Synthesis, Purification and Spectroscopic Characterization of Potential Impurities of Hexamethylmelamine

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The syntheses and spectroscopic properties (ir, ^{1}H nmr, ^{13}C nmr, uv and ms) of pure samples of 2-chloro-4,6-bis(dimethylamino)-*s*-triazine **1**, 4,6-dichloro-2-dimethylamino-*s*-triazine **2**, 4,6-bis(dimethylamino)-*s*-triazin-2(1*H*)-one **3**, 4-chloro-6-dimethylamino-*s*-triazin-2(1*H*)-one **4**, 6-dimethylamino-*s*-triazine-2,4(1*H*,3*H*)-dione **5**, and 2,4,6-tris(dimethylamino)-*s*-triazine (altretamine, HMM) are reported. Evidence for enol-keto equilibria are also presented for **3**, in which the enol form exhibits as an H-bonded dimer structure similar to the dimer of organic carboxylic acids.

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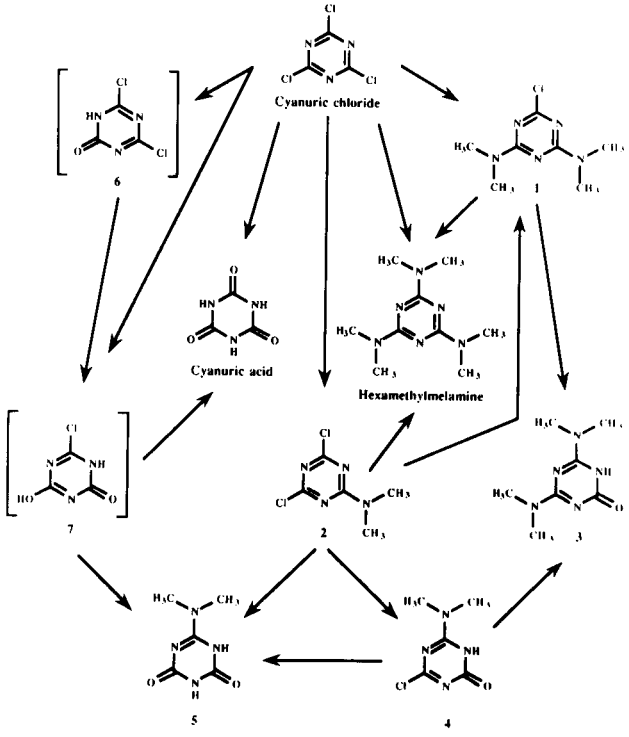
Introduction.

2,4,6-Tris(dimethylamino)-1,3,5-triazine, Hexamethylmelamine (HMM) is a synthetic antitumor agent with significant activity against ovarian, breast, the lymphomas, and small cell carcinoma [1-5]. It has recently been approved by FDA as a secondary line of treatment for ovarian cancer.

HMM is synthesized from cyanuric chloride and dimethylamine in an aqueous media. Due to the high reactivity of cyanuric chloride and hence its partial or complete hydrolysis in water, several potential by-products could therefore form during its manufacture (Scheme 1).

Scheme 1. Chemical pathway for formation of HMM byproducts.



The finished HMM could, therefore, potentially be contaminated with one or a combination of two or more of these impurities. It was therefore desirable that its purity be established by common standards acceptable to the FDA and all participating countries of the European Pharmacopoeia Convention.

To unequivocally determine the absence or presence of any of these potential impurities in the final HMM, it is essential to have a well characterized sample of each of these compounds as standards for hplc analyses. Although the synthesis for some of these compounds have previously been reported, their structural characterization are mostly limited to elemental analysis [6-9]. We wish to report the synthesis, in some cases with optimized yields, and structural characterization of compounds **1-5** by various spectroscopic techniques (ir, ms, ^{1}H-nmr, ^{13}C-nmr, and uv). Purity for each compound was determined by melting point, elemental analysis and hplc.

Results and Discussion.

