Studies on Methylazoxymethanol Synthesis of ^{14}C and ^{3}H labelled methylazoxymethyl-acetate *

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ABSTRACT

The synthesis of ^{14}C and ^{3}H labelled methylazoxymethylacetate, (MAM-OAc), a hepatotoxic and carcinogenic compound, was accomplished by the incorporation of a radioisotope labelled methyl group into N,N'-dimethylhydrazine with methyl iodide instead of dimethyl sulfate. Monosodium dibenzoylhydrazine was methylated with labelled methyl iodide and the second methyl group was subsequently added with unlabelled dimethyl sulfate to produce N,N'-dimethylhydrazine. This modification made it possible to prepare labelled N,N'-dimethylhydrazine with a small amount of total radioactivity and avoid an excessive waste of the methyl label. The labelled compound was then used in the synthesis of MAM-OAc by a previously published procedure. The incorporation of radioactivity into N,N'-dimethylhydrazine was in the range of 75-95 %. *Better yields were obtained for ^{3}H than for ^{14}C MAM-OAc.*

INTRODUCTION

The glucoside cycasin, (β-D-glucosyl-azoxymethane) [1,2] has been reported to be hepatotoxic and carcinogenic when administered orally to rats [3,4]. The toxic component has been demonstrated to be the aglycone,

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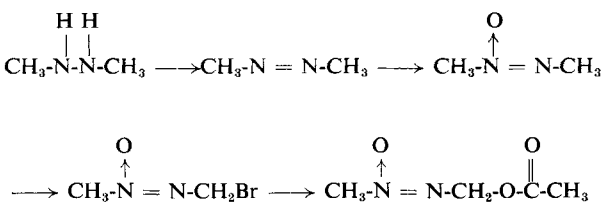
methylazoxymethanol (MAM) [5,6], and a chemical synthesis of the aglycone as the acetate ester has been reported [7]. The ester, unlike the glucoside, is toxic even when injected into animals [7,8] and thus is more convenient for use in biological experiments.

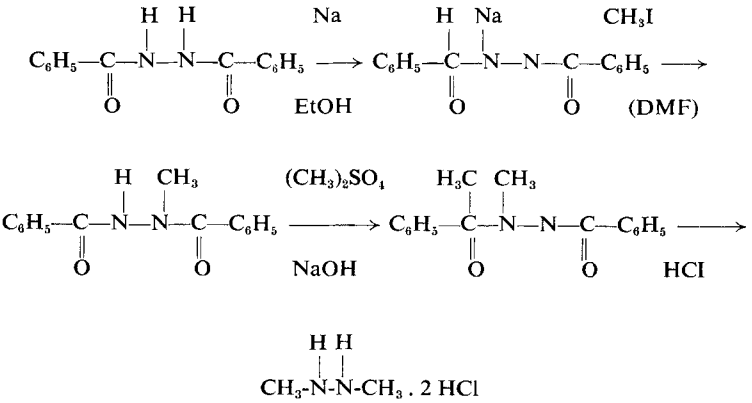
The synthesis of methylazoxymethyl-acetate (MAM-OAc) was accomplished by the oxidation of N,N'-dimethylhydrazine to azomethane then to azoxymethane, followed by bromination of azoxymethane in the allylic position by the Wohl-Ziegler reaction, and subsequent conversion to the acetate.



A trial synthesis of the ^{14}C labelled MAM-OAc starting with a commercially prepared ^{14}C labelled azomethane yielded only a small quantity of the product although the "cold runs" with the same quantities of materials provided satisfactory yields. There are indications that the poor yield may have been due to the effect of radiation on the compounds in the series of reactions.

An attempt to incorporate ^{3}H into the azoxymethane molecule also proved unsuccessful. Exposure of azoxymethane to one curie of tritium gas showed very little exchange, if any, while exposure to three curies of tritium produced considerable darkening of the compound [9].

Preparation of labelled MAM-OAc depends on the availability of high specific activity ^{14}C or ^{3}H labelled N,N'-dimethylhydrazine, the starting material. The hydrazine is usually prepared by methylation of N,N'-dibenzoylhydrazine with dimethyl sulfate, and thus the ^{14}C or ^{3}H label is placed in the methyl groups of dimethyl sulfate. It has been reported that ^{14}C labelled dimethyl sulfate can be prepared in 74 % yield [10]. However, only one of the methyl groups is reactive and in addition a three mole excess of the compound is needed for satisfactory methylation of N,N'-dibenzoylhydrazine. Therefore, this reaction not only results in an excessive waste of the radioisotope but because the overall yield of MAM-OAc is low it is necessary to start with a large total radioactivity, which makes the synthesis unsuitable for a biochemical laboratory. Consequently, it was necessary to develop a system for the preparation of N, N-dimethylhydrazine which required the use of a smaller quantity of radioactivity. This communication describes a synthesis of MAM-OAc with methyl iodide (iodomethane) as the agent for the incorporation of radioisotope labelled methyl group. The synthesis of N,N'-dimethylhydrazine was achieved according to the following series of reactions :



The dimethylhydrazine was used to synthesize MAM-OAc, and 75-95 % incorporation of the radioisotopic label into MAM-OAc was attained.

