SYNTHESIS OF CARBON-14 LABELED 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE[1]

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SUMMARY

The potent carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is present in tobacco and tobacco smoke. [Carbonyl^{14}C]NNK (6) was synthesized in 27% overall yield. [Carboxyl-^{14}C]nicotinic acid (1) was esterified with benzyl alcohol and the ester 2 was alkylated by 3-lithio-N-methylpyrrolidin-2-one (3). The resulting keto-lactam 4 was hydrolyzed and decarboxylated by treatment with boiling hydrochloric acid. Nitrosation at pH 4.0 gave [carbonyl-^{14}C]NNK (6). Carbonyl reduction of [carbonyl-^{14}C]NNK with either sodium borohydride or cultured rat liver slices gave [carbinol-^{14}C]4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol-(7).

Key Words: Carbon-14 labeled tobacco-specific N-nitrosamines

INTRODUCTION

The potent carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is present in mainstream and sidestream cigarette smoke, in chewing tobacco and in snuff tobacco (1,2). It is formed by nitrosation of nicotine during curing and burning of tobacco (3). Administration of NNK to A/J mice, F344 rats or Syrian golden hamsters resulted in high incidences of tumors (4,5,6). In F344 rats or Syrian golden hamsters, hydroxylation of the carbon α to the N-nitroso group is a major metabolic pathway which leads to the formation of DNA alkylating species (6,7). Enzyme-mediated reduction of the NNK carbonyl group gives the alcohol NNAl. This metabolic pathway was observed very early during fetal life of C57Bl

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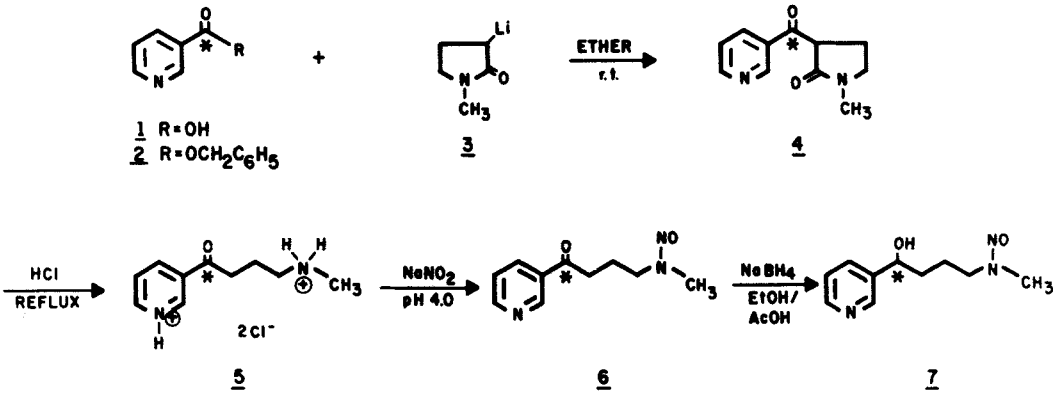
mice (8). It is also a major metabolic pathway of NNK in cultured human tissues (9). We now report the syntheses of carbon-14 labeled NNK and NNAl which were used in various metabolic studies (4,6,8,9,10).

RESULTS AND DISCUSSION

A previous synthesis of unlabeled NNK began with the alkylation of methyl nicotinate by the anion of N-methylpyrrolidin-2-one (11). In the present study, the volatile methyl nicotinate was replaced by the less volatile benzyl nicotinate (2). The alkylation of 2 by the lactam 3, the hydrolysis of the resulting keto lactam 4 and the nitrosation of the amino ketone 5 were carried out without the isolation of intermediates. Thus the present synthesis involves the purification of only one intermediate: [carbonyl-^{14}C] benzyl nicotinate (2). [Carbonyl-^{14}C]NNK (6) was obtained with an overall yield of 27% from commercially available [carboxyl-^{14}C] nicotinic acid (1). Sodium borohydride reduction of 6 gave the N-nitroso alcohol 7 in 95% yield.



EXPERIMENTAL SECTION

[Carbonyl-^{14}C]benzyl nicotinate (2)

[Carboxyl-^{14}C]nicotinic acid (1) (3 mCi, 4.2 mCi/mmol) was obtained from California Bionuclear Corporation (Sun Valley,

