TRITIUM GAS EXPOSURE AS AN ALTERNATIVE TO BASE-CATALYZED EXCHANGE FOR THE ONE-STEP TRITIATION OF NITROSAMINES

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Received December 13, 1976

SUMMARY

N-Nitrosodibutylamine (DBN) and fully deuterated N-nitrosodimethylamine (DMN-d_6) have been successfully tritiated using the Wilzbach procedure. Specific activities, after purification and transfer to aqueous solution for biochemical studies, were 180 and 14 mCi/mmole, respectively. ^{3}H-N-Nitrosodibutylamine (^{3}H-DBN) was rather sensitive to autoradiolysis, although the unlabelled derivative proved unusually resistant to base-catalyzed exchange with deuterium oxide. No decrease in deuterium content was observed during the preparation of ^{3}H-N-nitrosodimethylamine-d_6 (^{3}H-DMN-d_6), demonstrating that tracer quantities of tritium could be incorporated without significantly lowering the deuterium isotopic purity.

Until recently, the standard approach to preparing isotopically substituted N-nitroso compounds involved nitrosation of labelled precursors. Frequently, however, suitable starting materials were unavailable commercially, necessitating

0362-4803/78/0114-0091$01.00/0
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additional synthetic steps. Also, such procedures sometimes led to exchange loss of hydrogen label during workup.[1-3]

Attempts to shed light on this latter complication led to the discovery that the α-nitrosamino carbanion was considerably more stable than had previously been recognized[3,4], and a method for the labelling of nitrosamines by base-catalyzed exchange with solvent was soon developed. Numerous successful preparative applications[3,5,6] of this procedure revealed it to be efficient, inexpensive, and rather general. Nevertheless, base-catalyzed exchange also has certain limitations. For example, the nitrosamino function does not activate protons at carbon atoms more remote than the α position. Moreover, exchange reactivity is highly structure dependent even at the α carbon atom itself; for instance, deuterium incorporation was observed only in the methyl group of methylcyclohexylnitrosamine.[3] In addition, strong conformational preferences have been discovered which govern the formation of the requisite carbanion intermediate; thus C-H bonds perpendicular to the plane of the nitrosamino group (e.g., axial) and nearest the oxygen atom thereof (*syn*) are much more susceptible to exchange than those in the equatorial and/or *anti* positions.[7]

Compounds which we found impractical to prepare by either of the above procedures were the tritiated derivatives of dibutylnitrosamine (DBN), which proved surprisingly inert to base-catalyzed exchange (see Experimental), and dimethylnitrosamine-d_6 (DMN-d_6). The latter compound does undergo facile equilibration with solvent, but to obtain a product containing the desired high (>99%) proportion of deuterium isotope as well as tracer quantities of tritium, doubly labelled water containing negligible protium would be required as starting material.

Therefore, we attempted the Wilzbach procedure[8] to obtain these two compounds for planned studies of the metabolic activation of carcinogenic dialkylnitrosamines. The Wilzbach procedure, another approach to one-step exchange tritiation of

organic compounds, had apparently not been tried before with nitrosamines, possibly because of a presumed risk of partial reduction during exposure to tritium gas.[1] Our results, summarized in the procedures reported below for the preparation of ^{3}H-DBN and ^{3}H-DMN-d_6, demonstrate that the Wilzbach procedure may serve as a useful alternative to base-catalyzed exchange for the synthesis of tritiated nitrosamines.

EXPERIMENTAL

WARNING!

DBN AND DMN ARE POTENT CARCINOGENS IN LABORATORY ANIMALS. AVOID ALL HUMAN CONTACT WITH THESE COMPOUNDS AND THEIR VAPORS.

Dimethylnitrosamine-d_6 (DMN-d_6) was prepared as previously described[6], by base-catalyzed exchange of dimethylnitrosamine (DMN) with deuterium oxide. Dibutylnitrosamine (DBN) was purchased from Eastman Kodak Co., Rochester, N.Y. Ultraviolet (UV) spectra were recorded on a Varian Techtron UV-Vis Spectrophotometer, Model 635. Silica gel with fluorescent indicator was used for all thin layer chromatography (TLC), autoradiography, and radiochromatography; the developer used was either methylene chloride or 50:50 methylene chloride:cyclohexane for DBN and 9:1 benzene:ethanol for DMN-d_6. Radiochromatography was effected by spotting a plastic-backed TLC plate with an appropriate number of counts and allowing it to develop through about 10 cm; the finished chromatogram was cut into fractions 5 mm in width which were placed in scintillation vials and covered with 1 ml ethanol before the addition of 10 ml scintillation fluid for counting. Gas chromatography (GC) of DBN preparations employed 3% OV-17 on 80/100 Supelcoport in 6' x 1/8" stainless steel columns at an isothermal oven temperature of 120°C. For DMN-d_6, the mass spectrometer's GC inlet employed a 15% DEGS on 80/100 Chromosorb-WAW column at a temperature of 110°C while all other GC work with this compound was

done using 28% Pennwalt 223 + 4% KOH on 80/100 mesh Gas-Chrom R at 130°C. Helium flow rate was 30 ml/min. Mass spectra were obtained using a JEOLCO 01SG-2 mass spectrometer operating at 70 eV. Radioactivity measurements were done using a Beckman LS-250 Liquid Scintillation System. New England Nuclear Corporation (Boston, MA.) performed the Wilzbach procedures by exposing each compound to 15 Ci of tritium gas for 2 wk at room temperature.

