SYNTHESIS OF ^{14}C-LABELLED METHYLAZOXYMETHANOL ACETATE OF HIGH SPECIFIC ACTIVITY

Frederick D. Cazer*, Satish B. Barnela, Kultar S. Kumar, Prabhakar L. Kamat, George E. Milo and Donald T. Witiak**, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy and the Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

SUMMARY

The micro-scale synthesis of ^{14}C-labelled methylazoxymethanol acetate (^{14}C-MAM-acetate) having a specific activity of 110 mCi/mmol was accomplished in 25% yield from N,N'-[methyl-^{14}C]-dimethylhydrazine with a specific activity of 112.5mCi/mmol.

Key Words: Carbon-14; Methylazoxymethanol Acetate; Azomethane; Azoxymethane; Bromoazoxymethane; N,N'-dimethylhydrazine.

INTRODUCTION

Cycasin (β-D-glucosyl-azoxymethane)[1,2] is hepatotoxic and carcinogenic in rats[3,4] and this toxicity is attributable to the aglycone, methylazoxymethanol (MAM)[5,6], which is also a metabolite of 1,2-dimethylhydrazine. MAM-acetate has increased stability, reduced volatility, retains toxicity[7,8] and is a very potent transformer of human fibroblasts in culture (manucripts in preparation). Although the synthesis of ^{14}C-MAM-acetate was reported by Horisberger and Matsumoto[9], their synthesis of this compound, starting with commercially prepared ^{14}C-labelled azomethane, yielded only small quantities of labelled product. It was suggested that poor yields may reflect autoradiolysis. In contrast, we report here the micro-scale synthesis of ^{14}C-MAM-acetate of near theoretical specific activity and in significantly higher yields.

DISCUSSION

Using a modification of the synthetic scheme reported by Horisberger and Matsumoto[9] the synthesis of ^{14}C-MAM-acetate (5) was accomplished by oxidation of

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* Radiochemist of the O.S.U. Comprehensive Cancer Center

** To whom requests should be addressed.

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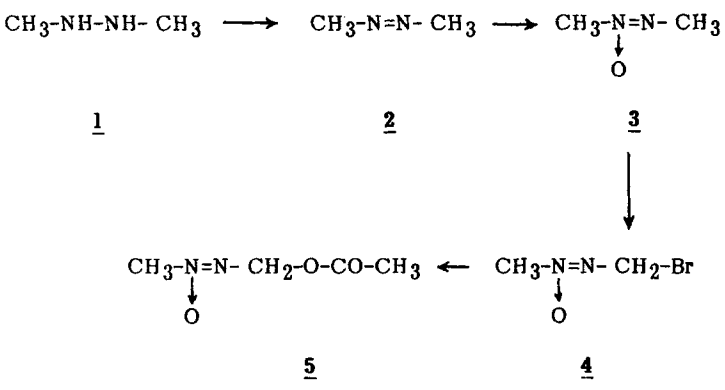
N,N'-[methyl-^{14}C]-dimethylhydrazine (1) to ^{14}C-azomethane (2) and subsequently ^{14}C-azoxymethane (3). Allylic type bromination followed by reaction with silver acetate afforded 5.



Although reaction conditions using cold material were optimized at each step, the reaction conditions for the radiolabelled material required considerable modification. Micro-scale production of high specific activity 5 was carried out using the described apparatus. The 25 ml reaction flask and 10 ml traps were critical; poor yields of 3 were obtained with unlabelled material on the micro-scale when these vessels were 50 and 25 ml, respectively.

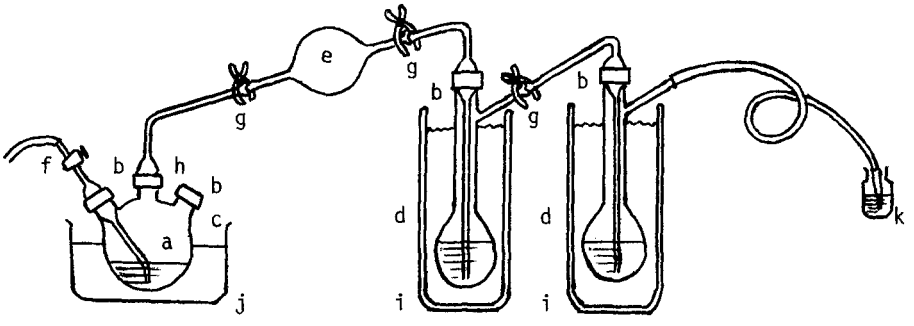
Under these conditions 1 was converted to 3 in 80% yield; a significant improvement over the 31% average reported by Horisberger and Matsumoto[9]. The argon flow rate of 5-6 ml/min is critical since a slower rate does not adequately carry the azomethane from the reaction flask and at faster flow rates azomethane escapes from the traps. Both traps were charged with the less polar methylene chloride solvent substituted for previously used ether[9]. Thus, the m-chloroperbenzoic acid is retained on the column during chromatographic purification on basic alumina.