The ir spectra of cyanuric chloride and compounds **1, 2,** and **4** (Table 1) show characteristic C-Cl stretching bands in the 855-833 cm^{-1} region. The carbonyl stretching of the amide moiety in cyanuric acid and compounds **3, 4,** and **5** show a strong absorption in the 1730-1677 cm^{-1} region. All of the triazine compounds of this work show C-N stretching absorption(s) between 1608-1302 cm^{-1}.

The ^{1}H and ^{13}C nmr chemical shifts for each of the triazine compounds are tabulated in Table 2. The assignment of the peaks for both ^{1}H and ^{13}C spectra was straightforward by using known substituent-induced chemical shifts and published data on related triazines [10-13]. The methyl protons of the dimethylamino groups appear as singlets between 3.09-3.17 ppm. The NH protons of the oxygen containing triazines (cyanuric acid and com-

Table 1

Selected IR Bands of HMM and its Potential Impurities

Compound	CH Strch. (cm^{-1})	CH In-plane bending (cm^{-1})	CH Outo-of-plane bending (cm^{-1})	CN Stretch (cm^{-1})	C-Cl Stretch/C=O amide (cm^{-1})
1	2940, 2870	1044, 988, 1408	791	1577, 1493	833
2	2942	1021, 960	790	1608, 1549	851
3	2933, 2863		789	1590, 1562	/1677
4	2997	994		1519, 1491	849/1698
5		1069, 1055	744	1595	/1730
HMM	2917, 2869	1215, 1053	806	1545, 1392, 1302	

Table 2

^{1}H and ^{13}C Chemical Shifts of HMM and its Potential Contaminants

Compound	NH	CH_3		2	4	6	solvent
		$^{1}H\delta$	$^{13}C\delta$				
Cyanuric Chloride	--	--	--	172.530	172.530	172.530	($CDCl_3$)
Cyanuric Acid	11.15 (br)	--	--	149.806	149.806	149.806	(DMSO)
1	--	3.130 (s)	36.369 (s)	165.033	165.033	169.000	($CDCl_3$)
2	--	3.231 (s)	37.056 (s)	164.839	169.933	169.333	($CDCl_3$)
3	--	3.167 (s)	36.960 (s)	157.190	160.092	160.092	($CDCl_3$)
				165.516			
4	11.329 (br)	3.125 (s)	38.406 (s)	150.653 (?)	153.432	150.653 (?)	(DMSO)
5	10.882	3.094 (s)	37.077 (s)	153.356	153.356	155.758	(DMSO)
	10.515						
HMM	--	3.097 (s)	35.782	165.913	165.913	165.913	($CDCl_3$)

pounds **3-5)** exhibited broad singlets, and in the case of **4** no peaks, in 10.5-11.4 ppm region. Amide protons are normally observed between 6-8 ppm. The downfield shifts and manifestation of broad signals for the NH protons of cyanuric acid and compounds **3** and **5** are indicative of intermolecular hydrogen bonding. This is confirmed by the upfield shift and narrowing of the NH peaks upon dilution. The NH protons of compound **5** are observed at 10.88 and 10.55 ppm. Based on the substituent-induced chemical shift, the peak at 10.88 ppm is assigned to the amide pro-

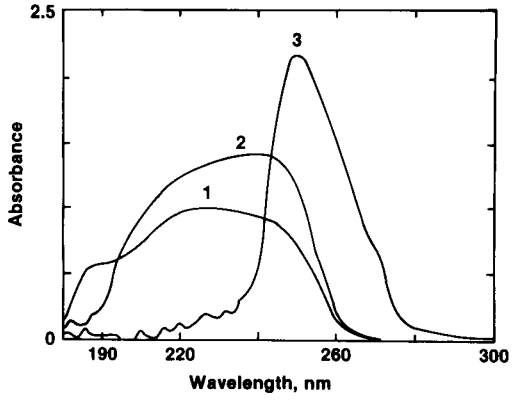


Figure 1. The uv spectra of compound **3** in (1) water, (2) methanol and (3) chloroform at 1.21 x 10^{-4} *M*.