Calif.) and was suspended in 3 ml of dry CH_2Cl_2 at room temperature. Chlorodiethylphosphate (364 mg, 2.1 mmol) was added in small portions followed by freshly distilled triethylamine (425 mg, 4.2 mmol). The mixture became clear before turning cloudy. The mixture was stirred for 30 min. After the addition of distilled benzyl alcohol (164 mg, 1.5 mmol), the mixture was stirred at room temperature overnight. About 2 ml of solvent was evaporated with a stream of nitrogen and the residue was chromatographed by TLC on silica gel using hexane:ethyl acetate, 1:1. The labeled benzyl nicotinate (2) was identical in R_f=0.4 (silica gel, methylene chloride: ethyl acetate 10:1) to the unlabeled compound obtained as a colorless oil by the same method. The infrared spectrum showed absorption at 1720 cm^{-1} (carbonyl), nmr ($CDCl_3$): 5.3 ppm (s, 2H, CH_2), 7.3 (m, C_6H_5 + 5-pyH, 6H), 8.2 (dt, 4-pyH, 1H), 8.6 (m, 6-pyH, 1H), 9.2 (m, 2-pyH, 1H), mass spectrum (70 eV) m/e (rel. intensity) 213 (34), 106 (100), 91 (90), 78 (25).

Anal. Calcd for $C_{13}H_{11}NO_2$: C, 73.22; H, 5.20; N, 6.57

Found C, 73.45; H, 5.24; N, 6.51

Litt. (12) b.p. 188-189° at 12 mm pressure.

[Carbonyl-^{14}C]NNK (6)

n-Butyllithium/hexane (4.1 ml, 9.8 mmol) was added dropwise to a stirred solution of freshly distilled N-methylpyrrolidin-2-one (1.2 g, 12 mmol) in dry ether (5 ml) at -78°. A 1.3 ml portion of this solution was transferred to a cold (-78°) solution of [carbonyl-^{14}C]benzyl nicotinate in dry ether (5 ml) via a syringe. The reaction mixture was allowed to warm slowly to room temperature and stirred overnight. The lithium salt of the keto lactam was precipitated with hexane (5 ml). The white solid was collected by fil-

tration and dissolved in concentrated HCl (5 ml). The mixture was heated under reflux for 2 days, and then was cooled to 0° and neutralized with 10N NaOH. Sodium nitrite (1 g, 11.8 mmol) was added in small portions and the pH was readjusted to 4.0. The reaction mixture was stirred at room temperature overnight. After adjusting the pH to 9.0, the reaction products were extracted 5 times with 3 ml portions of CH_2Cl_2. The combined extracts were dried over $MgSO_4$ and concentrated under N_2 and the residue was purified by TLC on silica gel (CH_2Cl_2:CH_3OH 20:1, 2 migrations).

A UV absorbing band having an R_f of 0.29-0.35 was extracted with a mixture of CH_2Cl_2 and CH_3OH. The radioactive substance and unlabeled NNK comigrated on silica gel TLC and coeluted on a (23.9 mm x 30 cm) μ-Bondapak-C_{18} column (Waters Associates, Milford, Mass) using a solvent system previously described (6). The isotopic purity was higher than 99% as determined by HPLC and the overall yield from [carboxyl-^{14}C]nicotinic acid (1) was 826 μCi (27%).

[Carbonyl-^{14}C]NNAl (7)

The N-nitrosamine 7 was obtained by reduction of 6 with $NaBH_4$. One hundred μl of glacial acetic acid was added to a solution of [carbonyl-^{14}C]NNK (20.7 μCi, 4.9 μmol) in ethanol (1ml). This solution was stirred at room temperature and treated with $NaBH_4$ (50 mg, 1.32 mmol). After the addition of 1 ml of methanol the reaction was stirred for 7 hr. The mixture was diluted with 2 ml of phosphate buffer (pH 7.0) and the ethanol and methanol were evaporated with a stream of nitrogen. HPLC of the residue indicated that 3% of [carbonyl-^{14}C]NNK had not been reduced. [Carbonyl-^{14}C]NNAl was purified by HPLC on a μ-Bondapak-C_{18} column using a linear gradient of 100% H_2O to 50% CH_3OH in H_2O in 50 minutes at a

flow rate of 1 ml per minute. [Carbonyl-^{14}C]NNAl eluted between 38 and 40 ml. The isotopic purity was 99.8% and the yield was 95%. Labeled and unlabeled NNAl coeluted from a μ-Bondapak-C_{18} column with a sodium acetate buffer under conditions described previously (6).

Alternatively, [carbonyl-^{14}C]NNK (6) could be reduced by cultured rat liver slices. Rat liver was perfused successively with Hanks medium and Williams medium E. Liver slices were cut approximately 1 mm thick from all lobes. Each dish contained liver slices (about 0.46 g) which were incubated for 48 hours with 5 ml of Williams medium E and 2.5 μCi (4.2 mCi/mmol) of [carbonyl-^{14}C]NNK. Separation of [carbonyl-^{14}C]NNAl from the other radioactive metabolites was achieved by HPLC on two μ-Bondapak-C_{18} columns as previously described (6). NNAl constituted 62% of all radioactive compounds. Twenty-one per cent of NNK remained unchanged.

Warning: NNK is a potent animal carcinogen and should be handled with gloves in a fume hood.

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