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Gas-phase reactions of alcohols with hexamethylene triperoxide diamine (HMTD) under atmospheric pressure chemical ionization conditions

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RATIONALE: Hexamethylene triperoxide diamine (HMTD) is a sensitive peroxide explosive first synthesized in 1885. HMTD exhibits an unusual gas-phase phenomenon in the presence of alcohols that has been previously observed, but incorrectly resolved. We are attempting to determine this specific mechanism.
METHODS: We used positive ion mode atmospheric pressure chemical ionization (APCI) as the interface to the mass spectrometer. HMTD was infused with various solvents including ^{18}O- and ^{2}H-labeled methanol in order to determine gas-phase reaction mechanisms.
RESULTS: Based on these labeled experiments, it was determined that, under APCI conditions, the alcohol oxygen attacks a methylene carbon of HMTD and releases H_2O_2. This was attempted with nine different alcohols and, in each case, the alcohol is fully incorporated into the molecule with the peroxide release. A mechanism for this reaction has been proposed.
CONCLUSIONS: This work appears to have confirmed the gas-phase reaction mechanism of HMTD with alcohols. As we continue efforts to characterize this unusual molecule, the information may prove useful in determining formation and degradation mechanism(s). In addition, this property of HMTD may find use in other fields of science.

Hexamethylene triperoxide diamine (HMTD)[1] is a sensitive peroxide explosive that is relatively easy to synthesize from hexamethylenetetramine (hexamine), hydrogen peroxide and catalytic levels of citric acid. Although it has never found use as a military explosive due to its poor thermal stability and high sensitivity to impact, friction and electrostatic charge, it has become more commonly used by terrorists.[2–5] Our efforts to successfully prevent the use or production of HMTD by terrorists require fundamental understanding of the mechanistic principles associated with its formation and decomposition.

Although HMTD was first synthesized in 1885 by Legler,[1] the structure was not proposed until 1967 by Urbanski[6] and not confirmed until 1985 by Schaefer *et al*.[7] using X-ray crystallography. Its structure is unusual in that there is a planar three-fold coordination about the two bridgehead nitrogen atoms rather than a pyramidal structure.[7] Ring strain in HMTD may account for the stability and sensitivity issues mentioned above. Despite a plethora of information on HMTD, a mechanism for its formation has only recently been tentatively proposed.[8]

Development of an analytical method for HMTD was investigated to identify potential, non-volatile decomposition products by liquid chromatography interfaced with mass spectrometry (LC/MS). Typical optimization for the LC/MS conditions of a new compound is initiated by directly infusing a solution (usually 50:50, v/v, acetonitrile/water at 1 to 10 μg/mL) of purified standard into an electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source. Further signal enhancement may be source- or solution-specific and is usually investigated in an iterative manner to improve detection limits depending on the analysis requirements. During solution optimization it is important to take future chromatography conditions into consideration. Although previous separation work reported for HMTD used methanol and water,[9] we preferred to perform initial testing using the aprotic organic solvent acetonitrile. When HMTD was later infused into the mass spectrometer in a methanol/water solution, the spectrum suggested that a gas-phase chemical reaction had occurred between a methylene carbon of HMTD and the alcohol. The purpose of this work is to help describe the behavior of HMTD in the gas phase. This information may aid present efforts to elucidate the formation and destruction mechanisms of this molecule. In addition, the ability of HMTD to react with alcohols under chemical ionization conditions may prove useful to other fields of research.

EXPERIMENTAL

Chemicals and reagents

Water, acetonitrile and methanol were all Optima HPLC grade solvents from Fisher Chemical (Fair Lawn, NJ, USA). Isopropanol, isobutanol, 1-butanol, cyclohexanol and anhydrous

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citric acid were ACS grade, also from Fisher Chemical. Hexamine, xylitol, 1-octanol and *tert*-butyl alcohol were purchased from Acros Organics (Morris Plains, NJ, USA). 2-Butanol was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). D-(+)-Glucose was obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide (50%) was purchased from Univar (Redmond, WA, USA). Ethanol (200 proof) was ACS grade obtained from Ultrapure (Darien, CT, USA). HMTD was produced in-house by standard methods reported in previous work.[10] Methanol labelled with ^{18}O, d_4-methanol and d_2-water were purchased from Cambridge Isotope Labs (Cambridge, MA, USA).