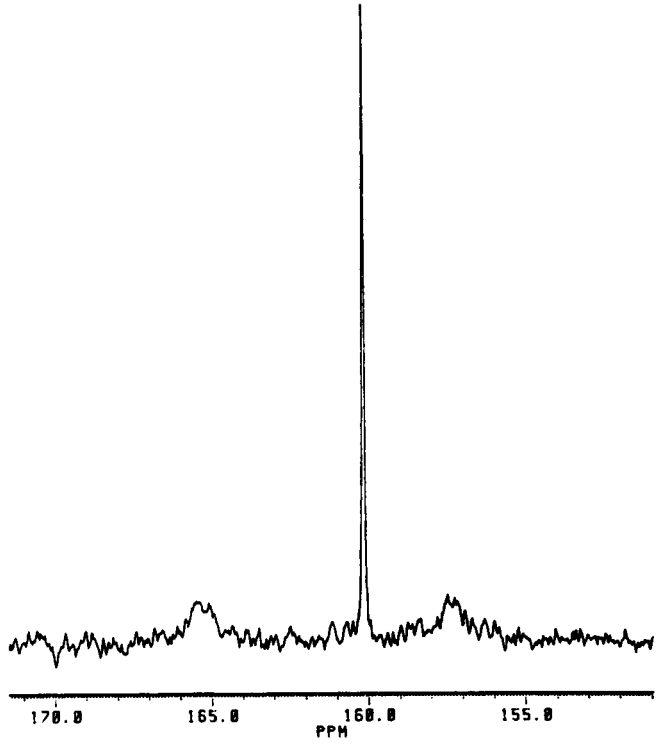
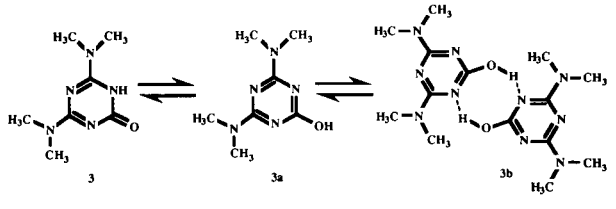


Figure 2. 75.46 mHz ^{13}C spectrum of compound **3** in deuteriochloroform; expanded carbonyl region in which the sharp peak is C_4 and C_6 and the two broad peaks at 165.6 and 157.2 ppm are C_2 of **3** and **3a** or **3b** respectively.

ton adjacent to the two carbonyl groups and the 10.55 ppm to the amide proton adjacent to the double bond and carbonyl groups. Amide protons of cyanuric acid and compound **4** are observed as broad singlets at 11.15 and 11.329 ppm, respectively. The proton spectrum of **3** did not show a peak for amide NH. This can be explained by a slow interchange between the keto and enol structures (**3** and **3a**). Compound **3** can theoretically exist in both enol-keto tautomers. It has been shown that the enolate isomer forms a dimer, **3b**, by intermolecular hydrogen bonding. Bishop *et al.* [15] suggested a four membered ring hydrogen bonded dimer but we favor an eight membered ring dimer **3b** which occurs in carboxylic acids. Further evidence for the existence of both tautomers comes from its hplc behavior [14], solvent dependent uv absorption spectra (Figure 1) and ^{13}C spectrum (Figure 2).

High pressure liquid chromatograms of **3** exhibited a concentration dependent retention time in the region of 1.90-3.95 minutes; its retention time decreased as its concentration increased [14]. Two factors could influence its retention time behavior. The degree of dimerization and the keto-enol equilibrium. Since the enolate form of compound **3** can form a hydrogen-bonded dimer (structure **3b** [15]) its degree of dimerization should increase as the concentration increases. The hydrogen-bonded dimer is expected to be less polar than its monomer, it should therefore elute at a later retention time in a reverse phased hplc mode. However, this is opposite to the retention time behavior displayed by compound **3** [14]. This suggests that compound **3** undergoes a concentration dependent tautomeric shift between an enol and a keto form. Since keto-isomer is more polar than the enolate form, the decrease in retention time for the more concentrated solution of **3** may indicate that, the keto isomer is favored as concentration increases.



