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ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · JANUARY 2006

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Chromatographic Separation and Identification of Products from the Reaction of Dimethylarsinic Acid with Hydrogen Sulfide

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Received August 16, 2005

The reaction of dimethylarsinic acid (DMA^V) with hydrogen sulfide (H_2S) is of biological significance and may be implicated in the overall toxicity and carcinogenicity of arsenic. The course of the reaction in aqueous phase was monitored, and an initial product, dimethylthioarsinic acid, was observed by using LC−ICP−MS and LC−ESI−MS. Dimethylarsinous acid was observed as a minor product. A second slower-forming product was identified, and the electrospray mass chromatograms for this species produced ions at *m/z* 275, 171, and 137 in positive mode. To aid in the identification of this slower-forming product, crystalline standards of sodium dimethyldithioarsinate and dimethylarsino dimethyldithioarsinate were prepared and re-characterized by using improved spectroscopic and structural analysis techniques. An aqueous solution of sodium dimethyldithioarsinate produced a single major chromatographic peak that matched the retention time (7.6 min) of the slower-forming product and contained similar molecular ions at *m/z* 275, 171, and 137 via LC−ESI−MS. The dimethylarsino dimethyldithioarsinate standard produced four aqueous phase species one of which coeluted with the slower forming product. This coeluting peak also produced the identical ESI−MS ions as the slower-forming product of $DMA^V + H_2S$. ESI−MS/MS experiments conducted on sodium dimethyldithioarsinate in deuterated water produced molecular ions at *m/z* 276, 173, and 137. Subsequent collisionally activated dissociation (CAD) experiments on *m/z* 276 did not produce a product ion at *m/z* 173. These data indicate that two different species are present in solution, while NMR data indicate that only dimethyldithioarsinic acid exists in aqueous solutions. This discrepancy was investigated by conducting NMR studies on the acidic solution of sodium dimethyldithioarsinate after taking this solution to dryness. The resolubilized solution produced a proton NMR signal characteristic of dimethylarsino dimethyldithioarsinate. Therefore, it was concluded that the ESI−MS ion at *m/z* 275 associated with the slowly forming second reaction product and the sodium dimethyldithioarsinate compound is a product of the ESI desolvation process.

Introduction

The reaction of dimethylarsinic acid (DMA^V)[1] with hydrogen sulfide (H_2S) was first reported in 1843 by Bunsen as part of his *Untersuchungen über die Kakodylreihe (Investigations of the cacodyl series)* (*1*). Bunsen describes purging a concentrated alcoholic solution of cacodylic acid (DMA^V) with H_2S which resulted in the formation of a white precipitate he named "kakodylsulfokakodylat." Subsequent investigations of this compound have been sparing (*2*−*5*), but recent studies indicate that this reaction may be highly relevant to the dietary exposure and metabolic transformation of arsenic.

Dimethylarsinic acid has been used extensively in agriculture as a contact herbicide and is commonly found in the environment. One major source of environmental DMA^V is the biological transformation of inorganic

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[1] Abbreviations: As^V, arsenate; As^{III}, arsenite; MMA^V, monomethylarsonic acid ($MeAs^V$); MMA^{III}, monomethylarsonous acid ($MeAs^{III}$); DMA^V, dimethylarsinic acid (Me_2As^V); DMA^{III}, dimethylarsinous acid (Me_2As^{III}); TMAO, trimethylarsine oxide (Me_3As^V); TMA, trimethylarsine (Me_3As^{III}); H_2S, hydrogen sulfide; $DMTA^V$, dimethylthioarsinic acid; $DMTA^{III}$, dimethylthioarsinous acid; DMDTA, dimethyldithioarsinic acid; NaS_2AsMe_2, sodium dimethyldithioarsinate; $Me_2As(S)SAsMe_2$, dimethylarsino dimethyldithioarsinate; TEAH, tetraethylammonium hydroxide; HCl, hydrochloric acid; FeS, iron sulfide; $CDCl_3$, deuterated chloroform; D_2O, deuterium oxide; TMS, tetramethylsilane; ICP, inductively coupled plasma; ESI, electrospray ionization; NMR, nuclear magnetic resonance; ATR, attenuated total reflenctance; CAD, collisionally activated dissociation.

10.1021/tx050227d CCC: $30.25
Published on Web 11/22/2005

arsenic by living organisms (*6*). This metabolic pathway involves a series of alternating reduction and oxidative methylation reactions as follows:

$$As^{V} \rightarrow As^{III} \rightarrow MeAs^{V} \rightarrow MeAs^{III} \rightarrow Me_2As^{V} \rightarrow Me_2As^{III} \rightarrow Me_3As^{V} \rightarrow Me_3As^{III}$$

In humans and other animals, inorganic arsenic exposure initiates enzymatic conversion and methylation of the arsenic (*7*, *8*) producing DMA^V as the major arsenical metabolite. DMA^V is also excreted as the major metabolite after consumption of naturally occurring arsenosugars (*9*). While DMA^V is less acutely toxic than inorganic arsenic (*10*), there is increasing evidence of a unique toxicity of DMA^V which Kenyon and coauthors have recently reviewed (*11*). DMA^V is indicated as a complete carcinogen and is a likely contributor to the overall toxicity of inorganic arsenic (*11*). The recognition of the toxicity of DMA^V, together with the finding that methyl arsenic species, $MeAs^{III}$ and Me_2As^{III}, are far more toxic than the As^V analogues (*12*), is now incorporated into the hypothesis that the metabolic pathway of arsenic is not a mode of detoxification but may be an aggravating factor in the carcinogenicity of arsenic.

The carcinogenicity and biotransformation of DMA^V are not well-understood, and it may be that DMA^V is not the species ultimately exhibiting the toxic effects. Prior to excretion, several chemical pathways are available for further transformation of DMA^V in vivo. Some bacteria are capable of arsenic−carbon bond cleavage (*13*), and demethylation of DMA^V by intestinal microorganisms has been suggested (*14*). Cullen et al. (*15*) showed evidence that arsenicals can be both methylated and demethylated in mouse ceca. Lu et al. (*16*) found that DMA^V can be converted to trimethylarsine oxide (TMAO) and ultimately be volatilized in the form of trimethylarsine (TMA).

