SYNTHESIS OF ^{14}C-LABELLED COMPOUNDS. III. SYNTHESIS OF N-NITROSO-O,N-^{14}C-DIMETHYLHYDROXYLAMINE.

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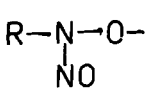
SUMMARY

C^{14}-labelled O,N-dimethylhydroxyurethane is synthesized from C^{14}-labelled dimethylsulfate and N-hydroxy-urethane. It is further converted without purification into O,N-^{14}C-dimethyl-hydroxylammoniumchloride, which was then nitrosated with sodiumnitrite in acidic medium to give N-Nitroso-O,N-^{14}C-dimethylhydroxylamine in 49% yield.

Key-Words: O,N-^{14}C-dimethylhydroxy-urethane, O,N-^{14}C-dimethylhydroxylammonium-chloride, N-Nitroso-O,N-^{14}C-dimethylhydroxylamine

INTRODUCTION

High carcinogenic activity of dimethylnitrosamine and its mode of action on rat liver are well established (1). The carcinogenicity of N-Nitroso-O,N-diethylhydroxylamine (1) is also reported (2). Along these lines the carcinogenicity and metabolic studies of N-Nitroso-O,N-dimethylhydroxylamine (2) are underway in our laboratory (3). However, ^{14}C-labelled N-Nitroso-O,N-dimethylhydroxylamine needed for the metabolic studies has so far not been recorded in the literature. We report here the synthesis of the above nitrosamine following a modified procedure used for the synthesis of unlabelled compound (5)



1 R=C_2H_5 2 R=CH_3

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according to figure 1.

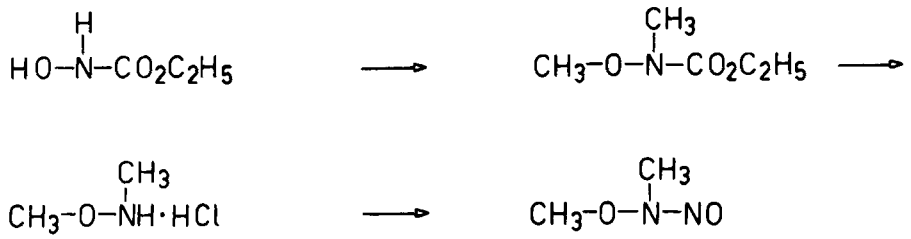

Fig. 1. Synthetic scheme for the synthesis of N-Nitroso-O,N-dimethylhydroxylamine.

RESULTS AND DISCUSSION

Labelled dimethyl sulfate was mixed with unlabelled dimethyl sulfate and then reacted with N-hydroxy-urethane in potassium hydroxide solution. O,N-dimethylhydroxyurethane thus obtained was isolated by ether extraction. After removing the ether the crude product was further hydrolysed by hydrochloric acid to give O,N-dimethylhydroxylamine hydrochloride which was dried in vacuo to give colourless needles. This hydrochloride was nitrosated by sodium nitrite in acidic medium to give the desired nitrosamine in good yield. When checked by thin layer chromatography the product was found to be one spot pure. The NMR - spectra of the cold compound prepared under identical conditions had shown traces of impurity of dichloromethane which was used as a solvent for extraction. This impurity would have been hazardous in animal experiments. Dichloromethane was therefore removed by vacuum distillation to obtain very pure N-Nitroso-O,N-dimethylhydroxylamine.

Nitrosation of O,N-dimethylhydroxylamine hydrochloride with nitrosyltetrafluoroborate (7) under anhydrous conditions also gave good yield of the desired nitrosamine; but nitrosation with sodium nitrite in slightly acidic medium was found to be a better procedure. C^{14}-nitrosamine and the cold analogue prepared under identical conditions showed spots on thin layer chromatography at the same Rf value. The structure was confirmed by the NMR-spectra of the cold product.

MATERIALS AND METHODS

General: ^{14}C-dimethylsulfate (specific activity 12.4 mCi/mmole) was purchased from Amersham Buchler, Bucks. England. Radioactivity was measured in a Nuclear Chicago Mark III scintillation counter.