To examine the distribution of the two isomers as a function of solvent polarity, uv absorption spectra of **3** were measured in water, methanol, and chloroform (Figure 1 and Table 3). In going from water to methanol to chloroform its λ max shifted from 232 to 252, a bathochromic shift, with increasing molar extinction coefficient. This is consistent with shift of this keto-enol equilibrium in favor of the enol tautomer **3b** which possesses a higher degree of conjugation, as the polarity of the solution decreases.

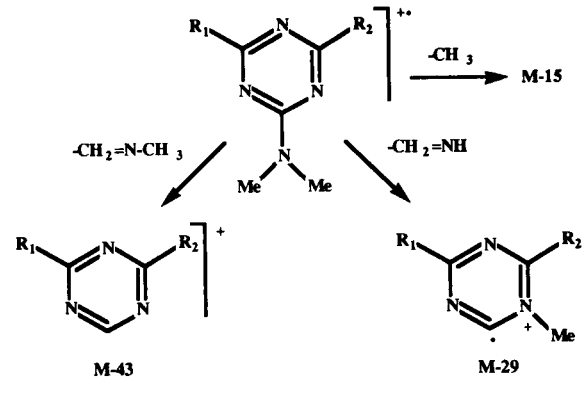
Table 3
UV Spectral Data of HMM and its Potential Contaminants

Compound	Solvent	Max (nm)	ε Max
1	[a]	232	35800
		272	3800
2	[a]	240	23300
3	[b]	244	27400
		274	9500
4	[a]	214	15700
		264	15500
5	[b]	234	7850
		276	3700
Cyanuric acid		216	870
		276	3700
HMM	[a]	228	49900
		276	4270

[a] CH_3CN, [b] CH_3CN:H_2O(98.7:1.3). [c] CH_3CN:H_2O (98:2).

Proton-decoupled ^{13}C nmr spectrum (Figure 2) of compound **3** showed two broad signals at 165.516 and 157.190 ppm and a sharp peak at 160.092. This observation further suggests the presence of a tautomeric exchange process between structures **3** and **3a**. The resonance signal at 160.092 ppm is assigned to carbons 4 and 6 and the two broad peaks at 165.516 and 157.190 ppm to carbon 2. The broadening of the signals is due to the slow interconversion of **3** and **3a** or **3b** on the nmr time scale. No temperature studies were done to confirm this as nmr time availability was restricted.

Direct-inlet electron impact (EI) mass spectra of the triazines of this study (Figure 3 and Table 4) show strong molecular ion (M^+) at their expected m/z value, which losses a methyl radical to give m/z M-15. The molecular ion peaks were base peaks for subsequent fragmentation in all compounds studied. They exhibit some of the same characteristics, but the presence of different substituents affect the relative abundance of the fragments. Elimination of a neutral fragment ($-CH_2=NH$) from one of the dimethylamino substituent with simultaneous migration of a methyl group to a ring nitrogen gives rise to ions m/z M-29. The