There is good reason to be concerned about the Bunsen reaction in that highly toxic Me_2As^{III} species could be produced by the reduction of DMA^V with sulfide ions. Sulfur compounds such as glutathione (GSH) play a central role in the metabolic pathway outlined for arsenic by acting as electron donors for the reduction steps. DMA^V has been shown to be reduced to Me_2As^{III} species by thiols (*17*), and complexes such as the thioarsenite $[Me_2As^{III}(GS)]$ are known to be highly toxic (*18*). At the DMA^V stage in the metabolism of arsenic, thiols may play a more complicated role than electron donor and may produce a deviation from the normal metabolic pathway that could prove to be further implicated in the toxicity of arsenic. Arsenic methyltransferase has been shown to catalyze methylation of DMA^V (producing TMAO); but in vitro, this conversion is inhibited with increasing glutathione concentration (*19*). This finding is surprising because the expected complex $[Me_2As^{III}(GS)]$ has been shown to rapidly convert to TMAO. The formation of an unknown GSH−DMA complex has been suggested as a factor in DMA^V-induced cellular apoptosis (*20*), and the depletion of cellular GSH has been shown to markedly reduce the cytotoxicity observed for DMA^V (*21*).

Speculation by Marafante et al. (*22*) in 1987 that a metabolite of DMA^V might be an unknown sulfur-containing species has resulted in the investigation of derivatization of DMA^V by thiols. Some portion of DMA^V has been shown to bind in vivo with sulfhydryl groups of proteins and enzymes to give reduced Me_2As^{III} derivatives (*23*), and this is an active area of interest in arsenic toxicology. Endo et al. (*24*) have recently identified an unknown DMA^V metabolite as sulfur-containing with a molecular mass of 154. This species was generated by intestinal bacteria in the presence of cysteine and discovered to be both highly genotoxic and cytotoxic (*25*). This discovery should be considered in a larger context in which a number of naturally occurring organoarsenicals have recently been identified in which the As=O group has been replaced with the As=S moiety. The mechanism of formation and the toxicity of these thio-As^V derivatives are topics of considerable interest (*26*), and this inquiry seems likely to be extended to DMA^V.

Besides amino acids, an important source of sulfur in humans and other mammals is hydrogen sulfide, and the reaction of H_2S with DMA^V is biologically relevant because both compounds can interact in mammalian systems (*27*). The in vitro reaction has been used to prepare analytical standards such as dimethylthioarsinous acid ($DMTA^{III}$) and dimethylthioarsinic acid ($DMTA^V$). These standards have been used in the chromatographic identification of unknown DMA^V metabolites: $DMTA^{III}$ $[Me_2As^{III}SH]$ in the liver of rats (*28*) and $DMTA^V$ $[Me_2As^V(S)OH]$ in an extract of sheep wool (*29*) and from an enzymatic extraction of rice (*30*). None of these studies reported the reduction of DMA^V by H_2S first observed by Bunsen or isolated the respective dimethylthioarsenicals.

During our studies of the $DMA^V + H_2S$ reaction, it became apparent that $DMTA^V$ is an initial product, but as the reaction progressed, $DMTA^V$ was slowly converted into one or more species which did not elute from a PRP-X100 anion-exchange column. The goal of this current study is to identify all the products of the reaction of DMA^V with H_2S and to develop chromatographic methods capable of distinguishing between these species. In a concurrent study (*31*), a synthetic standard of $DMTA^V$ has been isolated as the oxygen-bridged thioanhydride species $[Me_2As^V(S)OAs^V(S)Me_2]$ (*32*) and used to confirm the identification of $DMTA^V$ in aqueous solutions.

Materials and Methods

Reagents. Distilled deionized (DDI) 18 MΩ water (Millipore Corporation, Bedford, MA) was used for mobile phase makeup. DMA^V was purchased from Strem (Newburyport, MA) and was >98% pure. Dimethylarsinous acid (DMA^{III}) was prepared by reduction of DMA^V with sulfur dioxide (*33*) and used as a standard for identification of DMA^{III} based on LC−ICP−MS retention time match. Ammonium acetate, acetic acid, and methanol were used for mobile phase makeup and were purchased from Fisher Scientific (Pittsburgh, PA). Deuterated chloroform ($CDCl_3$) and deuterium oxide (D_2O) used for NMR sample makeup were purchased from Sigma-Aldrich (St. Louis, MO) and Cambridge Isotope Laboratories (Andover, MA), respectively. Hydrogen sulfide gas was generated from iron sulfide (FeS) purchased from Harshaw Scientific (Cleveland, OH) and hydrochloric acid (HCl) purchased from Fischer Scientific as described elsewhere (*34*). A gas cylinder of H_2S was used for the results summarized in Supporting Information SI-1. Tetraethylammonium hydroxide (20 wt %, Aldrich), malonic acid (BDH Chemicals, Toronto, Canada), and nitric acid (69%, sub-boiling distilled, Seastar Chemicals, Sidney, Canada) were used for mobile phase makeup used in the separation described in the Supporting Information SI-1.

Instrumentation and Chromatographic Separation. The Liquid Chromatograph (LC) was an Agilent 1100 (Palo Alto,

CA). The column used for reverse-phase chromatography was a Phenomenex (Torrance, CA) Intersil 5 μm ODS-2 (C-18) with a 10 mM ammonium acetate mobile phase adjusted to pH 4.6 with acetic acid and containing 10% methanol (w/w). The flow rate was 0.5 mL/min. Anion chromatography utilized a PRP-X100 column (Peek, 4.6 mm × 25 cm + guard column, Hamilton, Reno, NV) with a 20 mM $(NH_4)_2CO_3$ eluent at pH = 9.0 and a 1 mL/min flow rate (*30*). The autosampler vials used were Target DP (National Scientific Co., Duluth, GA). The autosampler vials were rinsed with 10% HNO_3, rinsed with four aliquots of 18 MΩ water, and air-dried prior to use. To compensate for sensitivity differences, injection volumes of 10 μL were used for detection by inductively coupled plasma (LC−ICP−MS), and 100 μL injections were used for electrospray analysis (LC−ESI−MS).

