Synthesis of ^{14}C-Labeled N-Nitrosobis(2-oxopropyl)amine

Summary

N-Nitrosobis(2-oxopropyl)amine (BOP), a potent and selective pancreatic carcinogen in the Syrian golden hamster was synthesized with a ^{14}C label in the alpha carbon. Oxidation of 2-hydroxypropyl-2-hydroxypropyl-1-^{14}C-amine followed by nitrosation with sodium nitrite and hydrochloric acid gave radiolabeled BOP in 60% yield.

Key words: N-nitrosobis(2-oxopropyl)amine, carbon 14, nitrosamine, pancreatic carcinogen.

Introduction

N-Nitrosobis(2-oxopropyl)amine (BOP)[1] has been shown to selectively induce pancreatic adenocarcinomas in the Syrian golden hamster by a single subcutaneous injection.[2] Moreover these chemically induced pancreatic neoplasms are morphologically similar to those found in man.[3] Currently pancreatic cancer is being studied at this institute and elsewhere using this animal model.

Proposed biochemical studies with BOP necessitated preparation of a radio-labeled compound. The use of a tritium label was discarded for the following reasons: (1) The chemical lability of the alpha protons of nitrosamines.[4] (2) The beta-oxo substituent has been shown to greatly increase the rate of exchange of the alpha and gamma protons with deuterium or tritium oxide *in vitro*.[5,6] Since this label was readily incorporated, it follows that the label might be readily exchanged *in vitro*.

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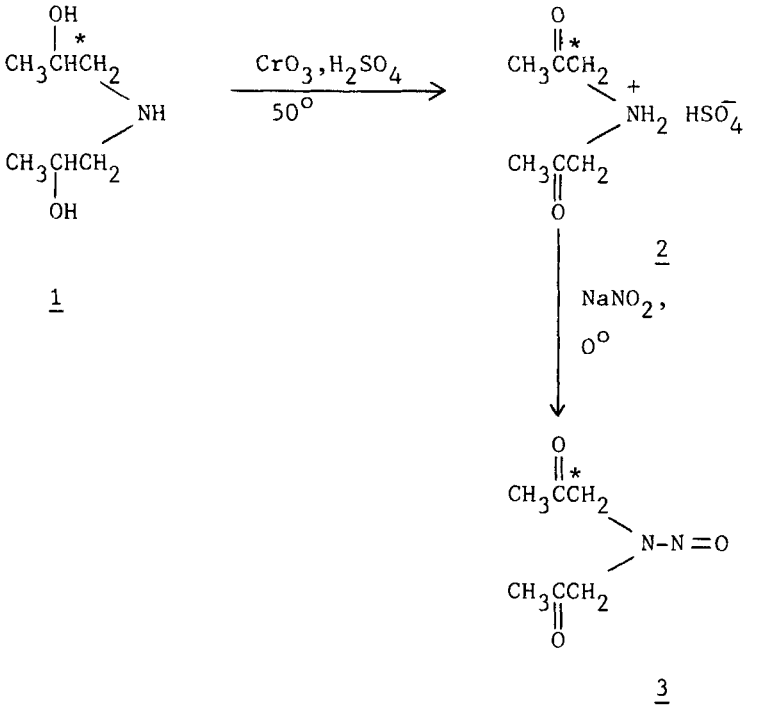
Results and Discussion

We have previously reported the synthesis of 2-hydroxypropyl-2-hydroxypropyl-1-^{14}C-amine (1) from commercially available d,1-sodium lactate-1-^{14}C.[7] BOP incorporating a ^{14}C label in the alpha carbon was synthesized by aqueous chronium trioxide oxidation of the hydrosulfate salt of 1 followed by nitrosation with sodium nitrite. The yield was 60% based on 1. The amine 2 is stable only as a salt, and attempts to isolate the free base led to extensive decomposition. Attempts to oxidize N-nitrosobis-(2-hydroxypropyl)amine directly with a wide variety of oxidizing agents including activated MnO_2, CrO_3/pyridine, DCC/DMSO and the Jones reagent led to low and irreproducible yields. Similarly, low yields were obtained by treatment of unlabeled 1 with these oxidizing systems followed by nitrosation.

