Journal of Labelled Compounds and Radiopharmaceuticals - Vol. IX, No. 4

SYNTHESIS OF N,N-[METHYL-^{14}C]-DIMETHYLHYDRAZINE OF HIGH SPECIFIC ACTIVITY

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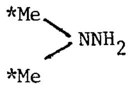
SUMMARY

N,N-[Methyl-^{14}C]-dimethylhydrazine hydrochloride (1) having a specific activity of 115 mCi/mmol, was synthesized in two steps. Dimethylation of acetophenone hydrazone 3 with ^{14}C-methyl iodide (specific activity = 58 mCi/mmol) in the presence of potassium in liquid ammonia followed by acid catalyzed hydrolysis of the hydrazone 4 afforded 1 in a yield of 60.0% (>97% radiochemical purity).

Key Words: Carbon-14; N,N-Dimethylhydrazine; Methyl iodide; Hydrazone.

INTRODUCTION

More than forty hydrazines and their derivatives have been found to be carcinogenic to laboratory animals.[1] This is of considerable concern owing to the high degree of human exposure to such agents.[2] Hydrazines have natural and synthetic sources[2] and the simple hydrazines including N,N-dimethylhydrazine are used as high-energy storage propellants by the U.S. Air Force.[3] Notably, N,N-dimethylhydrazine has been shown to transform human fibroblast cells in culture as assessed by anchorage-independent growth in soft agar and tumor production in athymic nude mice.[4] To further explore events leading to human cell transformation, which may involve macromolecular alkylation and subsequent DNA damage and repair, a facile synthesis for ^{14}C-labeled N,N-dimethylhydrazine was desired. Methodology based on the novel chemistry published by Kaiser et al.[5] is described in this article.



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0362-4803/82/040585-05$01.00

Received February 11, 1981
Revised December 7, 1981

RESULTS AND DISCUSSION

Synthetic intermediate hydrazone 3 was prepared from the corresponding N,N-dimethylhydrazone 2 by exchange with anhydrous hydrazine according to the method of Newkome and Fishel.[6] For radiolabelling purposes it is critical that hydrazone 3 be free of residual dimethylhydrazone 2 since contamination with unlabelled 2 would lead to lower specific activity of intermediate 4 and target 1. The exchange method, rather than direct reaction of acetophenone with hydrazine, was preferred since the latter method leads to tars and most importantly unwanted azines from rearrangement of acetophenone hydrazone.[6] Fractional distillation and monitoring by NMR analysis at $\delta 2.59$ ($-NMe_2$ for 2) served to ensure the purity of 3. Synthesis of 4 from 3 was based upon dialkylation of hydrazone N,N-dianions with alkyl halides.[5] Thus, hydrazone 3 was converted to its blue-green dianion using a slight excess of potassium in liquid ammonia. The solution color change from deep blue to blue-green served as a reliable indicator of dianion quality. Thus, if a brown color persists alkylation will be unsuccessful. On the other hand, a yellow solution treated with additional potassium rapidly developed the desired persistent blue-green color. Addition of 2.2 equivalents of ^{14}C-methyl iodide converted the blue-green solution to a pale yellow color signifying the quenching of the dianion by alkylation. Liquid ammonia, ether and any unreacted methyl iodide were distilled into a vented trap. The residue was triturated with ether and the yellow solution was removed by pipette from the crystalline potassium iodide. Radiolabelled hydrazone 4 was converted to 1 using ether saturated with gaseous HCl. Thin layer chromatographic analysis of the hydrolysate was carried out according to procedures developed by Fiala and Weisburger.[7]

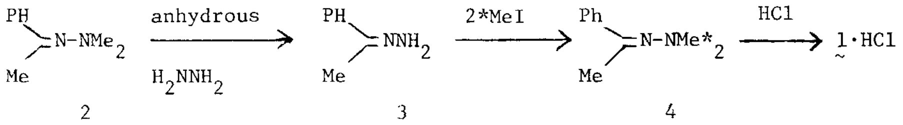

Figure 1: Synthesis of ^{14}C-N,N-Dimethylhydrazine (1)

EXPERIMENTAL SECTION

Nuclear magnetic resonance data were obtained using $CDCl_3$ as a solvent with a Bruker HX-90 spectrometer. Radioactive disintegrations were measured on a Beckman LS-355 liquid scintillation counter using Amersham PCS as a counting cocktail. Thin layer chromatography was carried out on glass plates precoated with Avicel cellulose (250μ thickness), purchased from Analtech. Plates were developed with 2-propanol-water-conc. HCl (130:40:30) and were visualized with Folin-Ciocalteau reagent obtained from Fisher Scientific.