The ICP−MS instrument used as a chromatographic detector was an Agilent (Tokyo, Japan) 4500 fitted with a concentric nebulizer (Precision Glassblowing, Englewood, CO). Chromatographic data were collected by monitoring *m/z* 75 and 77 with a 1 s dwell time. Chromatographic peaks were integrated using the Agilent software. The instrumental conditions were slightly detuned from optimal so that a 100 uL injection of a 100 ppb standard of arsenobetaine produced a maximum height of 100 000 counts. At the beginning and end of each sample run, a postcolumn injection of 100 μL of a 100 ppb arsenobetaine standard dissolved in eluent was made using a LabPro Rheodyne valve (Rohnert Park, CA). This valve was controlled by the Agilent 1100 LC and provided a means of monitoring instrument drift (*35*). Total arsenic determinations were made by flow injection analysis without the chromatographic column.

LC−ESI−MS analysis was carried out by attaching a ThermoFinnigan (San Jose, CA) LCQ Deca ion trap mass spectrometer equipped with an atmospheric pressure ionization source to the Agilent (Palo Alto, CA) 1100 LC. The LCQ was operated in positive-ion electrospray ionization mode with an ESI spray voltage of −3 kV, sheath gas of 80, auxiliary gas of 20, and heated capillary temperature of 300 °C. The mass range during LC/MS analysis was set to 50−2000 Da. LC−ESI−MS/MS analysis of eluting arsenicals provided structural confirmation by collisionally activated dissociation (CAD) of precursor ions.

The data shown in Supporting Information SI-1 used a LC system consisting of a Waters (Milford, MA) model 510 delivery pump and a Reodyne (Rohnert Park, CA) model 7010 injector valve with a 20 μL sample loop. The column was a reverse-phase GL Sciences (Tokyo, Japan) Inertsil ODS, 250 mm × 4.6 mm i.d. (C-18), equipped with a 2 cm C-18 guard column (Supelco, St. Louis, MO) as described elsewhere (*36*, *37*). The LC conditions used were 10 mM tetraethylammonium hydroxide (TEAH), 4.5 mM malonic acid, 0.1% methanol, pH = 6.8 (by using HNO_3) pumped at 0.8 mL/min. All samples and standard solutions were filtered (0.45 μm) prior to injection onto the column. Data collected for Supporting Information SI-1 utilized a double-focusing magnetic sector field ICP−MS (Element2, Thermo Finnigan, Germany) operated in low-resolution mode (R = 300) and equipped with a conical nebulizer. The LC system was connected to the ICP nebulizer via a PTFE tube (20 cm × 0.4 mm) and appropriate fittings. Arsenic compounds in the samples were identified by matching the retention times of the peaks in the chromatograms with those of known standards. Quantification was done by comparing peaks with those of matching standards.

High resolution mass spectrometry was used for the characterization of the standards: sodium dimethyldithioarsinate and dimethylarsino dimethyldithioarsinate. Crystal samples were dissolved in methanol and directly infused into a Waters hybrid Q-TOF2 quadrupole time-of-flight mass spectrometer from Micromass (Manchester, U.K.) with nitrogen as the nebulizing gas and the Z Spray ion source. The lock mass and mass calibration used polyalanine as an internal standard for mass accuracy. Acetic acid (1%) was added to the standard of dimethylarsino dimethyldithioarsinate.

Raman and Fourier transform infrared (FT-IR) spectroscopic data were collected for dimethylarsino dimethyldithioarsinate and sodium dimethyldithioarsinate standards using a LabRam HR dispersive Raman spectrometer system (JY Horiba, Irvine, CA). The Raman data collection parameters used were laser excitation wavelength 633 nm, 50× objective, slit width of 200 μm, 150 L/mm grating, and an integration time of 10 s averaged 2×. The FT-IR spectrum of dimethylarsino dimethyldithioarsinate was obtained using a Magna 550 FT-IR spectrometer system (Nicolet, Madison, WI) with a Durascope one reflection diamond-coated zinc selenide (ZnSe) attenuated total reflectance (ATR) attachment (Smiths Detection). The Magna 550 data collection parameters used were resolution 4 cm^{-1}, coadditions 64, gain of 1, and a TE-cooled DTGS detector. The FT-IR spectrum of sodium dimethyldithioarsinate was obtained using an Illuminator FT-IR microscope with a diamond-coated ZnSe ATR objective (Smiths Detection). The Illuminator data collection parameters used were resolution 4 cm^{-1}, coadditions 128, gain, and a liquid nitrogen-cooled MCT detector. The Raman spectroscopic data were collected over a spectral range of 50−4000 cm^{-1}, and the FT-IR spectroscopic data were collected over a spectral range of 4000−650 cm^{-1}. No sample preparation was required for any of the FT-IR ATR or Raman spectroscopic measurements. A small amount of powder was placed on a low E slide (Smiths Detection) and placed on the microscope stage for the Raman and Illuminator FT-IR measurements. For the Durascope ATR measurements, a small amount of powder was placed on the ATR internal reflectance element (IRE) and compressed using a load of 5. Clean background measurements were made prior to obtaining FT-IR spectra of the samples. Infrared and Raman spectra of standards are available as Supporting Information available with this manuscript (SI-2 and SI-3).

X-ray diffraction data for crystals of dimethylarsino dimethyldithioarsinate were collected using a Bruker AXS (Karlsruhe, Germany, and Madison, WI) SMART APEX CCD diffractometer at 100(2) K using monochromatic Mo Kα radiation with the omega scan technique. The unit cell was determined using SAINT+, and the structure was solved by direct methods and refined by full-matrix least-squares against F^2 with all reflections using SHELXTL (*38*). Experimental details of the X-ray analysis are available from the Cambridge Crystallographic Database by citing CCDC 268678.

