SYNTHESIS OF ^{14}C-LABELED N-NITROSOBIS(2-HYDROXYPROPYL)AMINE

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SUMMARY

N-Nitrosobis(2-hydroxypropyl)amine (BHP), a potent pancreatic carcinogen in the Syrian golden hamster, was synthesized with the ^{14}C-label in the 1-position of one of the propyl groups. Starting with lactic-1-^{14}C acid, BHP was prepared utilizing a four-step reaction sequence, with an overall yield of 45%. Intermediate purification was not necessary.

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

INTRODUCTION

N-Nitrosobis(2-hydroxypropyl)amine (BHP) has been shown to be a potent pancreatic carcinogen in the Syrian golden hamster.[1,2] Preliminary metabolism studies with BHP have indicated the necessity of a radio-labeled compound for further biochemical studies. Tritium incorporation was first considered due to cost and ease of introduction of the label. Several drawbacks, however, soon became apparent. The chemical lability of the alpha protons in nitrosamine is well-known.[3] Additional studies have shown that N-nitroso-2-hydroxypropyl-2-oxopropylamine is a metabolite of BHP.[4] Nitrosamines with a beta-oxo substituent greatly increase the rate of exchange of alpha and gamma protons with deuterium oxide or tritium oxide *in vitro*.[5,6] Since this label

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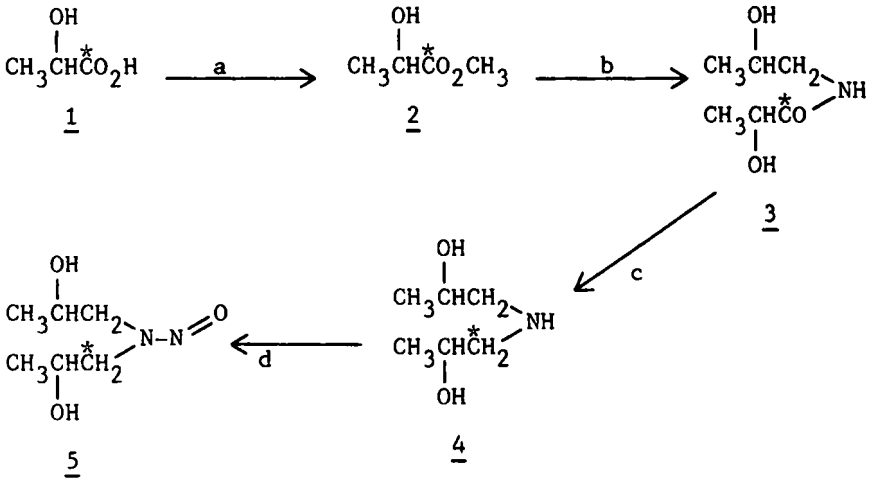
is readily incorporated, it follows that the label might be readily exchanged in vivo. These facts preclude the use of tritium radio-label in this compound for biological investigations.

Consequently a synthesis of BHP incorporating a ^{14}C label was undertaken. Krüger has shown that higher specific activities of rat liver RNA are obtained following administration of N-nitroso-2-hydroxypropyl-1-^{14}C-propylamine in comparison with the 2-^{14}C-labeled compound.[7] Hence we incorporated the label at C-1, although the procedure below is also applicable to both C-2 and C-3, since sodium lactate-^{14}C is commercially available with each position labeled.

EXPERIMENTAL

All chemicals were purified before use. Thin-layer chromatograms employed silica gel (Eastman Chromagram) and were developed with ethyl acetate. Autoradiography used Kodak SB54 X-ray film. CMR spectra were determined on a Varian CFT-20 spectrometer in deuterochloroform, proton decoupled and reported ppm downfield from tetramethylsilane. GLC was performed on a Varian 3740 chromatograph on a 3-m x 2-mm column of 8% SE-30 on Chromosorb HP at 180°C at a He flow of 30 ml/min. All intermediate products were characterized by comparison of spectral and chromatographic properties with synthetic standards.

The synthetic scheme is shown below:



[a]CH_2N_2, CH_3OH; [b]$CH_3CHOHCH_2NH_2$, CH_3OH, reflux; [c]$B_2H_6/(CH_3)_2S$, THF, reflux; [d]$NaNO_2$, HCl, H_2O, 0°

N-(2-Hydroxypropyl)lactamide-1-^{14}C (3).--We acidified 0.70 mg (750 μCi) of dl-sodium lactate-1-^{14}C in 2 ml methanol to pH 2 with methanolic HCl. An ethereal solution of diazomethane[8] was added dropwise until a yellow color persisted for 10 min. After the solution was filtered, 2.25 g (20 mmol) methyl lactate and 7.0 g (90 mmol) 1-amino-2-propanol in 10 ml methanol were added to the filtrate. The mixture was refluxed for 24 hr and the volatiles removed in vacuo (15 mm) at 80°. The yellow oil (2.2 g, 79% yield) was 95% pure, and contained 5% of 1-amino-2-propanol, as determined by carbon magnetic resonance (CMR) and gas-liquid chromatography (GLC). GLC retention times: N-(2-hydroxypropyl)-lactamide, 1.6 min; 1-amino-2-propanol, 1.0 min. An autoradiogram of a silica gel thin-layer chromatogram (R_f = 0.07) did not detect any radiochemical impurities. The product was used without

further purification.

N-Nitroso-2-hydroxypropyl-2-hydroxypropyl-1-^{14}C-amine (5).--The amide, 3 (2.2 g, 15 mmol), was dissolved in 125 ml dry THF and 15 ml (150 mmol) borane-dimethylsulfide added dropwise with stirring under nitrogen. After 12 hr reflux, the mixture was cooled to 0° and 45 ml methanol slowly added. After 24 hr reflux, 9.5 ml of 50% aqueous H_2SO_4 was added and the volatiles removed *in vacuo* (30°, 15 mm). The resultant aqueous solution was cooled to 0° and 2.1 g (30 mmol) $NaNO_2$ in 5 ml H_2O added. After 2 hr stirring at 0°, the mixture was extracted with ethyl acetate (5 x 10 ml), dried ($CaSO_4$) and concentrated *in vacuo* (30° mm). Chromatography on a 20 x 250-mm column of silica gel eluting with methylene chloride/ethyl acetate (4/1) yielded 1.58 g (65%) BHP. Thin layer chromatography (TLC) on silica gel (R_f = 0.22) and subsequent autoradiography indicated the isolated product was homogeneous. CMR: δ 66.5 and 65.5 (C_2, anti); 64.8 and 64.4 (C_2, syn); 61.1 and 60.7 (C_1, anti); 53.6 and 53.2 (C_1, syn); 20.9 (C_3, anti); 20.3 and 20.1 (C_3, syn).

DISCUSSION

Preparation of methyl lactate from sodium lactate, hydrochloric acid and diazomethane in methanol offers a significant advantage to generation of lactic acid in water, extraction with ether and subsequent treatment with diazomethane. This is due to the unfavorable partition coefficient of lactic acid between water and ether. The reduction of the amide 3 with borane/dimethylsulfide is superior to lithium aluminum hydride reduction, since the former yields significantly less C-N bond cleavage.

The CMR spectrum of radio-labeled BHP was identical with

the spectrum of an authentic sample.[1] TLC on silica gel gave identical R_f values for the labeled and authentic compounds. The measured specific activity was 26 μCi/mmol (overall radiochemical yield: 45%). ^{14}C-BHP has been stored as a dilute ethanol solution at 4°C for one month with no detectable decomposition.

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