Methods and results

The procedure described below was tested with low activity ^{14}C methyl iodide and repeated later with higher activity ^{14}C and ^{3}H methyl iodide. Each of the steps was first thoroughly studied with unlabelled material to determine the optimum conditions for the reaction. At each step a small sample was removed, thoroughly dried, and melting point determination and radioactivity count was made on the sample. The compound was dissolved in a toluene-alcohol mixture containing a phosphor (0.5 % PPO-POPOP (49 : 1) in toluene) and counted in a Beckman Scintillation Counter.

N,N'-dibenzoylhydrazine [11]

This compound, which was used as the starting material, was prepared according to the method described in *Organic Synthesis.*

Monosodium N,N'-dibenzoylhydrazine [11,12]

N,N'-dibenzoylhydrazine (25 g) was added to a liter Erlenmeyer flask containing ethanol-sodium ethoxide (2.4 g sodium in 500 ml absolute ethanol). The flask was attached to a condenser placed on a hot plate stirrer and the mixture was refluxed with stirring for 30 min, then cooled, and filtered. The salt was washed with ethanol (300 ml) followed with ether (500 ml) then dried in a vacuum desiccator over calcium chloride. The yield of monosodium N,N'-dibenzoylhydrazine was 25.2 g. (93 %)

Monomethyl N,N'-dibenzoylhydrazine

The monosodium N,N'-dibenzoylhydrazine (25.2 g) was transferred to a liter Erlenmeyer flask containing dimethylformamide (DMF) (100 ml) and

methyl iodide (1.42 g) and stirred for one hour. ^{14}C labelled methyl iodide (11·4 g, total activity : 0.440 mC) was then added and the mixture was stirred overnight at room temperature. Finally, another 1.42 g of unlabelled methyl iodide was added and the mixture was stirred until a drop of the liquid tested on Hydrion paper was neutral. The mixture was added to 2 liter of cold water and, after standing for three hours, the precipitate was filtered and washed with water.

The yield of monomethyl N,N'-dibenzoylhydrazine was 20.6 g (85 %). Melting point 134-37° C. Literature [13] : 145° C.

N,N'-dimethyl dibenzoylhydrazine [11]

The monomethyl N,N'-dibenzoylhydrazine (20.6 g) was transferred to a 500 ml Erlenmeyer flask with water and was mechanically stirred. The compound was methylated at 95° C with dimethyl sulfate (24 ml) added in two ml portions at 2 min intervals. Sodium hydroxide (50 %) was added at a rate which maintained the reaction in a slightly alkaline condition when tested with Hydrion paper. The solid was filtered, crushed in water, filtered again and dried.

The yield of N,N'-dimethyl dibenzoylhydrazine was 19.4 g (89 %) Melting point 82-85° C. Literature [14] : 85° C.

N,N'-dimethylhydrazine dihydrochloride [11]

The N,N'-dimethyl dibenzoylhydrazine was transferred to a liter Erlenmeyer flask, connected to a condenser, and refluxed with 32 % hydrochloric acid (70 ml) for two hours. The hydrolyzate was filtered through a Buchner funnel to remove the benzoic acid. The filtrate was extracted with ether-benzene (1 : 1) to remove the remaining benzoic acid in solution. The water was evaporated under reduced pressure and the solid was dried with absolute ethanol. The compound was recrystallized from ethanol by adding equal volumes of ethyl ether and petroleum ether. The yield of N,N'-dimethylhydrazine dihydrochloride was 9.3 g (97 %). Melting point 147-155° C. Literature [11] : 165-167° C.

Azomethane and azoxymethane [7]

These compounds were prepared in sequence without separating azomethane. Slight modifications in procedure were made from that reported earlier [7]. Azomethane was prepared by oxidizing N,N'-dimethylhydrazine with yellow mercuric oxide [15]. The azomethane generator was connected through a drying tube containing calcium chloride to a cylinder (250 ml) which was attached to a Dewar condenser filled with an acetone-dry ice mixture. The cylinder contained m-chloroperbenzoic acid dissolved in ether (200 ml) and the azomethane was bubbled through the liquid. Nitrogen gas was passed through the system while the azomethane was generated.