^{1}H and ^{13}C NMR spectra were obtained on a 300 MHz Avance (Bruker) spectrometer. NMR results in $CDCl_3$ were calibrated relative to TMS (δ = 0.00 ppm), and D_2O spectra used ethanol as a reference (*39*).

Synthetic Procedures. Arsenic-containing compounds are potentially toxic and carcinogenic. All reactions and subsequent procedures were carried out in a well-ventilated fume hood and gloves were worn when handling arsenicals. All glassware coming in contact with arsenicals or H_2S were rinsed to waste in the fume hood prior to cleaning.

Sodium Dimethyldithioarsinate, Na(S_2AsMe_2). This compound is a known complexing agent and was prepared according to a procedure described by Förster et al. (*40*). DMA^V (2.02 g, 14.6 mmol) and NaOH (0.58 g, 14.5 mmol) were dissolved in 25 mL of boiling ethanol. Hydrogen sulfide was bubbled into the boiling solution for 30 min, and a white solid precipitated. This solid was triturated under ethanol 3 times and dried to give sodium dimethyldithioarsinate as stable, colorless crystals (2.61 g). Mp > 200 °C. Synthesized crystals had a higher melting point than those in the original report (*40*), and this difference may be due to differing degrees of hydration or purity. ^{1}H NMR (300 MHz, D_2O): δ 1.99 (s) ppm. ^{13}C NMR (100 MHz, D_2O): δ 29.5 ppm. HRMS: *m/z* calcd for $Na_2S_2AsC_2H_6^+$ (M + 2Na$^+$), 214.8922; found, 214.8916. FT-IR ATR (neat): 3296 cm^{-1} (br, s), 3006 cm^{-1} (w), 2913 cm^{-1} (w), 2141 cm^{-1} (m), 1645 cm^{-1} (m), 1621 cm^{-1} (m), 1411 cm^{-1} (m), 1252 cm^{-1} (m), 909 cm^{-1} (m), 881 cm^{-1} (s), 850 cm^{-1} (w), 823 cm^{-1} (w). FT-IR values are in agreement with those reported by Casey et al. (*41*) and Silaghi-Dumitrescu et al. (*42*). Raman (neat): 3319 cm^{-1} (br, w), 2989 cm^{-1} (m), 2909 cm^{-1} (s), 1269 cm^{-1} (w), 624 cm^{-1} (m), 602 cm^{-1} (s), 428 cm^{-1} (s), 221 cm^{-1} (br, m), 151 cm^{-1} (w).

Dimethylarsino Dimethyldithioarsinate, $Me_2As(S)SAsMe_2$. Preparation of this compound was carried out according to a procedure described by Zingaro and coauthors (*2*). Briefly, DMA^V (1.11 g, 8.0 mmol) was dissolved in water (1.25 g), and ethanol (3.75 mL) was added. Hydrogen sulfide was bubbled into this mixture for 1 h, and the resulting solution was filtered and the solvent removed. Recrystalization of the resulting residue from hot ethanol gave white crystals (0.98 g, 88%). Mp 70−72 °C. ^1H NMR (300 MHz, CDC13): δ 2.16 (s), 1.55 (s), 1.40 (s) ppm. ^1H NMR analysis is in agreement with originally reported values (*2*). ^{13}C NMR (100 MHz, CDC13): δ 27.7, 15.7 ppm. HRMS: *m/z* calcd for $C_4H_{13}AsS_2^+$ ($M + H^+$), 274.8891; found 274.8886. FT-IR ATR (neat): 2981 cm^{-1} (w), 2903 cm^{-1} (w), 1404 cm^{-1} (m), 1251 cm^{-1} (m), 917 cm^{-1} (m), 899 cm^{-1} (m), 877 cm^{-1}(s), 839 cm^{-1} (s), 823 cm^{-1} (m), 616 cm^{-1} (m). Raman (neat): 2986 cm^{-1} (w), 2907 cm^{-1} (w), 579 cm^{-1} (w), 478 cm^{-1} (w), 407 cm^{-1} (w), 351 cm^{-1} (s), 221 cm^{-1} (w), 141 cm^{-1} (w), 100 cm^{-1} (w). X-ray structural analysis: Crystal data for $C_4H_{12}As_2S_2$. $M_r = 274.12$, crystal size $0.4 \times 0.39 \times 0.07$ mm^3, triclinic, space group $P\bar{1}$, unit cell dimensions: $a = 6.2765(5)$, $b = 7.0576(6)$, $c = 11.1732(9)$ Å, $\alpha = 100.432(2)$, $\beta = 95.402(2)$, $\gamma = 90.312(2)°$; $V = 484.47(7)$ $Å^3$, calculated density = 1.879 Mg/m^3, $Z = 2$, $T = 100(2)$ K, $2\theta_{max} = 56.54°$, 5659 independent reflections, $R_1 = 0.0293$ for 2295 reflections with $I > 2\sigma(I)$, and $wR_2 = 0.0934$, 77 parameter, Goodness-of-fit on $F^2 = 1.113$. CCDC 268678 contains the supplementary crystallographic data for this compound. Structural data are in agreement with reported values (*3*). These data can be obtained at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Center (CCDC), 12 Union Road, Cambridge CB2 1EZ, U.K.; fax, +44(0)1223-336033; e-mail, deposit@ccdc.cam.ac.uk].

Results

The reaction between H_2S and DMA^V has recently been shown to produce $DMTA^V$ and a secondary product (*29*), and the potential production of sulfur analogues of DMA^V was a mutual interest to the laboratories in Vancouver and in Cincinnati. The Vancouver laboratory conducted the studies summarized in Supporting Information SI-1. An H_2S gas cylinder was used to deliver a reproducible H_2S sparge, and a C-18-based chromatographic separation interfaced to an ICP−MS was used to separate and quantify the reaction products as a function of H_2S sparge time. The use of LC−ESI−MS was precluded by the mobile phase which included tetraethylammonium hydroxide and malonic acid as ion-pairing reagents (*36*). The data in Supporting Information SI-1 clearly indicates the formation of an initial product (Product 1) and the formation of a secondary product (Product 2). Some reduction to DMA^{III} (Product 3) is observed. SI-1 also indicates the reaction in ethanol water mixtures produces the same products relative to pure water, but the reaction is more rapid in the ethanol mixture. This observation is significant because the synthetic standards used in this study were prepared in ethanol or ethanol−water mixtures.