The desirable temperature of trap #1 is 10 - 12°C. Too low a temperature results in precipitation of m-chloroperbenzoic acid and this in turn decreases conversion of azomethane (2) to azoxymethane (3). Gas flow and evolution of radioactive gas was routinely monitored by bubbling the trap effluent through scintillation cocktail. During 20 - 30 mCi reactions only 3 - 10 μCi of ^{14}C could be detected in the scintillation vial. A

significant difference in reaction time was required for bromination using labelled vs. unlabelled azoxymethane. For unlabelled material bromination was complete in 2 - 3 hr, whereas azoxymethane of high specific activity always required a 5 - 6 hr reaction time (4 experiments). Although it is tempting to propose an isotope effect to explain these results, further work is necessary to substantiate such a possibility.

Miligram quantities of MAM-acetate (5) were purified by thin layer chromatography (TLC) and visualized under UV light. The MAM-acetate band was scraped and eluted with methylene chloride. Caution should be exercised during solvent evaporation so as not to lose product. TLC-purified ^{14}C-MAM-acetate was analyzed by HPLC under conditions markedly different than those reported by Fiala et al.[11] Neither HPLC methodologies may be utilized for preparative purification owing to difficult product recovery from the eluting solvent (approx. 12% MeOH/H_2O). Long range spin coupling (J=1.46) between N-CH_3 and NCH_2 functions confirmed the structural assignment.

Assembly for the Micro-scale Preparation of Azoxymethane



a. 25 ml three necked flask. b. 14/20 "Thread-Tite" joint. c. "Thread-Tite" teflon/silicon septum cap. d. 10 ml bulb long necked trap with side arm and including a gas bubbler that extends close to the bottom of trap. e. 10 ml bulb filled with anhydrous $CaCl_2$. f. Gas inlet tube extending close to the bottom of 3 necked flask. g. 12/5 ball and socket joint secured with a clamp. h. gas outlet tube secured to reaction flask. i. dewar flask j. ice bath k. scintillation vial with 10 ml cocktail and a pipet bubbler.

EXPERIMENTAL SECTION

NMR data were obtained in 100% $CDCl_3$ using an IBM NR/80 spectrometer. Radioactive disintegrations were measured on a Beckman LS-355 liquid scintillation counter using Amersham PCS or NEN formula 963 as a counting cocktail and dpm were determined using OXI-TEST internal standard from Radiomatic Instrument and Chemical Co Inc. Standard N,N'-dimethylhydrazine dihydrochloride was purchased from Aldrich; m-chloroperbenzoic acid and unlabelled MAM-acetate were purchased from Sigma. TLC plates were silica gel GF, 10 x 20 cm, 250 micron, glass plates purchased from Analtech. HPLC was carried out using a Laboratory Data Control (L.D.C.) Gradient System controlled by a Commodore PCM 1611 control module. The column effluent was monitored using a L.D.C. Spectromonitor III variable wavelength UV detector and the radioactivity was measured by radioactive flow detector FLO-ONE model HP using Flo-Scint II cocktail purchased from Radiomatic Instrument and Chemical Co. The column was a L.D.C. Excalibar Spherisorb ODS 5μ, 4.6 x 250 mm. All glassware utilized had "Thread-Tite" 14/20 joints and caps purchased from Reliance Glass Work, Inc.

N,N'-[Methyl-^{14}C]-dimethylhydrazine dihydrochloride (1):

Dimethylhydrazine (1), having a specific activity of 112.5 mCi/mmol, was prepared in 50% yield by the method of Kumar et al. [10] The purity of the compound was determined by comparing its TLC with that of standard N,N'-dimethylhydrazine dihydrochloride.