Instrumentation and methods

Using an Exactive Orbitrap mass spectrometer (Thermo Electron, Franklin, MA, USA) with an APCI interface, positive ions were produced and introduced into the instrument. The tune conditions for infusion experiments (10–20 µL/min flow) were as follows: spray voltage, 5000 V; capillary temperature, 140 °C; sheath gas (N_2), 25; auxiliary gas (N_2), 3; heater temperature 160 °C; capillary voltage, 40 V; tube lens voltage, 160 V; and skimmer voltage, 15 V. The units for sheath and auxiliary gas flow rates are arbitrary. The mass spectrometer source conditions for chromatographic analysis were optimized by increasing the sheath gas flow rate to 30 and the auxiliary gas flow rate to 15 to provide better desolvation at higher liquid flow rates (200–250 µL/min flow). Liquid chromatography was performed using a Thermo Electron Accela quaternary pump. Sample injections were performed using a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland). Initial reversed-phase chromatography used a Hypersil C-18 column (2.1 × 100 mm, 5 µm; Thermo Scientific, Franklin, MA, USA) with binary delivery of a gradient mobile phase. Ultimately, the HPLC system developed for optimum analysis of HMTD and hexamine employed an Advantage PFP column (100 × 2.1 mm, 5 µm; Analytical Sales and Service, Pompton Plains, NJ, USA). In order to gain some retention of hexamine, neutral pH conditions were preferable, but this caused broadening of the HMTD peak shape. To remedy this problem, three different mobile phase solvents were used to provide both pH and solvent strength gradients. Initially, 95% solvent A (10 mM ammonium acetate, pH 6.8) and 5% solvent C (acetonitrile) were held for 3 min following injection to retain hexamine. The system was then rapidly ramped to 85% solvent B (0.1% acetic acid), 5% solvent A and 10% solvent C over the next 3 min. The organic levels were increased slowly for 9 min to 35% C, 60% B and 5% A, then rapidly for 3 min to 90% C and 5% of both A and B. This was held for 2 min before returning to the initial conditions and re-equilibrating for 5 min prior to the next injection. Data collection and analysis were performed with Thermo Xcalibur software (version 2.2, SP 1.48).

RESULTS AND DISCUSSION

Infusion of HMTD in 50:50 (v/v) acetonitrile/water into the APCI source operated in positive ion mode produced abundant protonated molecules $[M+H]^+$ at *m/z* 209 (±5 ppm from theoretical *m/z* 209.0768). Fragment ions of *m/z* 191, 179, 145 and 117 were produced in the source and are depicted in Fig. 1 (structures are also consistent with later hydrogen/deuterium (H/D) exchange data, not shown). Initially, acetonitrile was used as the organic phase while the aqueous phase contained pH modifiers of either 0.1% acetic acid (pH ~3.2) or 10 mM ammonium acetate (pH ~6.8). Due to the lack of retention or reasonable peak shape, even under highly aqueous conditions, it was decided to switch to methanol (MeOH) as the organic phase. This was consistent with methods described by Crowson and Beardah,[9] who also employed APCI positive ion mode conditions, but with an isocratic method using 5% MeOH. Although retention was improved by this alteration, the peak shape was inconsistent and unacceptably broad. In addition, a new peak at *m/z* 207 was observed in the spectrum obtained for HMTD (also reported by Crowson and Beardah[9]). To assure that this was not an impurity from MeOH,