The title reaction was concurrently studied (in Cincinnati) using FeS + HCl to generate H_2S and a PRP-X100 column (commonly utilized in arsenic speciation research) with ICP−MS detection. These studies indicated that the amount of chromatographable arsenic on a PRP-X100 column (see Materials and Methods, Instrumentation and Chromatographic Separation) would decrease with reaction time and with longer H_2S sparging. Therefore, alternative reverse-phase chromatographic conditions were developed (C-18 column, without malonic acid; see Materials and Methods) which produced a good mass balance between the number of nanograms of As injected and the sum of the chromatographic concentrations. Figure 1 utilizes this separation to characterize the arsenic-containing products from the sparging of a 100 ppm aqueous DMA^V solution with H_2S for 30 min (H_2S produced from 4 g of FeS). The 1 h data shown in Figure 1 indicates the predominant arsenic-containing species (Product 1) has a retention time of 9.1 min and produces a protonated molecule at *m/z* 155 corresponding to C_2H_8AsSO using ESI−MS. These MS results support an identification of this species as $DMTA^V$ and are consistent with Hansen et al. (*29*) and the unknown metabolite reported by Endo et al. (*24*).

The reaction vessel was sealed (allowing residual H_2S to react), and after 20 h, the reaction mixture was sampled again. In this case, most of Product 1 ($DMTA^V$) was converted to the slowly forming Product 2 identified with a retention time of 7.6 min. Chromatographic recoveries for both experiments were high (87%, 1 h; 93%, 20 h) indicating that all major products were eluting from the column. These data agreed with the findings in Vancouver (see Supporting Information, SI-1), and the LC mobile phase was compatible with ESI−MS which should allow structural verification. The LC−ESI−MS analysis (positive mode) of the slowly forming second product indicates the production of three major ions at *m/z* 137, 171, and 275. The production of an ESI−MS signal at *m/z* 171 is consistent with the findings of Suzuki et al. (*28*) and Hansen et al. (*29*). Suzuki et al. (*28*) have deduced this species to be $Me_2As(S)SH$ (molecular weight = 170) via an electrospray signal at *m/z* 169 (negative mode). Hansen et al. (*29*) identified a similar product and observed a major ion at *m/z* 171 (positive mode electrospray). However, Figure 1 indicates that *m/z* 171 is chromatographically unresolved from *m/z* 275 on the column used which could be interpreted as (1) a coelution of multiple species, (2) adduct formation from *m/z* 171, or (3) an incomplete thermal degradation of *m/z* 275 to *m/z* 171. Of these possibilities, adduct formation is unlikely because the mass-to-charge difference between 275 and 171 cannot be assigned to a mobile phase constituent. The potential for thermal degradation of *m/z* 275 is investigated in Supporting Information SI-4. The SI-4 figure indicates that a lower capillary temperature did produce an increase in *m/z* 275 relative to 171, but this increase is marginal over the temperature window studied (180−300 °C). Therefore, coelution was considered the most likely possibility.

A potential coeluting species based on the *m/z* 275 signal in Figure 1 is dimethylarsino dimethyldithioarsinate ($Me_2As(S)SAsMe_2$, MW = 274). Another reason to suspect this compound as a coeluting species is that synthesis of Bunsen's "cacodyl disulfide" ($Me_2As(S)SAsMe_2$) by Zingaro et al. (*2*) utilizes reaction conditions similar to those used in Figure 1 and SI-1. The expected product created by double sulfur exchange on DMA^V is dimethyldithioarsinic acid (DMDTA), but this product apparently reacts further to give $Me_2As(S)SAsMe_2$ as the major product (*1*, *2*) of the reaction. This species was confirmed by X-ray crystallographic analysis of a single crystal as shown in Figure 2. The structure was first reported by Cameron and Trotter (*3*), and our measurement at 100 K is in general agreement with the less precise results these authors reported at room temperature in 1964. The unusual structure contains one pentavalent and one trivalent As atom in

