

ACYLHYDROPEROXIDE OXIDATIONS OF THE ANTICANCER AGENT HEXAMETHYLMELAMINE

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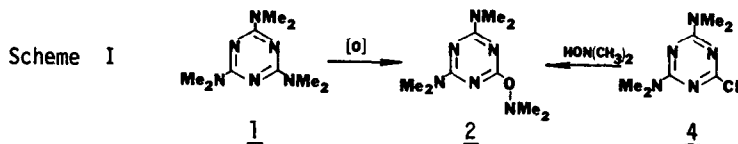
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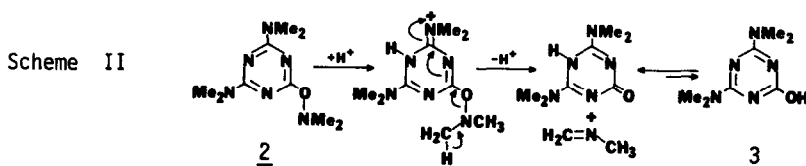
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Summary: The clinically effective anticancer agent hexamethylmelamine undergoes an unusual acylhydroperoxide oxidative transformation to give the novel product 2,4-bis(dimethylamino)-6-[(dimethylamino)oxy]-1,3,5-triazine.

Hexamethylmelamine (1) is an established antitumor agent which is effective against several human malignancies (1). We have reported previously that N-demethylation is the major route of microsomal metabolism which is mediated by cytochrome P-450 (2,3). More recently, we have turned our attention to other oxidative pathways which may be important to the required metabolic activation of this agent. Since acylhydroperoxide oxidations are known to model flavin-oxidase (e.g. amine oxidase) mediated metabolism of substituted heteroaromatic compounds, we undertook and now report our studies on acylhydroperoxide oxidations of hexamethylmelamine.



Previous studies reported in the literature (4) indicated the potential for N-oxidation at heterocyclic nitrogen of 1. We have found that 1 instead undergoes a facile as well as an unusual acylhydroperoxide oxidative transformation which has not been reported for this class of heterocycle. Oxidation of 1 by various peracid reagents gives in good yield (Table 1) 2,4-bis(dimethylamino)-6-[(dimethylamino)oxy]-1,3,5-triazine (2). A typical preparation of 2 was conducted as follows: To a solution of 1 (10 g, 47.6 mmole) in chloroform (100 mL) was added a solution of m-chloroperoxybenzoic acid (16.7 g, 57.1 mmole, 58.96 ± 2.21% by iodometric titration) in chloroform (400 mL). After stirring at 50 °C for 24 hr, the cooled reaction mixture was extracted with aqueous carbonate (5% Na<sub>2</sub>CO<sub>3</sub>, 3 X 150 mL) and the organic layer dried with sodium sulfate. Removal of drying



agent by filtration and solvent *in vacuo* afforded a white crystalline solid from which 2 (4.09 g, 38%, m.p. 79.5–80 °C) was obtained after chromatography (silica, ethyl acetate) and recrystallization (pentane).

Table 1 Oxidation of HMM with peroxide reagents.

<u>Peroxide</u>	<u>Solvent</u>	<u>Temp.</u>	<u>Time</u>	<u>1</u>	<u>2</u>	<u>3</u>
MCPBA	HCCl <sub>3</sub>	25°C	24 hr	86% <sup>a</sup>	4%	5%
MCPBA	HCCl <sub>3</sub>	50	8	64	14	19
MCPBA	HCCl <sub>3</sub>	50	24	17	38	41
p-NO <sub>2</sub> PBA	HCCl <sub>3</sub>	50	24	21	27	49
H <sub>2</sub> O <sub>2</sub>	CH <sub>3</sub> CO <sub>2</sub> H	25	13	45(73) <sup>b</sup>	5(8)	12(19)

<sup>a</sup> isolated yields

MCPBA m-chloroperoxybenzoic acid

<sup>b</sup> yields determined by NMR

p-NO<sub>2</sub>PBA p-nitroperoxybenzoic acid

Also obtained from the reaction mixture was recovered 1 (1.68 g, 17%) and an acid catalyzed rearrangement product of 2 which was identified by spectral analysis and independent synthesis as 2-hydroxy-4,6-bis(dimethylamino)-1,3,5-triazine (3). The <sup>1</sup>H-NMR of 2 shows two singlets (3.140 ppm, 12H and 2.902 ppm, 6H). Electron impact mass spectral analysis shows parent ion (226 m/z) and major fragments at 183, 168, 154, 139, and 96 m/z. Structure assignment of 2 was unambiguously confirmed by x-ray crystallographic analysis (5) and independent synthesis (Scheme 1). Independent synthesis of 2 was conducted as follows: To a stirred, room temperature solution of N,N-dimethylhydroxylamine hydrochloride (242 mg, 2.48 mmole, 10 equiv.) and barium hydroxide (380 mg, 2.48 mmole, 10 equiv.) in freshly distilled triethylamine (8 mL) was added 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine (4) (50 mg, .248 mmole, prepared by the procedure of Pearlman and Banks (6)). After stirring at 60° for 17 hr, the cooled mixture was filtered with the aid of diethyl ether. Chromatography of the clear oil, (silica, ethyl acetate) obtained after removal of TEA and ether afforded after recrystallization (pentane) 2 (32%), which was identical in all respects to 2 obtained from peracid oxidations of 1.