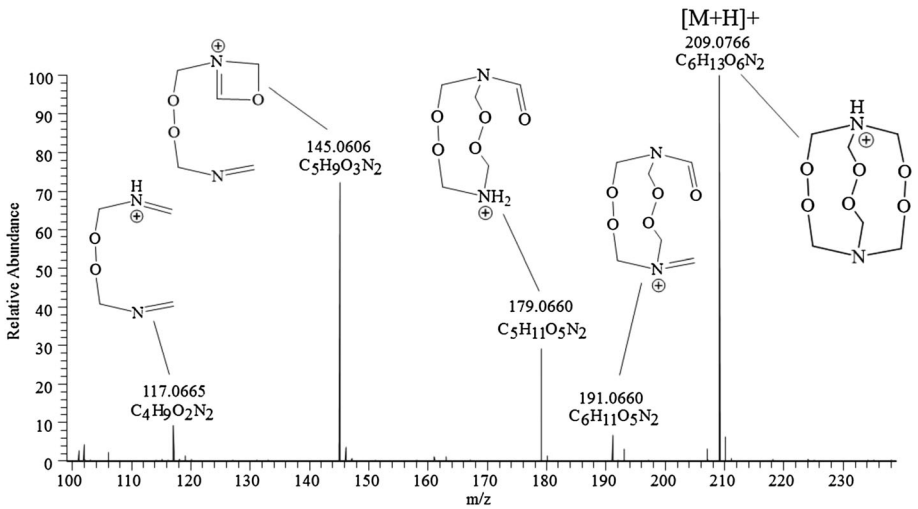


Figure 1. HMTD and tentatively identified fragment ions produced in the source using APCI+, with acetonitrile/water with 0.1% acetic acid mobile phase.

additional infusion experiments were performed using various solutions. When 100% MeOH was infused, the presence of impurities was ruled out. Infusion of HMTD in 100% MeOH provided ion signals of both *m/z* 209 and 207 in roughly equal abundances. Using an aqueous solution with 10% MeOH yielded only a small amount of *m/z* 207 (roughly 10% relative abundance, Fig. 2). Initially, the *m/z* 207 ion was thought to result from protonated HMTD losing two hydrogen atoms (H_2 gas) in the gas phase, as previously reported.[9,11,12] Crowson and Beardah,[9] using a nominal mass, quadrupole instrument, attributed the ion at *m/z* 207 to a fragment of HMTD, but did not specifically designate the fragment ion structure. In 2004, Xu *et al.*,[11] using a TSQ7000 triple quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA, USA) (nominal mass instrument), reported *m/z* 207 as being the $[M-1]^+$ ion. The exact mass for the ion formed by loss of H_2 from $[M+H]^+$ is *m/z* 207.0611, which was reported by Kinghorn *et al.*,[12] using an accurate mass time-of-flight (TOF) instrument. Although we did observe this ion, it was at approximately 5% relative abundance. The major part of the *m/z* 207 ion detected in our lab had an exact mass of *m/z* 207.0981, consistent with the unlikely loss of an oxygen and the gain of a methyl group. Since this phenomenon occurred using MeOH and not acetonitrile, an alcohol solvent adduct of a fragment ion appeared to be the likely culprit. To test the theory, additional