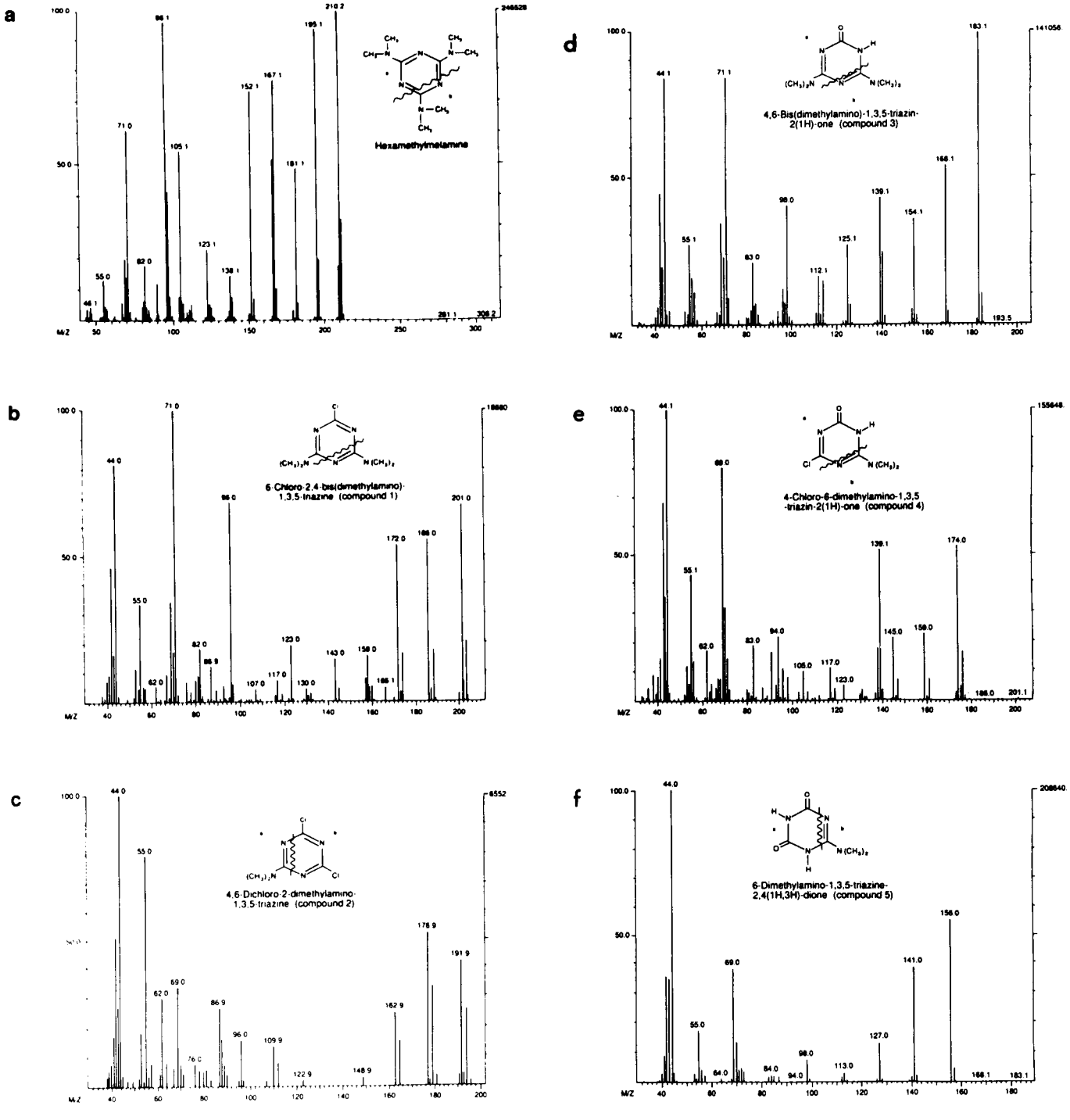


Figure 3. EI-MS spectra of HMM (a), compound **1** (b), compound **2** (c), comound **3** (d), compound **4** (e) and compound **5** (f).

M-43 mass ions appear to be a result of CH_3 and H rearrangement with losses of $[N(CH_3)CH_2]$ from the molecular ion peaks. But, the loss of $[N(CH_3)CH_2]$ fragment in compound **3** is a radical loss and not a rearrangement. The masses at m/z M-69 and 69 appear also to be rearrangement ions [a + H] and [b − H] as illustrated in Figure 3a-f. Similarly, fragments at mass M-71 and 71 could be explained by the ions [a − H] and [b + H].

