Uptake was monitored manometrically and was approximately 50% complete after 30 min. After 12 h, H_2 was introduced at 1 atm and the mixture was stirred for 3.0 h. Solvent and labile tritium were removed by codistillation with 2X2 ml of ethanol under reduced pressure. The residue was dissolved in ethyl acetate, filtered through Celite, and the solvent evaporated to give 15, 1.3 Ci, 52%, as a yellow oil. The oil was used in the next step without further purification.

[5-^{3}H]N'-Nitrosonornicotine 16

A solution of $NaNO_2$ (35 mg, 0.5 mmole) in 0.2 ml of H_2O was added dropwise over 15 min to a stirred solution of 1.3 Ci of 15 in H_2O. Unlabelled nornicotine (5mg) and 1.5 ml of 3N HCl were then added to the reaction mixture. The mixture was allowed to stand at room temperature overnight, then made alkaline with dilute KOH solution and extracted with 3x10 ml of CH_2Cl_2. The crude [5-^{3}H]N'-nitrosonornicotine (1.1 Ci) was purified by chromatography on a silica gel column using CH_2Cl_2 and 2% methanol in CH_2Cl_2 to elute 15, 11 mg, 0.062 mmol, 46%, 610 mCi (specific activity 9.8 Ci/mmol), TLC silica gel 60, CH_2Cl_2/CH_3CN 5:2, R_f=0.48 and CH_2Cl_2/methanol 20:1, R_f=0.52. It was 97% pure (chemical and radiochemical) by UV and radio-TLC in comparison with unlabelled standard.

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