The radiochemical purity of the product was measured on thin layer chromatograms by an LB 2723 thin layer scanner II (Berthold, Wildbad, F.R.G.). Precoated Silica gel plates (5 x 20, F-254, E. Merck, Darmstadt, F.R.G.) (developed in the solvent system dichloromethane/ether/n-hexane 10:7:5) were used for thin layer chromatography. The packing of the HPLC column (500 x 3 mm) was Li Chrosorb Si 60 (E. Merck), methanol was used as solvent at 60 bar. Optical density was determined at 230 nm . NMR-spectra were taken by a 90 Mhz, Brucker HX 90, with TMS as internal standard.

SYNTHESIS OF O,N-^{14}C-DIMETHYLHYDROXYURETHANE

In a 100 ml round bottomed two necked flask with attached water condenser were put 0.01 mole N-hydroxy-urethane (1.05 gm) (6) and stirred with 15% potassium hydroxide solution (10 ml) under cooling for thirty minutes. ^{14}C-dimethylsulfate (25.4 mg, 2500 μCi) was made up to 2.51 gms (0.02 mole) by mixing with cold dimethylsulfate. This was then introduced drop by drop through a dropping funnel in the stirred reaction mixture while maintaining the reaction temp. at 25°. The stirring was further continued for 24 hours and the reaction mixture was then acidified with dilute sulfuric acid to pH 0.1 (8) and under cooling with ice water stirred again for 1.5 hours. The mixture was then extracted four times with ether (total 100 ml) and washed three times with 20% potassium hydroxide solution (total 2 ml). After drying over sodium sulfate the ether was removed without vacuum to yield O,N-^{14}C-dimethylhydroxyurethane (1.10 gms; yield 82.7%) as a colourless thick oil which was then used for hydrolysis without further purification. Cold O,N-dimethylhydroxyurethane prepared under identical conditions was purified by vacuum distillation (0.05 Torr, oil bath 140°, b.p. 65°C) and checked by NMR.

NMR - ($CDCl_3$) δ =1.30 (d,3H); δ =3.05 (s,3H); δ =3.90 (s,3H); δ =4.15 (q,2H);

SYNTHESIS OF O,N-^{14}C-DIMETHYLHYDROXYLAMINE HYDROCHLORIDE

The crude O,N-^{14}C-dimethylhydroxyurethane (1.10 gm; 0.0082 mole) was taken in a 100 ml round bottomed three necked flask. A water condenser was then attached to it in which ice cold water was circulated. Concentrated hydrochloric acid (25 ml) was added drop by drop through a dropping funnel. The temperature was raised to 100° by keeping the reaction vessel in an oil bath for 1 hour. After this the reaction product was dried on a rotary evaporator in the same reaction vessel when a colourless semisolid was obtained. Absolute ethanol (25 ml) was added and then the ethanol was removed on the

rotary evaporator. This was repeated three times when colourless needles of O,N-^{14}C-dimethylhydroxylamine hydrochloride (0.723 gm; yield 74.1%) were obtained. This hydrochloride was used for nitrosation without further purification. Spectroscopic studies were carried out on cold hydrochloride prepared under identical conditions and purified by crystallisation from ethanol.

NMR - (d_6-DMSO) δ =2.73 (s,3H); δ =3.80 (s,3H); δ =9.70 (2H,broad)

SYNTHESIS OF O,N-^{14}C-DIMETHYLHYDROXYLAMINE

O,N-^{14}C-dimethylhydroxylamine hydrochloride (0.723 gm; 0.0044 mole) in the three necked flask was dissolved in water (5 ml). This was then covered with methylene chloride (50 ml). Sodium nitrite (0.612 gms; 0.012 mole) in water (2 ml) was added dropwise to the above stirred solution under cooling in an ice water bath. The pH