^{14}C-Methyl iodide (58 mCi/mmol) was purchased from Amersham as a custom preparation prepared to our specifications by aqueous base extraction and P_2O_5 drying and used without dilution or further purification. Transfer of ^{14}C-methyl iodide was carried out under reduced pressure in a Pope glass vacuum manifold. All glassware were predried under reduced pressure and flushed with ammonia (KOH-dried) for several minutes to obtain an ammonia atmosphere. Ammonia was condensed in an auxiliary flask, dried with Na and distilled into a reaction flask.

Synthesis of ^{14}C-N,N-Dimethylacetophenone Hydrazone(4). Approximately 12 mg (0.3 mg atom) of potassium was added to about 8 ml of dry liquid ammonia in a 10 ml two necked round bottom flask. To this stirred blue solution was added 20.1 mg (0.15 mmol) of acetophenone hydrazone dissolved in 1 ml of dry ether. The flask was cooled with liquid N_2 and ^{14}C-methyl iodide (20 mCi at 58 mCi/mmol, 0.34 mmol) was added under reduced pressure. The reaction was kept sealed as it was warmed to -78°C (dry ice/acetone). The reaction proceeded as the mixture was allowed to thaw and reach -33°C (liquid NH_3 bp) and was vented through a trap as it exceeded atmospheric pressure. Following venting of the ammonia, ether and residual ^{14}C-methyl-iodide, a yellow oil containing white crystals of potassium iodide was obtained. The oil was dissolved in dry ether (2 ml) and an aliquot taken for counting. The ether solution was found to contain 10.8 mCi (this is a crude radiochemical yield of 54%). The hydrazone **4** was immediately hydrolyzed as described below.

N,N-[Methyl-^{14}C]-dimethylhydrazine Hydrochloride (1). To the dry ether solution (2 ml) containing hydrazone 4 was added 2 ml of ether saturated with gaseous HCl. The reaction mixture was allowed to stand overnight at room temperature and was centrifuged to pack the white crystalline solid. The supernatant was removed, the crystals washed with dry ether, and centrifuged again as above. The ether supernatant was removed and the crystals dried under reduced pressure (0.5 hr, 0.1 mm Hg) and overnight in a nitrogen atmosphere desiccated with P_2O_5. The dried sample was shown to be pure by tlc using authentic 1 as standard. The radiochemical purity was determined to be >97% by scraping and counting each zone. Aliquots were weighed in a dry nitrogen atmosphere, dissolved in methanol and counted in PCS. The specific activity was determined to be 106.0 mCi/mmol. However, from elemental analysis of unlabeled samples prepared under identical conditions we have found that such samples contain about 0.5 mol of water of hydration. When we consider this water of hydration we calculate the specific activity to be 114.8 mCi/mmol. The chemical yield of this reaction sequence was 10.02 mg (0.09 mmol, 60% based on starting hydrazone 3) and the radiochemical yield was 10.5 mCi (51%). In some experiments it was necessary to purify 1 using 20x20 cm Avicel plates. The radioactive band corresponding to 1 was scraped and exhaustively extracted with anhydrous methanol·HCl.

We have observed that at this high specific activity 1 is 80% decomposed after standing 3 months in the dark in 0.1N HCl at 4°C. The shelf life of this material can be lengthened by storing it as the solid hydrochloride salt, desiccated, in the dark at -70°C. Under these conditions we have found no measurable decomposition (by tlc) over a period of 6 months.

ACKNOWLEDGEMENT

We are grateful to the U.S. Airforce Office of Scientific Research for support of this work under grant number F49620-80-C-0086. We also thank the National Cancer Institute (P-30-CA-16058) and the Comprehensive Cancer Center of The Ohio State University for support of the Radiochemistry Laboratory without whose resources this work and the biological studies to follow could not have been accomplished.

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