Table 4

Selected Mass Spectral Fragmentation of HMM and its Potential Impurities

Compound	M+	M-CH_3	M-($NHCH_2$)	M-($NH(CH_3)CH_2$)	[a+H (-H)]	[b+H (-H)]	M-Cl
1	201	186	172	158	132 (130)	71 (69)	166
2	192	177	163	149	123 (121)	71	
3	183	168	154	139	114 (112)	71 (69)	
4	174	159	145	131	105 (103)	71 (69)	139
5	156	141	127	113	87 (85)	71 (69)	
HMM	210	195	181	167	141 (139)	71 (69)	

Compounds **1**, **2**, and **4**, which possess chlorine atoms, showed characteristic chlorine isotope peaks at two mass units higher than their respective molecular ion peaks and fragments containing chlorine atoms (Figure 3b, c, and e). The relative isotopic abundance of fragments bearing chlorines were consistent with the number of chlorine atoms present. Compounds **1** and **4** are found to undergo facile loss of chlorine atom to give M-35 fragments. The mass spectrum of compound **2**, on the other hand, does not show a peak for the loss of a chlorine atom from the molecular ion peak illustrating the influence of substituents on the fragmentation pattern of triazines.

EXPERIMENTAL

Measurements.

Melting (decomposition) points, uncorrected, were determined on a Gallenkamp MF-370 apparatus equipped with ATKINS J. Thermocouple Model 33033-JC. The ir spectra were run as potassium bromide pellets on a Perkin Elmer-FT Spectrometer Model 1710 IR Spectrometer. The 75.46 MHz ^{13}C, and 300.13 MHz ^{1}H nmr spectra were run on a Bruker AM-300 WB spectrometer using a 5-mm probe for both ^{1}H, and ^{13}C and an internal lock on the deuterium solvents, deuteriochloroform or DMSO. The ^{13}C nuclei were composite pulse proton-decoupled (CPD). Chemical shifts are reported in ppm δ units, and are relative to internal tetramethylsilane (TMS). The mass spectra were obtained on a Finnigan MAT 312 with a magnetic double focusing and a reverse Neir-Johnson configuration equipped with a Data General Nova-4 with Finnigan "INCOS" software. The instrument was calibrated with perfluorokerosene, high boiling (Peninsular Chemresearch, Inc.). The ionization mode was electron impact at 70eV. The scan range was 35 to 800 amu at a scan rate of 5 s/scan. Resolution was approximately 700. The electron multiplier voltage was 1600 emV; emission current, 1 mA; accelerating voltage 3000 V; source temperature, 250°; inlet temperature program, 30 to 450°. HPLC assays were carried out with a high-pressure liquid chromatography system consisting of a Waters 6000A pump, a Waters U6K injector and a Water Lambda-Max 480 detector (Waters Associates, Milford, Massachusetts) connected to a Waters Model 730 Data Module. The system was operated at 226 nm using a 5-μm Beckman Ultrasphere C_{18} (25 cm x 4.6 mm) column equilibrated with the mobile phase (isocratic, water-acetonitrile, 50:50) at a flow rate of 1.5 ml/min. The elemental analyses were carried out at Oneida Research Services, One Halsey Road, Whitesboro, NY 13492.

Chemicals.

Water (hplc grade) was obtained from Fisher Scientific (Fisher Scientific Co., Fair Lawn, New Jersey); hplc grade acetonitrile (J. T. Baker, Phillipsburg, New Jersey); cyanuric acid (98%) and cyanuric chloride (99%) (Aldrich Chemical Co., Milwaukee, Wisconsin); HMM (Pharm-Eco, Simi Valley, California, lot #3244).

Syntheses.

6-Chloro-2,4-bis(dimethylamino)-1,3,5-triazine (**1**), was synthesized in 55% yield according to the procedure of Pearlman and Banks [6] 68-69° (lit [6] 66-68°).