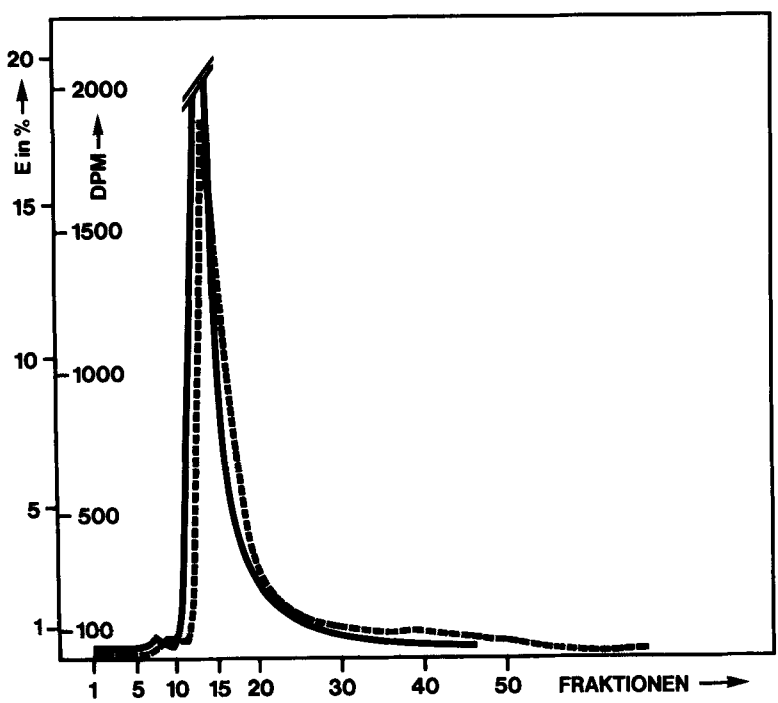

Fig. 2. Determination of optical density (---) and radioactivity (—) of the samples derived from elution by HPLC

of the reaction mixture was maintained between 2-4 by adding a few drops of hydrochloric acid when necessary. After the addition was complete, the mixture was further stirred fourty minutes at the same temperature. The methylene chloride layer was then separated and the aqueous phase was extracted three times with methylene chloride (20

ml) and the combined methylene chloride extracts were then dried for ten hours over anhydrous sodium sulfate. Methylene chloride was then very carefully removed without vacuum. Since N-Nitroso-O,N-dimethylhydroxylamine is very volatile, great care had to be taken while removing the solvent. It was further vacuum distilled at 30 mm in a specially prepared micro-distillation assembly to remove traces of methylene chloride. The distilled product was a yellow oil (0.441 gm; yield 49%; 1225 µCi). The thin layer chromatogram revealed that the product was one spot pure. For testing the radiochemical purity the ^{14}C labelled nitrosamine was analyzed by HPLC. Subsequent UV-measurement and scintillation counting showed the identity of the two curves (Figure 2). The specific activity determined by liquid scintillation counting (corrected) was 119 µCi/mmole and agreed with 125 µCi/mmole, which was calculated from the specific activity used initially and the yield of the final product. Spectral studies were carried out on the cold compound prepared under identical conditions.

NMR - ($CDCl_3$) δ =3.70 (s,3H); δ =3.75 (s,3H);

Analysis: Required C, 26.66%, H, 6.66%, N 31.11%
Found C, 27.00%, H, 6.91%, N 31.50%

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References:

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3) N-Nitroso-O,N-dimethylhydroxylamine was tested in BD-rats and found to be non carcinogenic (4). Because the analogous Diethyl-compound was tested in SD rats (see ref. 2) we decided to test the dimethyl compound once more in SD rats. This investigation is not finished up to now. Lijinsky reported (Z. Krebsforsch. 89: 31 (1977)) that N-Nitroso-O,N-Dimethylhydroxylamine is non carcinogenic in SD rats.
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The hydrochloride was synthesized according to "Methoden der organischen Chemie" (Houben-Weyl) Band X/1 p. 1198, Georg Thieme Verlag, Stuttgart, 1971, edited by E. MÜLLER.

6) Crude N-Hydroxyurethane prepared according to "Methoden der organischen Chemie", p. 1195 (see ref. 5) was distilled in 5 gr portions only.

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H.T. NAGASAWA, P.S. FRASER and D.L. YUZON, - J. Med. Chem. 16: 583 (1973)

8) We have learned that from solutions, not so extremely acidified O,N-dimethyl-hydroxyurethane can not be removed completely.