^{14}C-Azomethane (2) and ^{14}C-Azoxymethane (3):

These compounds were prepared in sequence without separating 2. Thus, in a 25 ml 3-necked round bottom flask fitted with a teflon/silicon rubber septum and a gas inlet tube, was placed 223 mg of Amberlite IRA-93 previously washed 4-5 times with water. Yellow mercuric oxide 56 mg, (0.26 mM) and 1 ml of water was added. Two cooled traps each containing 10 ml of methylene chloride were utilized. To trap #1 was added 50 mg of m-chloroperbenzoic acid. Trap #1 was cooled to approx. 10°C with cold water and trap #2 was cooled in an ice-water bath (approx. 0°C). The reaction flask was cooled in an ice bath and 24.36 mg, (0.178 mM, 20 mCi, 112.5 mCi/mmol) of N,N'-[methyl-^{14}C]-dimethylhydrazine dihydrochloride (1) dissolved in 1 ml of water was added by syringe through the septum. The ice bath was removed after 30 minutes and the reaction mixture was stirred at room temperature for 2 hours. Argon was then bubbled through the reaction

mixture at a flow rate of 5-6 ml/min and the temperature of the oil bath was gradually raised from room temperature to 80-85°C. The reaction was stirred for an additional 2-3 hours at this temperature. Heating and gas flow were stopped and the solutions from the two traps were combined and stored at -4°C for 24 hrs. The solution was brought to room temperature and passed over basic alumina (2 gm) using a 1 x 15 cm column. The column was washed with methylene chloride (5 ml) and the eluent was distilled at 70°C using a 15 cm vacuum jacketed vigreaux column. The total radioactivity in the residue was 16 mCi. The yield of ^{14}C-azoxymethane (3) was 80% based on 1 (NMR of the residue from unlabelled 3 exhibited NMR ($CDCl_3$) δ 3.18 (bs, 3H,=NCH_3), 4.05 (bs, 3H, CH_3 -NO=).

^{14}C-Bromoazoxymethane (4) was prepared by placing 16 mCi (0.145 mM) of 3, 70 mg (0.39 mM) of N-bromosuccinimide and 3 ml of carbon tetrachloride in a 10 ml pear-shaped flask fitted with a water condenser protected by a calcium chloride tube. The mixture was stirred at 50-55°C under a 60 watt lamp held at a distance of 2 cm. Development of a light orange color in the mixture indicated reaction completion (5-6 hrs.). The mixture was cooled to room temperature and filtered through a disposable pasteur pipet plugged with glass wool. The filtrate containing 4 was immediately converted to ^{14}C-MAM-acetate without further purification.

^{14}C-Methylazoxymethanol acetate (5): To the solution of 4 in carbon tetrachloride was added 85 mg (0.50 mM) of silver acetate. The mixture was protected from light, stirred at room temperature overnight, and filtered using a pasteur pipet plugged with glass-wool. The solvent was removed at 77°C using a short path distillation head affording a residue containing 10 mCi ^{14}C. The crude product was purified (TLC) on silica-gel using ethyl acetate:hexane (1:2) as eluting solvent. The band which chromatographed with unlabelled standard 5 was scraped, eluted with methylene chloride, and evaporated on a rotary evaporator at room temperature (caution must be exercised to avoid loss of 5) to afford 4.03 mCi (20.1% yield) of pure 5 exhibiting a specific activity of 110.3 mCi/mmol (determined from a weighed aliquot). The chemical and radiochemical purity of TLC-purified 5 was analyzed by HPLC. The only detectable contaminant was 3 (2.8%). TLC purified 5 was eluted using a 5-15% methanol linear gradient at 0.8 ml/min over 67 minutes. Compounds 3 and 5 were detected by monitoring both absorbance at 235 nm (0.05 AUFS) and dpm at 85% static efficiency using a 3:1 cocktail to eluent ratio and a 0.5 ml flow cell.

Retention times were 19.4 and 33 min. for **3** and **5**, respectively. NMR ($CDCl_3$) for both labelled and unlabelled standard exhibited δ 2.15 (s, 3H, CH_3CO), 4.06 (t, 3H, J = 1.46 Hz, CH_3NO) 5.37 (q, 2H, J=1.46 Hz,=NCH_2O). The distillate from crude **5** contained 6 mCi of **3**. This distillate on rebromination, acetylation and purification by TLC afforded 900 μCi of pure **5**. The total yield of **5** was 4.93 mCi (24%) based on **1**.

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