Anal. Calcd. for $C_7H_{12}ClN_5$: C, 41.69; H, 6.00; N, 34.73; Cl, 17.58. Found: C, 41.40; H, 5.99; N, 34.99; Cl, 17.54.

4,6-Dichloro-2-dimethylamino-1,3,5-triazine (**2**).

This compound was prepared according to the procedure of Pearlman and Banks [6] in comparable yield as very long white thin needles, mp 123-124° (lit [6] 122.5-123.5°).

Anal. Calcd. for $C_5H_6Cl_2N_4$: C, 31.11; H, 3.13; N, 29.02; Cl, 36.73. Found: C, 31.22; H, 2.81; N, 29.08; Cl, 33.01.

4,6-Bis(dimethylamino)-1,3,5-triazine-2(1*H*)-one (**3**).

This compound was synthesized by modified literature procedures [15,11] as colorless thick needles, mp 292-293° (lit [10,11] 285-288°; 290-292°).

Anal. Calcd. for $C_7H_{13}N_5O$: C, 45.89; H, 7.15; N, 38.22. Found: C, 45.98; H, 7.05; N, 38.20.

4-Chloro-6-dimethylamino-1,3,5-triazin-2(1*H*)-one (**4**).

To a cooled stirred solution of sodium hydroxide (1.0*N*, 12.95 ml, 12.95 mmoles) containing acetonitrile (15 ml) was added 2,4-dichloro-6-(dimethylamino)-1,3,5-triazine (1.0 g, 5.181 mmoles) in one portion. After a homogeneous clear solution was effected, sodium hydroxide (1.0*N*, 7.77 ml, 7.77 mmoles) followed by compound **2** (1.0 g, 5.181 mmoles) were added. This sequence of addition of base and compound **2** was repeated until a total of 44 ml (44.03 mmoles) of 1.0*N* sodium hydroxide solution and 5.0 g (25.9 mmoles) of compound **2** had been added. Finally, 7.77 ml (7.77 mmoles) of 1.0*N* sodium hydroxide was added and the mixture allowed to stir at room temperature overnight. After filtration, the cooled solution was treated with 1.0*N* hydrochloric acid (50 ml until the solution reached *p*H 3.0). The white precipitate that formed was filtered, washed sequentially with copious amount of cold water, ethanol, acetone, and chloroform and dried in a desiccator under high vacuum yielding 2.38 g of a white powder, mp 198° dec.

Anal. Calcd. for $C_5H_7ClN_4O$: C, 34.39; H, 4.04; N, 32.09; Cl, 20.30. Found: C, 34.24; H, 3.90; N, 32.40; Cl, 20.30.

6-(Dimethylamino)-1,3,5-triazine-2,4(1*H*,3*H*)-dione (**5**).

A suspension of 4-Chloro-6-dimethylamino-1,3,5-triazin-2(1*H*)-one (5.0 g) in water (80 ml) was refluxed for 15 minutes. After cooling to room temperature, all of the solvent was removed under reduced pressure. The resulting solid residue was triturated with boiling methanol and filtered while hot to yield 4.285 g of a white powder of the desired compound **5**, mp 327-328° dec.

Anal. Calcd. for $C_5H_8N_4O_2$: C, 38.46; H, 5.16; N, 35.88. Found: C, 38.15; H, 5.16; N, 35.34.

Compounds **6** and **7**.

Attempted synthesis and isolation of compounds **6** and **7** failed. The chlorines in compounds **6** and **7** are highly reactive. Using Horrobin's procedure [8], compound **7** was synthesized as a monosodium salt but its hplc showed some impurities. Purification of compound **7** by means of crystallization from several different solvents and solvent mixtures resulted in the total loss of its chlorine and yielded cyanuric acid. **CAUTION!** We have experienced a minor explosion after storage in a closed vessel of what we thought was compound **6**, at 4° for 48 hours. We believe that it decomposes to give off hydrogen chloride.

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