The ether solution was transferred to a flask, capped, and kept for two

days at 10° C then distilled. Azoxymethane boiling between 90-100° C at atmospheric pressure was collected.

The yield of azoxymethane was 1.61 g (31 %).

Methylazoxymethyl-acetate (MAM-OAc) [7]

The azoxymethane (1.61 g) was placed in a 100 ml round bottom flask and carbon tetrachloride (50 ml) containing N-bromosuccinimide (3.95 g) was added. The flask was connected to a condenser and irradiated [16] with an infra-red lamp for 45 min. The heat of the lamp was sufficient to reflux the mixture. The mixture was cooled and the succinimide was filtered off and the filtrate was returned to the flask. Silver acetate (5 g) was added and the mixture was stirred overnight with the flask covered with aluminium foil to exclude light. The silver bromide was filtered off and the solvent was removed at atmospheric pressure then the MAM-OAc was distilled at 45-49° C at 0.45 mm of mercury.

The yield of MAM-OAc was 0.772 g (27 %) and the compound had a specific activity of 0.0039 mC/mM. The infrared spectrum of the compound was identical with that of pure MAM-OAc.

The yields, specific activities, and percentages of ^{14}C or ^{3}H incorporated into four preparations of MAM-OAc with methyl iodide of higher total radioactivities than that used in the procedure described above are shown in Table I.

TABLE I. Yield, specific activity, and percentage of ^{14}C or ^{3}H incorporated into methylazoxymethyl-acetate.

Activity of Methyl Iodide Added mC		Yield of MAM-OAc g	Yield of MAM-OAc (%)	Specific Activity mC/mM	Incorporation [a] %
25	(^{3}H)	1.83	(14.6)	0.23	95
100	(^{3}H)	1.77	(14.1)	0.83	86
0.44	(^{14}C)	1.17	(9.4) [b]	0.0032	75
8.7	(^{14}C)	0.165	(5.2)	0.075	90

[a] Based on monosodium N, N'-dibenzoylhydrazine.
[b] Average of two experiments.

DISCUSSION

A modification of the methylation procedure in the preparation of N,N'-dimethylhydrazine makes it possible to synthesize a radioisotope labelled MAM-OAc starting with a reasonable level of total activity. Methylation

by the dimethyl sulfate procedure can incorporate only approximately 6-8 % of the total radioactivity added, whereas the new procedure incorporates about 87 % of the added activity and thus only one-tenth as much radioactivity is required. There is a wide variation in the percentages of activity incorporated, in the four experiments reported in Table I, and this is probably due primarily to the effect of radiation on the intermediate compounds in the synthesis.

The number of steps in the synthesis could have been reduced and MAM-OAc with a higher specific activity could have been obtained if the reaction of methyl iodide with disodium dibenzoylhydrazine were satisfactory. The disodium compound, prepared by the reaction of sodium and N,N'-dibenzoylhydrazine refluxed in toluene, was reacted with methyl iodide in benzene at 140° C for three days but no product was formed. The reaction was also carried out in dimethylformamide (DMF) but only a small yield of dimethyl dibenzoylhydrazine was obtained. The methylation of monosodium dibenzoylhydrazine, prepared by the sodium ethoxide method, was more reliable and convenient. Monosodium dibenzoylhydrazine is hygroscopic and difficult to purify, therefore, a small quantity of unlabelled methyl iodide was used first to react with the contaminants before the labelled material was added. The last quantity of unlabelled methyl iodide was added to be certain that the methylation was complete.

An important factor in the methylation with methyl iodide was the use of DMF as the solvent. Little or no reaction occurred with some of theo ther solvents tried, or the methyl iodide reacted with the solvent. The addition of water to the reaction mixture separated the bulky monomethyl N,N'-dibenzoylhydrazine and simplified the removal of the solvent.

The specific activity of ^{3}H MAM-OAc probably can be increased, without changing any condition of the synthesis, simply by using more compound in the second addition of methyl iodide. There is some question as to whether or not the specific activity of ^{14}C MAM-OAc can be increased without affecting the yield of the compound. The yield of MAM-OAc is lower for the ^{14}C than for the ^{3}H labelled compound and this may be due to the effect of stronger radiation of ^{14}C on the intermediate compounds in the synthesis. However, the quantities of material used produced the minimum quantity of MAM-OAc distillable with the equipment used and on the basis of experience with unlabelled synthesis it is probable that if larger quantities of compounds are used the yield and specific activity of ^{14}C MAM-OAc can be improved.

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