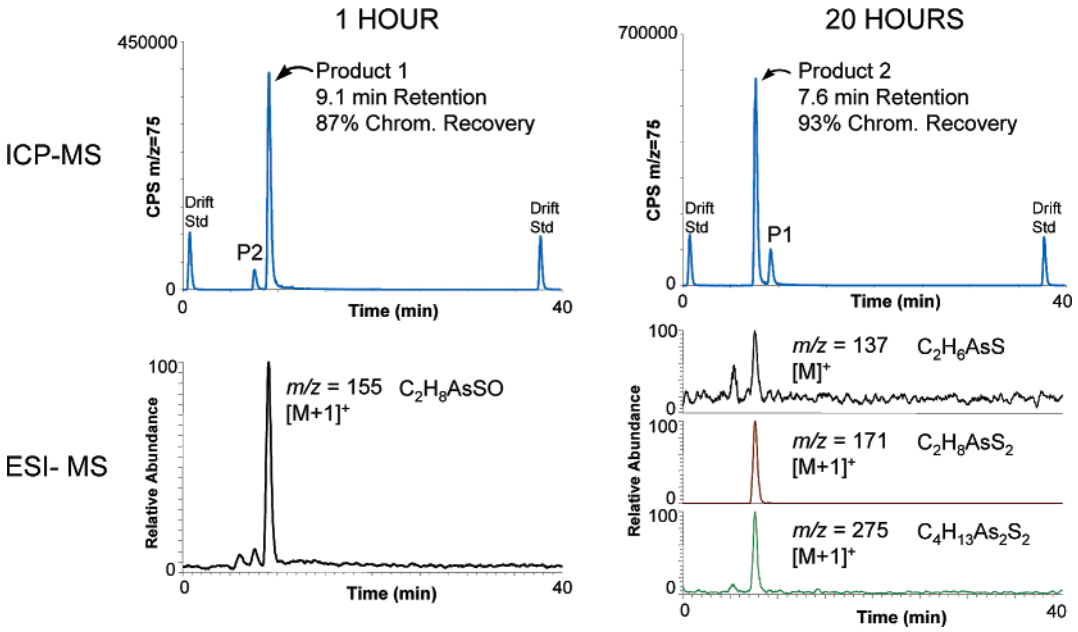

Figure 1. Characterization of reaction products of DMA (100 ppm) + H_2S in time as monitored by LC−ICP−MS and LC−ESI−MS. The reaction of DMA^V with H_2S was monitored using reverse-phase chromatography with ICP−MS and ESI−MS detection. DMA^V was sparged with H_2S gas for 30 min, and the 1 h data shown indicates the predominant arsenic-containing species (Product 1) has a retention time of 9.1 min and produces a protonated molecule at *m/z* 155 corresponding to C_2H_8AsSO. After 20 h of reaction time, the mixture was sampled again, and most of the Product 1 (DMTA^V) was converted to the slowly forming Product 2 identified with a retention time of 7.6 min. The LC−ESI−MS analysis (positive mode) of the slowly forming second product indicates a characteristic production of three major ions at *m/z* 275, 171, and 137. Chromatographic recoveries for both experiments were high (87%, 1 h; 93%, 20 h) indicating that all major products were eluting from the column. Column: intersil 5 μm ODS-2 (C-18). Solvent: 10 mM ammonium acetate mobile phase adjusted to pH 4.6 with acetic acid and containing 10% methanol (wt/wt), 0.5 mL min^{-1}.

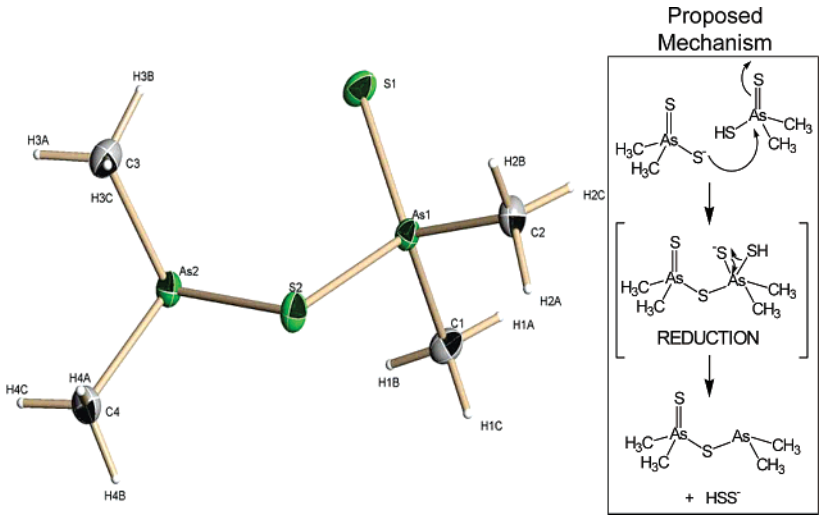

Figure 2. X-ray structure of $Me_2As(S)SAsMe_2$. X-ray diffraction data for crystals of dimethylarsino dimethyldithioarsinate were collected using a Bruker AXS SMART APEX CCD diffractometer at 100(2) K and monochromatic Mo Kα radiation with the omega scan technique. The unit cell was determined using SAINT+, and the structure was solved by direct methods and refined by full-matrix least-squares against F^2 with all reflections using SHELXTL (*1*). Experimental details of the X-ray analysis are available from the Cambridge Crystallographic Database by citing CCDC 268678.

the molecule with both As atoms connected by an As−S−As bridge. It is worth noting that the production of a mixed valence compound would be toxicologically important because of the genotoxic activity demonstrated by trivalent arsenicals (*12*). A bimolecular mechanism for the formation of $Me_2As(S)SAsMe_2$ from DMDTA likely involves loss of a transient disulfide (*43*) as shown in Figure 2. Silaghi-Dumitrescu et al. have done extended Hückel MO calculations in describing the formation and an observed sulfotropic rearrangement of a similar species (*5*). NMR analysis demonstrated the expected nonequivalence of the methyl groups attached to pentavalent versus trivalent arsenic centers. The HRMS results showed a ($M + H^+$) of 274.8891 and indicated $Me_2As(S)SAsMe_2$ could be the identity of Product 2 based on the observation of *m/z* 275 for Product 2 (see Figure 1). Additional characterization of $Me_2As(S)SAsMe_2$ was conducted and is summarized in Materials and Methods.

The above data indicate that the isolable product from the reaction of DMA^V with H_2S in aqueous ethanol is $Me_2As(S)SAsMe_2$. The product DMDTA (identified by Suzuki et al. (*28*) and Hansen et al. (*29*)) cannot be isolated as the free acid and was found to be isolable only in the presence of an appropriate metal. Instead of producing