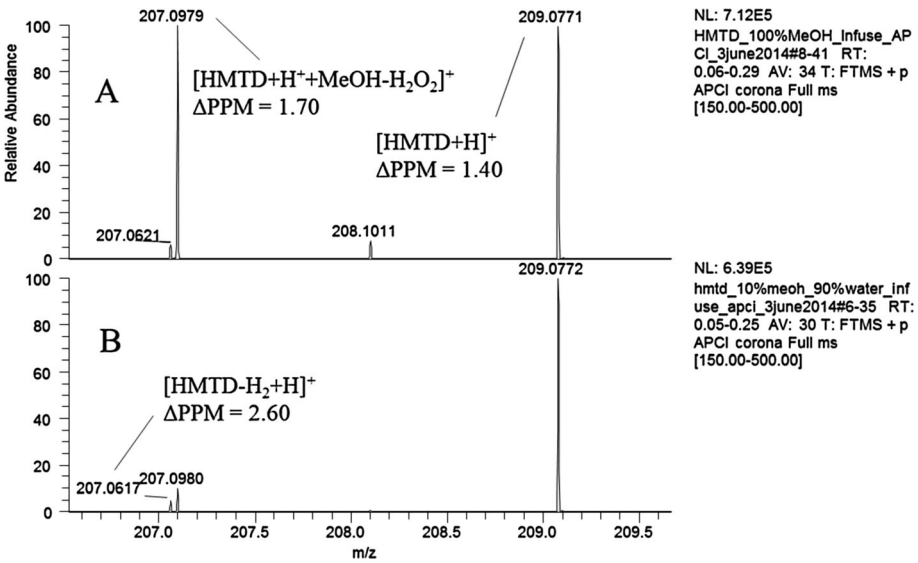


Figure 2. Infusion of HMTD standard solutions (5 µg/mL) in (A) 100% methanol and (B) 10% methanol/90% water. Note that, with the 10% methanol solution, the peak associated with $[HMTD–H_2+H]^+$ is ~50% of the $[HMTD+H^+ +MeOH–H_2O_2]^+$ peak, but only 5% relative abundance in both spectra.

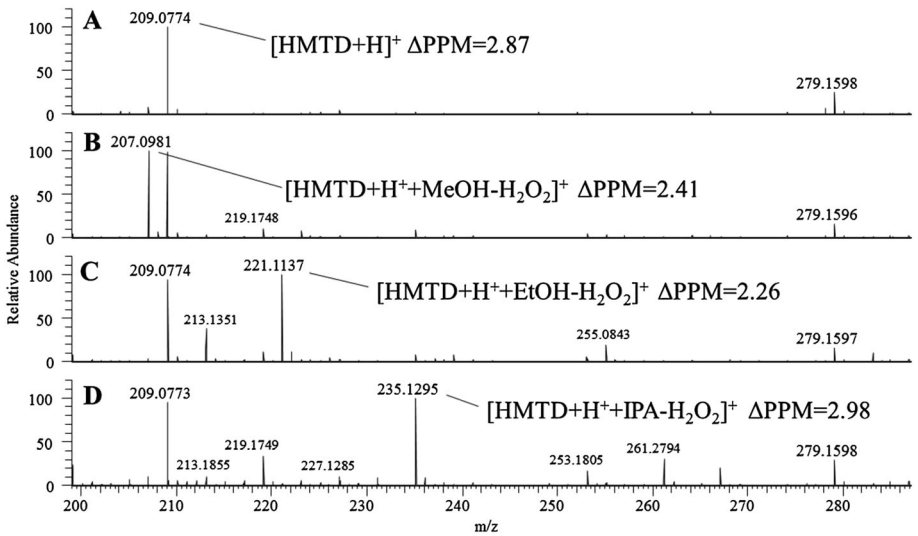


Figure 3. Infusion of HMTD standard solutions (5 µg/mL, 50:50 v/v) in (A) acetonitrile/water, (B) methanol/water, (C) ethanol/water, and (D) isopropanol/water.

infusion experiments were performed with ethanol (EtOH) and isopropanol (IPA) compared with acetonitrile. As expected, use of different alcohols resulted in the addition of ions with corresponding mass (EtOH → *m/z* 221 and IPA → *m/z* 235) verified by exact mass measurements (Fig. 3).

Cotte-Rodrıguez *et al.*[13] used a nominal mass Thermo Electron LTQ ion trap instrument with a variation of the Desorption Electrospray Ionization (DESI) source called DAPCI, which provides APCI-like results. This direct analysis technique combined with alkali metal (sodium or potassium) doped solvents was able to detect the sodium adduct of the stable HMTD-methanol product $[M+CH_3OH+Na]^+$ at *m/z* 263. Since the fragmentation pathway was inconsistent with a normal solvent adduct, they proposed a mechanism in which one peroxide bond of HMTD reacts with MeOH by a homolytic mechanism consistent with peroxide reactions, forming a methyl ether with the loss of water and formaldehyde.[13]

The mechanism of Cotte-Rodrıguez *et al.*[13] involves cleavage of the methanol oxygen and subsequent loss of that oxygen as water. If this mechanism is correct, the oxygen from the alcohol would be lost as water rather than being incorporated into the HMTD molecule upon gas-phase ionization. Therefore, we performed the experiment by infusing HMTD in [^{18}O]-methanol/[^{16}O]-water (50:50 v/v). The results (Fig. 4) show the addition of 2 *m/z* units (*m/z* 209.1022) to the *m/z* 207.0976 ion observed with [^{16}O]-methanol, indicating that the oxygen from methanol is incorporated into

