SYNTHESIS OF N-HYDROXYMETHYLPENTAMETHYLMELAMINE (RING ^{14}C), A CYTOTOXIC-ALKYLATING METABOLITE OF HEXAMETHYLMELAMINE[1]

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SUMMARY

The ^{14}C-ring labeled carbinolamine N-hydroxymethylpentamethylmelamine, a metabolite of the clinically useful anticancer agent hexamethylmelamine, was prepared in good yield from cyanuric chloride (ring, 2,4,6,-^{14}C). Amination of cyanuric chloride with dimethylamine gave 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine-2,4,6-^{14}C. Further amination with anhydrous methylamine gave pentamethylmelamine-2,4,6-^{14}C. Condensation of pentamethylmethylmelamine with excess aqueous formaldehyde gave N-hydroxymethylpentamethylmelamine in 54% overall yield.

Keywords: Hexamethylmelamine, metabolite, carbinolamine, radiolabeled.

INTRODUCTION

The carbinolamine N-hydroxymethylpentamethylmelamine (4, Scheme I) has been identified as an *in vitro* metabolite of the anticancer agent hexamethylmelamine (1). Hexamethylmelamine is an investigational antitumor agent with demonstrated activity in combination and as a single agent against several human cancers including ovarian carcinoma, lymphomas and small-cell carcinoma of the lung (2,3,4).

There is evidence that metabolism is required for antitumor activity of hexamethylmelamine, but the mechanism responsible for antitumor activity is not known. Our studies, as well as others, show that hexamethylmelamine is oxida-

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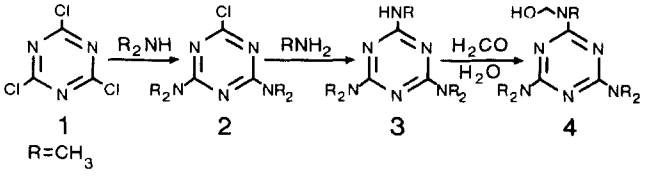
tively metabolized to demethylated products with production of formaldehyde (5,6,7). We have recently reported that hexamethylmelamine undergoes oxidative metabolic transformation to reactive intermediates as measured by covalent binding to microsomal protein and exogenously added DNA (8,9). Nicotine and other tertiary aliphatic amines have also recently been shown to undergo oxidative metabolic transformation to carbinolamine intermediates which can be trapped as cyanide or protein adducts (10,11). These studies have indicated that carbinolamine intermediates undergo two metabolically important reaction pathways: retro-aldol reaction leading to demethylated products and formaldehyde and β-elimination to iminium intermediates which are reactive electrophiles. To further characterize the metabolic activation and covalent binding characteristics of hexamethylamine, we required the radiolabeled carbinolamine N-hydroxymethylpentamethylmelamine.

Scheme 1



Synthesis of 4 in low yield from pentamethylmelamine (3, Scheme I) and aqueous formaldehyde has been previously reported (12). Synthesis of 3 and ^{14}C-ring labeled 3 by amination of cyanuric chloride have also been reported (13). For our synthesis of ^{14}C-ring labeled 4 (Scheme I), we have modified these procedures to increase yields of products and improve methodology of product isolation and purification. We now report synthesis of the carbinolamine N-hydroxymethylpentamethylmelamine (4) in 54% over all yield from ^{14}C-cyanuric chloride.

EXPERIMENTAL

^{14}C-Cyanuric chloride was obtained from Pathfinder Laboratories, Inc., St. Louis, MO. All starting materials, reagents and solvents were purified prior to use. All reactions were conducted under an argon atmosphere. Removal of

solvent under reduced pressure was carried out using a rotary evaporator with aspirator vacuum, unless otherwise noted. Samples were dried in vacuo (0.01 mm) at ambient temperature. Electron impact mass spectra were obtained with LKB 2091 and Kratos MS-50 GC/MS spectrometers. ^{1}H-NMR spectra were obtained with an IBM NR-80 FT spectrometer in $DCCl_3$ solvent. Chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. Infrared and ultraviolet spectra were obtained with Beckman Acculab-8 and UV-5260 spectrophotometers, respectively. Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. Analytical thin-layer chromatography was carried out on Merck precoated silica gel F-254 plates (.25 mm).

Synthesis of 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine (2)

Amination of cyanuric chloride (50 mg, 271 μmoles) with aqueous dimethylamine (2.0 ml, 804 μmoles) as previously reported (14) afforded, after recrystallization from pentane, monochloro-triazine 2 (53.3 mg, 264 μmoles, 97% yield); m.p. 67-68°C, lit. m.p. 66-68°C (14).