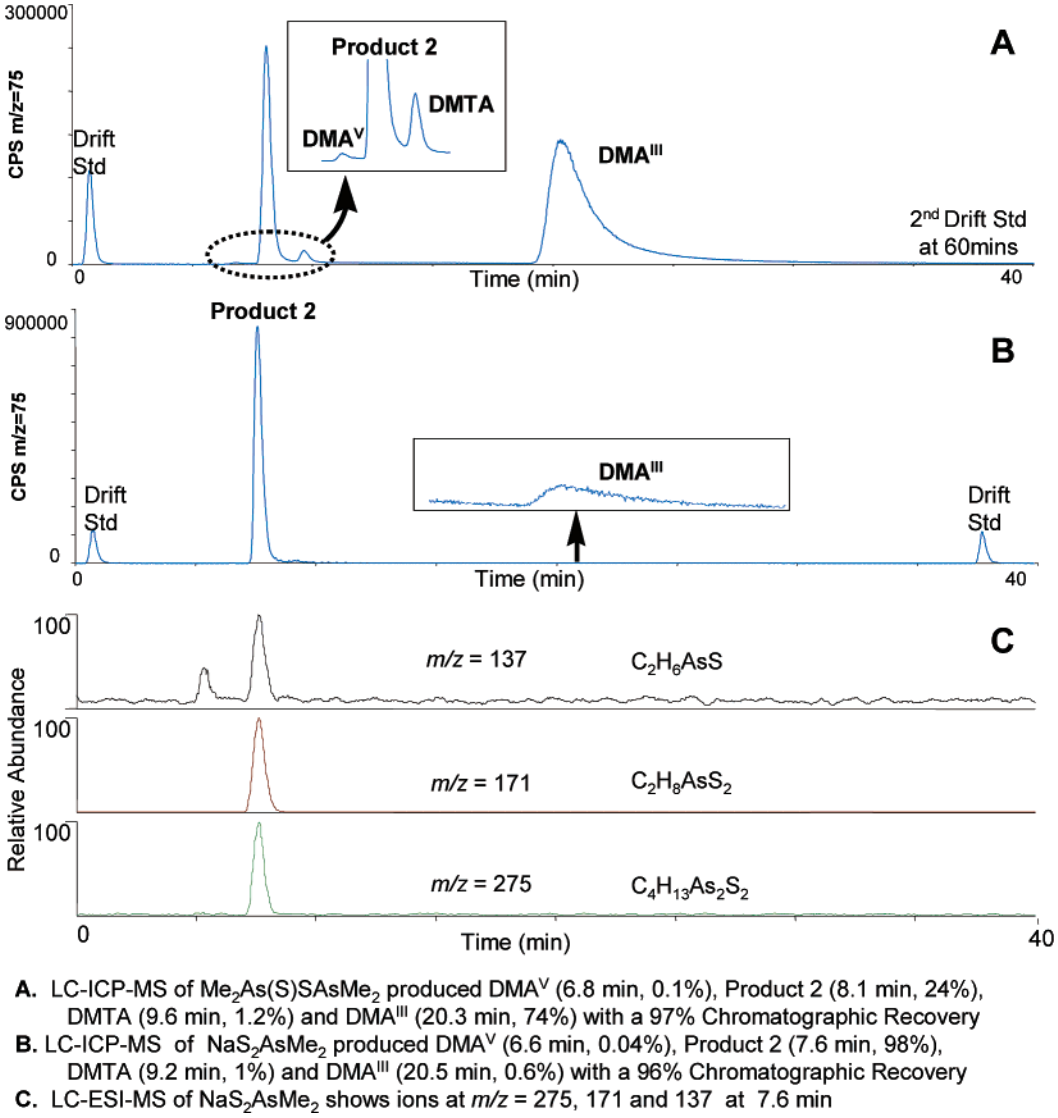

Figure 3. ICP−MS chromatograms of $Me_2As(S)SAsMe_2$ and NaS_2AsMe_2 and ESI−MS chromatogram of NaS_2AsMe_2: (A) the chromatogram resulting from dissolving crystals of $Me_2As(S)SAsMe_2$ in eluent; (B) the chromatogram from NaS_2AsMe_2 dissolved in eluent. $Me_2As(S)SAsMe_2$ is observed to degrade and produces four chromatographic peaks: DMA^V, $DMTA^V$, Product 2, and DMA^{III}. The integrated areas in panel A indicate that Product 2 and DMA^{III} represent 24% and 74% of the chromatographic arsenic, respectively. Chromatographic recoveries of 97% (A) and 96% (B) demonstrates that nearly all the arsenic species resulting from the dissolution of these standards are eluting from the column. (C) The figure gives the LC−ESI−MS results for the dissolution of NaS_2AsMe_2 and demonstrate the characteristic presence of ions at *m/z* 137, 171, and 275 representative of Product 2.

$Me_2As(S)SAsMe_2$, the transient dimethyldithioarsinate can be trapped as the metal complex affording structures such as $K[S_2AsMe_2]\cdot 2H_2O$ (*44*). In solution, it is likely that DMDTA and $Me_2As(S)SAsMe_2$ coexist, and it is the precipitation of $Me_2As(S)SAsMe_2$ or KS_2AsMe_2 that is the driving force in the formation of these products. In the present study, NaS_2AsMe_2 was prepared as described in the literature (*40*) and evaluated for use as a chromatographic standard. Our standard of NaS_2AsMe_2 was hydrated as evident by the presence of IR absorbance bands in the FT-IR ATR spectrum of the salt associated with waters of hydration (3296, 1645, and 1621 cm^{-1}). Additional characterization of NaS_2AsMe_2 is summarized in Materials and Methods.

The synthetic $Me_2As(S)SAsMe_2$ and NaS_2AsMe_2 materials were used to investigate the hypothesis that two coeluting species are producing the ICP−MS and ESI−MS signals associated with Product 2. Figure 3A shows the chromatogram resulting from dissolving crystals of $Me_2As(S)SAsMe_2$ in eluent, while Figure 3B shows the chromatogram from NaS_2AsMe_2 made up in eluent. $Me_2As(S)SAsMe_2$ is observed to degrade and produces four chromatographic peaks DMA^V, $DMTA^V$, Product 2, and DMA^{III}. The integrated areas in Figure 3A indicate that Product 2 and DMA^{III} represent 24% and 74% of the chromatographable arsenic, respectively. A 97% chromatographic recovery demonstrates that nearly all the arsenic species produced by the dissolution of $Me_2As(S)SAsMe_2$ are eluting from the column. Therefore, the integrated areas provide an indication of the distribution of the degradation products. Figure 3B shows that NaS_2AsMe_2 produces detectable quantities of the same four species, but Product 2 represents 98% of the chromatographable area. Similar to Figure 3A, the 96% chromatographic recovery in Figure 3B shows the integrated areas are indicative of the distribution of species present in solution. Finally, in Figure 3C, the LC−ESI−MS results for the dissolution of NaS_2AsMe_2 indicate the presence of ions at *m/z* 137, 171, and 275. The dissolution of $Me_2As(S)SAsMe_2$ produced similar ESI−