Scheme I



Experimental

^{1}H nmr was determined on a Varian CFT-20 spectrometer and is reported in ppm (δ) downfield from tetramethylsilane. IR spectra were measured on a Beckman IR-9 spectrophotometer. Analytical TLC was carried out on Brinkman MN silica gel G-25 UV_{254} plates. UV spectra were measured on a Cary 14 spectrophotometer. Elemental analysis was performed by Micro Tek, Skokie, Illinois. Radioactivity was measured in a Beckman Model LS-335 liquid scintillation system using Aquasol (New England Nuclear) as the scintillation cocktail. Radiochromatogram scans were determined using silica gel TLC plates and a Packard Model 385 Radiochromatogram Scanner.

N-Nitroso-2-oxopropyl-2-oxopropyl-1-^{14}C-amine (3).--The amine 1 (1.98 mCi, 0.90 mmol) was dissolved in 1.0 ml of H_2O, cooled to 5° and 0.45 ml of 36 N H_2SO_4 added dropwise with stirring. To this solution was added 250 mg of CrO_3 in 0.2 ml of H_2O. The dark green solution was heated at 50° for 1 hr.* Methylene chloride (5 ml) was added to the reaction mixture. The two phase mixture was cooled to 5° and a solution of 900 mg of $NaNO_2$ in 1.2 ml of H_2O added dropwise. After stirring 0.5 hr, the CH_2Cl_2 layer was separated and the aqueous layer extracted (2X) with equivalent volumes of CH_2Cl_2. The CH_2Cl_2 volumes were combined,

*At this point the extent of the oxidation may be monitored by the following procedure: A 1 μl aliquot of the reaction mixture is added to a 5 x 40 mm tube containing 10 μl CH_2Cl_2, 1 μl 40% $NaNO_2$ in H_2O, and 10 μl MeOH. The tube is capped, vortex mixed, the organic layer spotted on a silica gel TLC plate and developed with 20% ethyl acetate in CH_2Cl_2. If the oxidation is incomplete N-nitrosobis(2-hydroxypropyl)amine (R_f = 0.08) and N-nitroso-2-hydroxypropyl-2-oxopropylamine (R_f = 0.30, 0.24) will be present. More chromium trioxide and sulfuric acid may be added at this point and the reaction mixture heated an additional hour.

dried (Na_2SO_4), 5 ml of anhydrous methanol* added and concentrated in vacuo (30°, 25mm). The residue was dissolved in 1.0 ml of CH_2Cl_2, spotted on 2 preparative TLC plates (20 cm x 20 cm x 2 mm, silica gel MN, Brinkman) and developed with methylene chloride/ethyl acetate (4/1). The bands corresponding to BOP (R_f = 0.44) were scraped off and eluted (2 x 25 ml) with ethyl acetate. Yield 83 mg, (1.12 mCi). Overall yield, based on 1, was 60%. Recrystallization from ethyl ether/methylene chloride yielded colorless platelets mp 54-55°. A radiochromatogram scan indicated a product purity of >98%. ^1H NMR ($CDCl_3$) δ 5.10 (s, 2H, CH_2 *anti*), 4.35 (s, 2H, CH_2 *syn*), 2.23 (s, 3H, CH_3 *anti*), 2.12 (s, 3H, CH_3 *syn*). IR ($CHCl_3$) $\nu_{C=O}$: 1735 cm^{-1}. UV (CH_2Cl_2: ε_{243}, 5846; ε_{359} 99.

Anal. cal'd for: $C_6H_{10}O_3N_2$: C, 45.57; H, 6.37; N, 17.72; found: C, 45.53; H, 6.42, N, 17.77.

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*The methanol is added to destroy any nitrite esters of N-nitrosobis(2-hydroxypropyl)amine of N-nitroso-2-hydroxypropyl-2-oxopropylamine which may be present in the reaction mixture.

References

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