Attempted base-catalyzed exchange of DBN--A suspension of 37 mg DBN in 1.0 ml of deuterium oxide containing 62 mg of 40% sodium deuteroxide solution was made homogeneous by the addition of 0.65 ml of methanol-O-d. The resulting solution was refluxed for 4 hr and concentrated by fractional distillation of the methanol. The remaining aqueous reaction mixture was extracted twice with ether. The ether extracts were carefully concentrated and subjected to preparative TLC. The fluorescence-quenching band at $R_f \approx 0.5$ was removed from the plate and the adsorbent was extracted with ether to remove the nitrosamine. The gc/mass spectrum failed to reveal any trace of deuterium incorporation, i.e. the relative intensities of peaks at m/e 159 and 158 did not significantly change (initial ratio = 0.14; final ratio = 0.10) as a result of exposure to the deuterated solvent.

Wilzbach tritiation of DBN--After exposing 40 mg of DBN to tritium gas, the nitrosamine was taken up in ether and extracted with 1N sulfuric acid to remove labile tritium as well as any basic degradation products. The ether extract was subjected to preparative TLC and the UV-quenching band at R_f 0.3-0.6 was eluted from the silica gel with 50 ml ether. Recovery was found by UV spectrophotometry to be only 1.2 mg (3% overall yield), but no impurities could be detected by radiochromatography.

Autoradiolysis of ^{3}H-DBN--After the above ether solution had been stored in a sealed ampul at 3°C for two months, TLC and spectrophotometry revealed the presence

of UV-absorbing impurities. Radiochromatography showed two peaks, with 24% of the total activity appearing in the slower moving fraction near the origin. This impurity was reduced to 4% of the total activity by filtering the ether solution through a 15 mm silica column (5 mm diameter). The resultant solution contained 0.8 mg of ^{3}H-DBN with specific activity of 180 mCi/mmole. Half of this solution was carefully evaporated, and sufficient water was added to dissolve the remaining labelled nitrosamine. The aqueous solution also appeared to suffer autoradiolysis; a radiochromatogram run after the aqueous mixture had been stored for one year showed that the proportion of total counts appearing at the origin had again increased from 4% to 27%.

^{3}H-Dimethylnitrosamine-d_6--After subjecting 783 mg of DMN-d_6 to Wilzbach conditions, the crude product was taken up in 1 ml of an aqueous solution containing 600 mg of sulfuric acid and immediately extracted with three 1 ml portions of methylene chloride. The combined organic extracts were dried over magnesium sulfate. Autoradiography revealed the presence of several labelled impurities. The organic solution was extracted with hydrochloric acid and again dried over magnesium sulfate and filtered. The remaining yellow methylene chloride solution was placed in a test tube, covered with 1 ml water, and warmed gently while a stream of argon was bubbled in from the bottom of the tube. As the lower layer evaporated, the labelled DMN was transferred efficiently to the aqueous phase, while most of the yellow color remained as a water-insoluble gum which was later removed by decantation and extraction with a few drops of methylene chloride. Gas chromatography of the aqueous solution revealed two major peaks, which had the same retention times as DMN and methylene chloride (integrating for 98% and 2% of the total flame ionization detector response, respectively). Several additional peaks appeared when the attenuation was lowered; the largest integrated for 0.2% of the total area, while the others were even smaller ($\leq$ 0.05%). The size of the methylene

chloride peak could be diminished as desired by blowing a stream of argon over the surface of the liquid while the solution was warmed gently.

The aqueous solution was found by UV spectrophotometry and scintillation counting to contain 118 mg of ^{3}H-DMN-d_6 at a specific activity of 14 mCi/mmole. The molecular ion region of the mass spectrum (GC inlet) revealed that the peak at m/e 79 (DMN-d_5) was smaller than the $M^+ + 1$ ion (m/e 81), and the deuterium isotopic purity was calculated to be 99.3%.

ACKNOWLEDGEMENTS

Supported by the National Cancer Institute, in part under contract number NO1-CO-25423 with Litton Bionetics, Inc. We thank Ms. Catherine Bradley of New England Nuclear Corp. for conducting the Wilzbach exposures and supervising removal of labile tritium from the crude products. Performed in part during the first author's tenure as a Visiting Research Chemist and Guest Worker in the Analytical Chemistry Section, Carcinogen Metabolism and Toxicology Branch, National Cancer Institute, 1974-1975.

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