Synthesis of pentamethylmelamine (3)

A solution of the monochloro-triazine 2 (50 mg, 248 μmoles) in ether (3 ml) was added to a 25 ml two-neck round bottom flask equipped with a dewar condenser, gas bubbler, magnetic stirring bar and sealed with rubber septa. Methylamine gas (approximately 15 ml) was condensed and the mixture stirred at reflux for 15 hr. Methylamine and ether were then removed with the aid of a stream of argon and warm water bath. After trituration of the crystalline residue with 3 portions of ether (5 ml each) and removal of solvent, a white crystalline material was obtained. Low temperature (-70°C) recrystallization from pentane gave pentamethylmelamine 3 (51 mg, 243 μmoles, 98% yield); m.p. 100-102°C lit. m.p. 98-103°C (12).

Synthesis of N-hydroxymethylpentamethylmelamine (4)

To a solution of pentamethylmelamine (40 mg, 190 μmoles) dissolved in THF (1.35 ml) in a 15 ml round bottom flask was added freshly prepared aqueous formaldehyde (2.70 ml, approximately 30%, prepared by pyrrolysis of paraformaldehyde).

After stirring at room temperature 12 hr most THF was removed *in vacuo* and the resulting mixture lyopholized. Recrystallization of the dried residue twice from pentane gave 4 (24.5 mg, 108 μmoles, 57% yield) as a white crystalline solid (prisms) with m.p. 119-121°C lit. m.p. 119.5-123°C (12). ^{1}H-NMR ($CDCl_3$): 3.10 s 12H, 3.18 s 3H, 4.2 br s 1H, 4.97 s 2H. IR (KBr): 2940 cm^{-1}, 1545, 1395, 1050, 775. MS (m/z): 226 (m+), 209, 196 (100%), 181, 167, 153, 135.

Synthesis of radiolabeled N-hydroxymethylmelamine (4)

^{14}C-labeled 4 was prepared according to the procedures described for unlabeled material with the exception that intermediate products were used immediately and without further purification.

^{14}C-cyanuric chloride (13.16 mg, 7.14 mCi/mmole) was diluted with freshly sublimed cyanuric chloride (33 mg, 179 μmoles). The carbinolamine product 4 obtained was recrystallized 3 times from pentane and then dried *in vacuo*. ^{14}C-4 (30.39 mg, 134 μmole, 2.42 mCi/mmole) was thus obtained in 54% overall yield with m.p. 119.5-121°C. Admixture with authentic unlabeled 4 did not depress or broaden the melting point.

Treatment of 4 with 1 N HCl followed by basification and extraction with chloroform gave 3 as identified by thin-layer chromatography (ethyl acetate), gas chromatography and mass spectral analysis.

RESULTS AND DISCUSSION

The ^{14}C-ring radiolabeled carbinolamine N-hydroxymethylpentamethylmelamine (4) has been prepared conveniently and in 54% overall yield from ^{14}C-cyanuric chloride. Pentamethylmelamine, an intermediate product in the synthesis of 4 was prepared by an improved procedure and in greater yield than that previously reported (15).

Our previous studies have shown that the anticancer agent hexamethylmelamine is metabolically activated by hepatic microsomal preparations to reactive intermediates which covalently bind to protein and calf thymus DNA (8,9). Incubation

of ^{14}C-radiolabeled 4 prepared as described in this article with microsomal protein or with calf thymus DNA (in the absence of an NADPH-generating system) results in covalent binding of radiolabel to these macromolecules (9). Following incubation of hexamethylmelamine with fortified preparations or incubation of 4 directly with macromolecules, hydrolysis of isolated DNA and protein pellets yields the retro-aldol product 3 (9). We suggest that NADPH-dependent covalent binding of hexamethylmelamine to tissue macromolecules may be explained by metabolic formation of N-hydroxymethylpentamethylmelamine (4) and subsequent β-elimination to the iminium intermediate which reacts with nucleophilic moieties of DNA and protein. This hypothesis is consistent with data reported by Nguyen et al (11) and Ziegler et al (15) on their metabolism studies with nicotine and methapyrilene. Formation of cyanide and protein adducts during metabolic N-demethylation of these compounds was best explained by formation of carbinolamine and iminium intermediates.

In continuing studies with 4, we have documented that incubation of this metabolite inhibits colony formation of human tumor cells in culture (16). Similar toxicity with hexamethylmelamine is observed only when NADPH-fortified hepatic preparations are co-incubated with drug and tumor cells (16). We are continuing our studies on the mechanism of antitumor activity of hexamethylmelamine and 4.

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