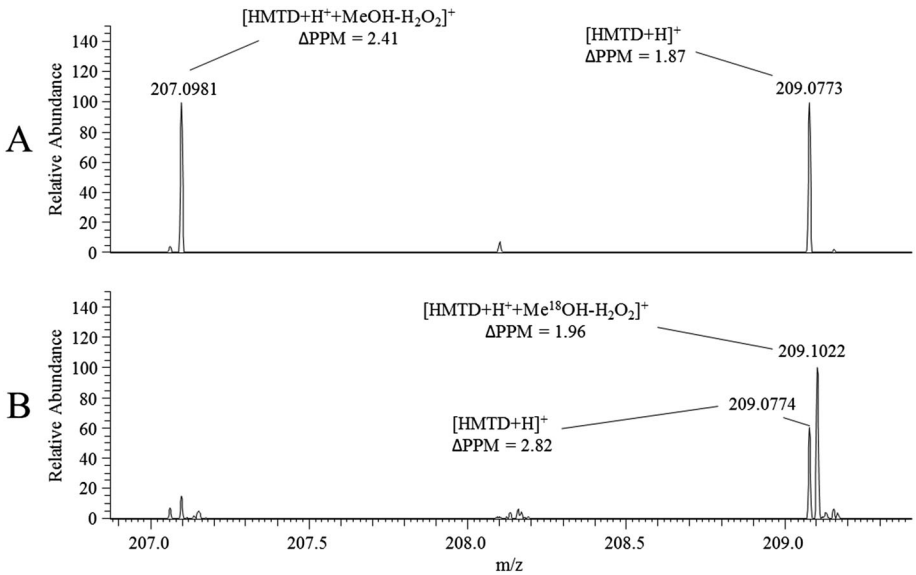


Figure 4. Infusion of HMTD standard solutions (5 µg/mL, 50:50 v/v) in (A) methanol/water and (B) [^{18}O]-methanol/water. Circled areas are expanded insets within each spectrum.

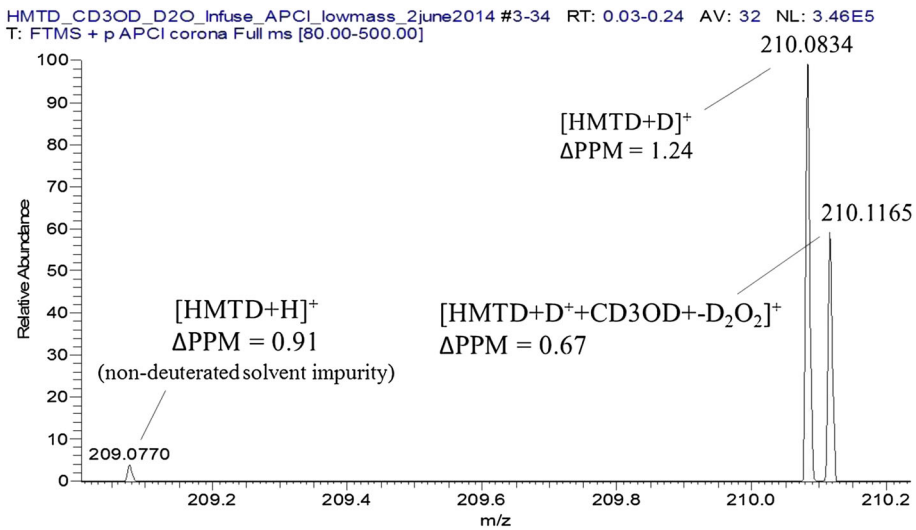


Figure 5. Infusion of HMTD standard (5 µg/mL, 50:50 v/v) in d_4-methanol/D_2O.