Rearrangement (Scheme II) of 2 to triazine 3 was found to occur during oxidations of 1, upon treatment of 2 with acid catalysts and by thermolysis (Table 2). Reductive cleavage of 2 with either sodium borohydride or 5% Pd/C in the presence of hydrogen gave 3 in near quantitative yield. Loss of a dimethylamino fragment from 2 was also observed during mass spectral analysis. Loss of mass 43 (m/z) is a predominant process and results in the spectrum's base peak. Structure assignment of the amine fragment has not been determined. The proposed imine fragment from rearrangement of 2 would be expected to be a reactive alkylating agent which would be capable of reversible covalent binding to tissue macromolecules.

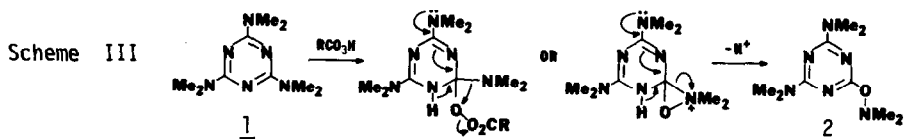
Table 2. Rearrangements of amino-oxy triazine 2.

<u>Solvent</u>	<u>Temp.</u>	<u>Time</u>	<u>2</u>	<u>3</u>
HCCl <sub>3</sub>	25°C	26 hr	97% <sup>a</sup>	3%
	50	26	63	37
	135 <sup>b</sup>	20	0	100(97) <sup>c</sup>
HCCl <sub>3</sub> /H <sup>+d</sup>	25	26	34	66
	50	26	3	97
	135 <sup>b</sup>	20	0	100(98)
HCCl <sub>3</sub> /H <sup>+e</sup>	50	26	0	100
Pentane	135 <sup>b</sup>	20	0	100(99)
H <sub>2</sub> O/H <sup>+f</sup>	25	4	21	79
	50	4	0	100
H <sub>2</sub> O/H <sup>+g</sup>	25	4	0	100(98)
H <sub>2</sub> O	25	24	100	0
	50	24	91	9

a Yields determined by NMR. b Sealed tube reaction. Solutions degassed with argon and then sealed under vacuum. c Isolated yield. d 0.1 equiv. m-chlorobenzoic acid. e 0.1 equiv. p-nitrobenzoic acid. f 0.05 M HOAc/NaOAc pH 5. g 0.01 M HCl.

Structure assignment of 3 was determined from its spectral data and by independent synthesis. The <sup>1</sup>H-NMR of 3 shows one singlet (3.163 ppm, 12H) and a broad peak (about 10.5 ppm, 1H). From the infrared spectrum of 3, it appears that the favored tautomer of 3 is the keto form (strong absorption at 1660 cm<sup>-1</sup>). Electron impact mass spectral analysis shows parent ion (183 m/z) and major fragments at 168, 154, 139 and 98 m/z. Independent synthesis of 3 was conducted as follows: To a solution of sodium methoxide (26.2 mg, 1.14 g-atoms Na) in methanol (8 ml) was added in three portions, 4 (114.9 mg, .569 mmoles). After stirring at 50° C for 13 hr. the cooled reaction mixture was diluted with 5% sodium chloride (30 ml) and extracted with chloroform. Chromatography (silica, ethyl acetate) of the concentrated extract gave after recrystallization (pentane) 2-methoxy-4,6-bis(dimethylamino)-1,3,5-

triazine 5 (83.3 mg, 78% yield, m.p. 89-90°C) Heating (steam bath) 5 (40 mg, .203 mmole) with 6 N hydrochloric acid (1.5 ml) for 20 min. gave after basification, extraction, (chloroform) removal of solvent and recrystallization (pentane) 23.7 mg (63%) of material which was identical to 3 in all respects.



At this time the oxidative reaction mechanism remains ambiguous for the transformation of 1 to 2. However, we propose the tentative reaction mechanism shown in Scheme III to describe this unusual transformation. This transformation is of further interest in view of work reported by Suschitzky and coworkers (7). Their studies demonstrate that peracid oxidations of N,N-dialkylsubstituted-2-amino derivatives of tetrachloro and tetrafluoro pyridines also give the corresponding N,N,O-trisubstituted hydroxylamines. However and in contrast to our oxidation of 1, they also found that introduction of an electron-releasing substituent, such as hydroxy and N,N-dialkylamino at the 4-position leads to the stable exocyclic N-oxide. While endocyclic and exocyclic N-oxide products have not been detected during the peracid oxidations of 1, we cannot preclude the intermediacy of these products at this time. Further chemical and biological studies are in progress to investigate this unusual transformation. Preliminary *in vitro* cytotoxicity and *in vivo* antitumor studies have shown 2 possesses greater antitumor activity than the parent drug (8). In conclusion, acylhydroperoxide oxidation of 1, which is a model reaction of several *in vivo* flavo-protein oxidases, provides an unusual oxidation product that may be important to the mechanism of action of the clinically useful anticancer agent hexamethylmelamine.

#### REFERENCES

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6. W. M. Pearlman and C. K. Banks, *J. Am. Chem. Soc.*, **70** 3728 (1951).
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8. Against A-549 lung carcinoma *in vitro*, 2 has ID<sub>50</sub> of 40 µg/ml. Against P-388 lymphocytic leukemia *in vivo*, 2 has T/C 130%. These and other metabolism-disposition studies will be reported elsewhere.

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