the HMTD. This species, with *m/z* 209.1022, was clearly resolved from the protonated parent molecule at *m/z* 209.0774 using a moderate level of resolution (25 000) for an Orbitrap system. The minute amount of *m/z* 207.0985 observed in this experiment suggests some contamination of unlabeled MeOH and not the operation of multiple mechanisms. To further confirm alkoxy incorporation, infusion of HMTD was performed in a 1:1 mix of deuterated aqueous methanol (CD_3OD/D_2O). The results (Fig. 5) suggest the formation of the ion for the deuterated molecule at *m/z* 210.0834 and the CD_3O adduct with the loss of D_2O_2 at *m/z* 210.1165, not the loss of DOH. These data confirm that the mechanism involves the loss of H_2O_2 (or D_2O_2), with all oxygen atoms originating from the HMTD peroxide, while the oxygen and carbon from the methanol are completely incorporated into HMTD as an ether. Formation of an ether rather than a primary alcohol is postulated. If an alcohol were formed, a carbon from HMTD would need to attack the methanol carbon, and this species would be subject to a facile loss of water in the source which was not detected.

To determine whether the reaction of HMTD with methanol was occurring in solution under ambient conditions, HMTD was allowed to sit in 100% MeOH and 50:50 (v/v) MeOH/water for 5 days at room temperature in an amber HPLC vial at a concentration of 5 μg/mL. These samples (10 μL) were then analyzed on the optimized HPLC system. The lack of any significant signal at *m/z* 207.0976 suggests that this phenomenon occurs rapidly in the gas phase and not as a consequence of a chemical reaction in solution (Fig. 6).

Since both protonated HMTD (*m/z* 209.0768) and $[HMTD+H^+ + CH_3^{18}OH–H_2O_2]^+$ (*m/z* 209.1022) were observed in similar abundance in the [^{18}O]-methanol experiment, this suggests that an intermediate is formed that may be converted into either species. The proposed mechanism, depicted in Fig. 7, is consistent with a chemical ionization mechanism where the protonated solvent molecule (MeOH or water) transfers a proton to a compound with higher gas-phase basicity such as HMTD.[14,15] The mechanism shows an intermediate (undetected) where either the charged water or alcohol molecule aligns with the solvent oxygen proximal to one of the six carbon atoms of HMTD. Two competing mechanisms can then proceed from that point. In pathway A (Fig. 7), the lone pair of electrons from the nitrogen removes a proton from the solvent to produce the $[M+H]^+$ ion and a neutral solvent molecule. Pathway B may proceed by the abstraction of the solvent proton by a peroxide oxygen, allowing these electrons to attack the electron-poor carbon of the HMTD peroxymethyl amine, breaking the carbon-peroxide bond. This intermediate (also not detected) can rapidly lose H_2O_2, as shown in Fig. 7. When water is the solvent, pathway B is not favored since the product of this mechanism (*m/z* 193.0819) is only about 1% of the relative abundance. This mechanism allows six possible intermediates for reaction initiation as opposed to only three intermediates by a homolytic reaction mechanism of the peroxide.[13]

Compared with ESI, the protonated molecule of HMTD was produced in far greater abundance using APCI, but it was still detected by ESI. To determine whether the reported reactions were driven by chemical ionization, the methanol/water solution was infused under positive ESI conditions. Small amounts of *m/z* 209 were observed until the electrospray voltage was increased to 6000 V or higher. At this point, where corona discharge could be physically observed, both *m/z* 209.0786 and 207.0976 (as seen in APCI, Fig. 2(A)) were observed at very high levels. This is consistent with a chemical ionization mechanism, but is not a desirable result for ESI conditions since this can lead to destruction of the electrospray tip.

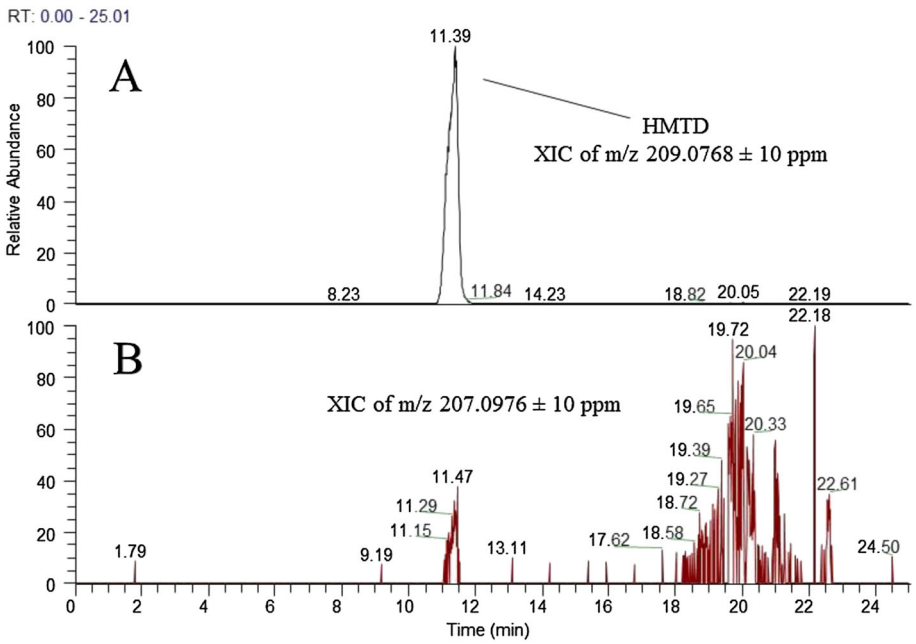
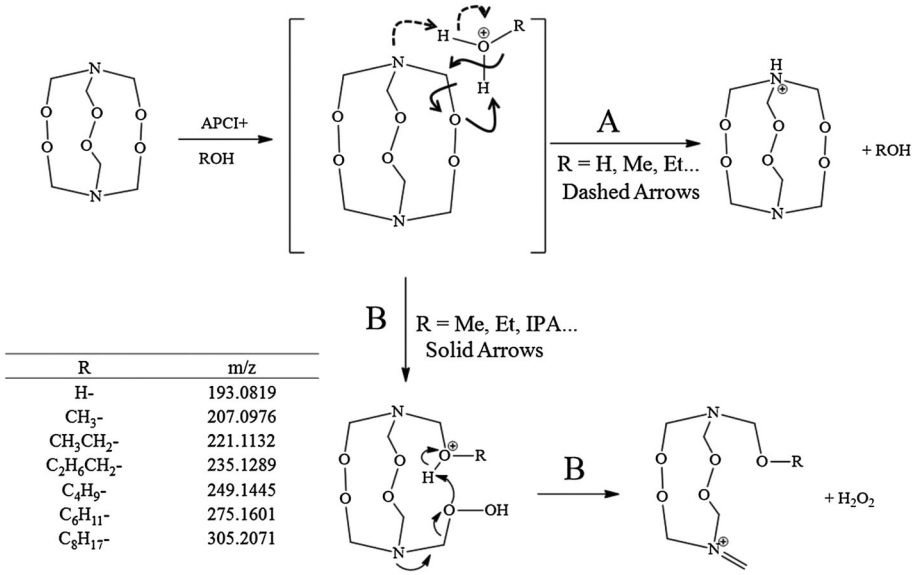


Figure 6. HPLC analysis of HMTD stored in methanol/water (50:50 v/v) under ambient conditions for 5 days. Extracted ion chromatograms (XICs) for (A) HMTD (~3×10^6 height counts) and (B) the methanol adduct (*m/z* 207.0976, ~4×10^3 height counts).



R	m/z
H-	193.0819
CH_3-	207.0976
CH_3CH_2-	221.1132
$C_2H_6CH_2$-	235.1289
C_4H_9-	249.1445
C_6H_{11}-	275.1601
C_8H_{17}-	305.2071

Figure 7. Proposed mechanism for the formation of (A) the protonated molecule and (B) the various alcohol adducts.

Infusion of HMTD with 100% methanol produced $[M+H^+ + CH_3OH - H_2O_2]^+$ ions of *m/z* 207.0976 in equal or greater abundance than the protonated precursor $[HMTD+H]^+$ (Fig. 2). When HMTD was added (10 µg/mL) to a mixture containing equal volumes of methanol, ethanol, isopropanol and individual isomers of butanol, each alcohol added to HMTD with a general trend of increasing abundance for larger alcohols (with equal signals for ethanol and isopropanol). This was true for n-butanol, 2-butanol and isobutanol, each producing a large signal at *m/z* 249.1445. Interestingly, in the case of n-butanol, the abundance of the methanol adduct dropped below that of the $[M-H_2+H]^+$ ion (*m/z* 207.0612). However, for the *tert*-butanol, the signal intensity of *m/z* 249.1445 was below 20% relative abundance (Fig. 8, 2-butanol not shown due to large impurities in this solvent). This is consistent with the proposed mechanism since the steric effects of the tertiary butyl group would prevent the alcohol from reacting with HMTD. Steric effects may also account for the similar abundance obtained for ethanol and isopropanol. Both 1-octanol and cyclohexanol were also infused with HMTD and formed corresponding products associated with each (*m/z* 305.2071 and 275.1601, respectively). In addition, xylitol and glucose (10 µM) were both added to solutions of HMTD (10 µg/mL in acetonitrile or methanol) and infused on the same system. Neither sugar reacted with HMTD in the manner of other alcohols. This may be caused by the electron-rich HMTD peroxides repelling the many oxygen atoms of these sugar molecules to prevent proximity to the methylene groups. Alternatively, the sugars, which are generally poorly ionized under APCI in positive ion mode, may resist reacting with HMTD. Additional work on other alcohols is ongoing.

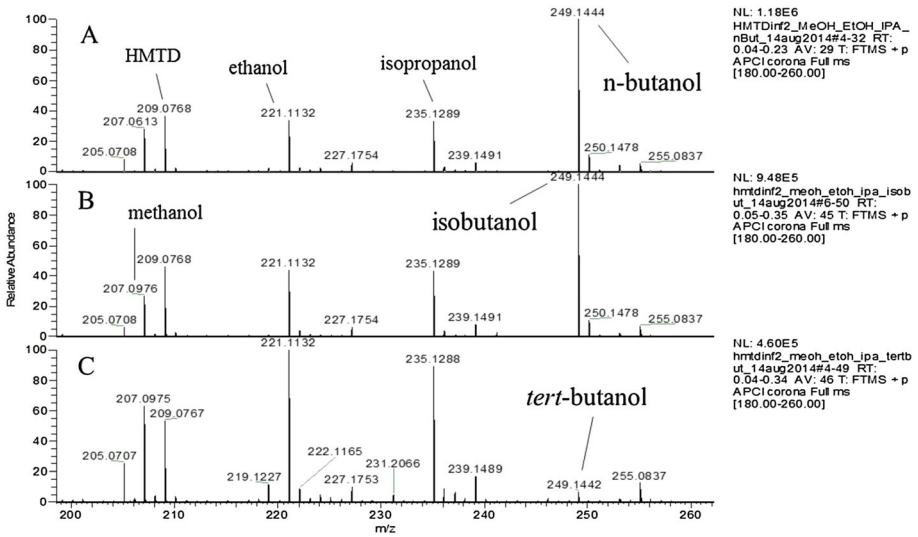


Figure 8. HMTD infused with alcohol mixtures of methanol, ethanol, isopropanol and (A) n-butanol, (B) isobutanol and (C) *tert*-butanol. 2-Butanol was not included due to the high number of impurities found in this alcohol, but the trend for HMTD adducts was similar to n-butanol and isobutanol.

CONCLUSIONS

Despite the considerable body of work performed on HMTD over the years, it still possesses many secrets. The work presented here shows that a gas-phase chemical reaction occurs with HMTD in the presence of alcohols to produce a hemiaminal ether under APCI conditions. We are hoping that this unusual behavior may be exploited to provide insight into the formation and degradation mechanism(s) of HMTD, neat, in solution and in the gas phase. The idea that the methylene groups of HMTD may be more reactive than the peroxides is an interesting prospect when considering the behavior of this molecule. An added benefit of this study is that it provides a method for quick characterization of various alcohols in solution; a property of HMTD that may find use in other fields of science, possibly as a probe substrate. Research efforts into HMTD mechanisms are ongoing in our lab.

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