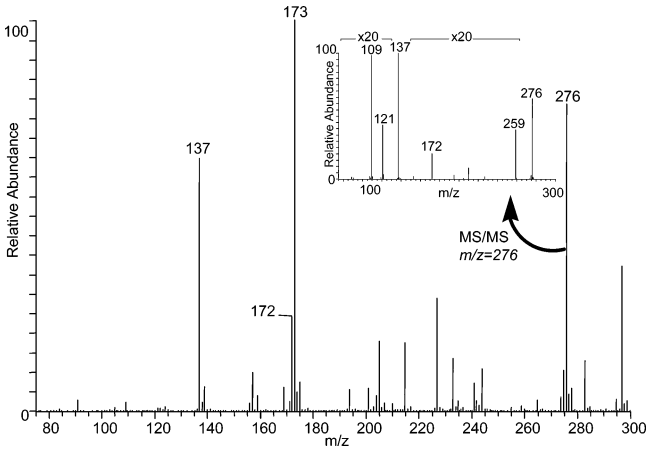

Figure 4. ESI−MS of NaS_2AsMe_2 in D_2O (1% formic acid) and ESI−MS/MS of m/z = 276. Dissolving NaS_2AsMe_2 in D_2O (1% formic acid) allowed an ESI−MS analysis (direct infusion) that demonstrated a mass shift in the deuterated solvent. This indicates that the species at m/z 173 has two exchangeable protons and the ion at m/z 276 has one exchangeable proton. Subsequent CAD analysis of the ion at m/z 276 gives a fragment ion at m/z 172 but does not yeild an ion at m/z 173 which was observed as the major peak in the ESI−MS spectrum of the standard in acidic deuterated water.

MS spectra for this eluting peak, and therefore, LC−ESI−MS is unable to unequivocally identify the source of Product 2.

To resolve the identity of Product 2, a sample of NaS_2AsMe_2 was made up in D_2O so that both ESI−MS/MS experiments and NMR data could be collected. The use of deuterated water was designed to aid in determining if the m/z 171 and 275 are independent species in solution, while NMR analysis could be used to clearly differentiate between the two standard materials. Dissolving NaS_2AsMe_2 in D_2O produced an ESI−MS analysis (direct infusion) with only one peak at m/z 215 [corresponding to $Na^+(NaS_2AsMe_2)$], and these results are consistent with the characterization of NaS_2AsMe_2 in nonacidic conditions (see Materials and Methods). To mimic the electrospray mobile phase, the mixture was acidified by addition of formic acid, and ^1H and ^{13}C NMR analyses were completed. NMR resonance signals (see Materials and Methods, Sodium Dimethyldithioarsinate, $Na(S_2AsMe_2)$) did not shift with the addition of acid, and from this, it was inferred that there was no chemical reaction. Figure 4 shows the ESI−MS analysis of the NaS_2AsMe_2 in D_2O (1% formic acid) and the MS/MS analysis of the ion at m/z 276. Mass shifts in the deuterated solvent indicate that the species at m/z 173 has two exchangeable protons and the ion at m/z 276 has one exchangeable proton. Subsequent CAD analysis of the ion at m/z 276 gives a fragment ion at m/z 172 but does not yield an ion at m/z 173 observed as the major peak in the ESI−MS spectrum of the standard in acidic deuterated water. Therefore, the m/z 173 ion observed in the ESI−MS is not produced by the species that produces m/z 276, and this result supports the hypothesis that both species are coeluting at 9.6 min. This is in contrast to the NMR data which supports a single-component peak hypothesis based on the observance of a single resonance.

Both observations could be correct if the concentration of $Me_2As(S)SAsMe_2$ was below the detection limit of NMR or if the species were undergoing a change induced by the electrospray process. The second possibility was investigated by taking an acidic solution of NaS_2AsMe_2 to dryness under argon and then holding the sample under vacuum to approximate the desolvation process associated with ESI−MS. The resulting residue was dissolved in $CDCl_3$, and the ^1H NMR contained characteristic methyl signals [δ 2.17 (s), 1.56 (s) ppm] which supported the partial conversion of NaS_2AsMe_2 to $Me_2As(S)SAsMe_2$ and DMA^{III}. The formation of DMA^{III} was confirmed by a LC−ICP−MS retention time match. The possibility of chloroform inducing the transformation was considered, but $Me_2As(S)SAsMe_2$ and DMA^{III} were not observed when NaS_2AsMe_2 was added to $CDCl_3$ or when an aqueous acidic solution of NaS_2AsMe_2 was extracted with $CDCl_3$. These observations support the hypothesis that Product 2 is undergoing a chemical change induced by the electrospray desolvation process that generates a protonated molecule at m/z 275.

Discussion

The aqueous reaction of DMA^V with H_2S produces first DMTA^V and then a second major reaction product with a retention time of 7.6 min as characterized by LC−ICP−MS and the production of LC−ESI−MS ions at m/z 275, 171, and 137. The identification of the second product as DMDTA is tentative because of the difficulty in interpreting the ESI−MS spectra of this eluting species. The argument for assignment as DMDTA is fourfold. First, the dissolution of NaS_2AsMe_2 in acidic mobile phase produces an LC−ESI−MS spectrum that is characteristic of Product 2. The NMR analysis of this solution indicated one proton singlet and one carbon resonance which is consistent with the equivalent methyl groups on DMDTA and not $Me_2As(S)SAsMe_2$ which exhibits two nonequivalent methyl groups. Second, the strongest data for the potential coelution of DMDTA and $Me_2As(S)SAsMe_2$ are the CAD experiments conducted in deuterated water (see Figure 4). These data clearly indicate that the production of the ion at m/z 171 is independent of the m/z 275 ion. The presence of $Me_2As(S)SAsMe_2$ can be refuted by the lack of supporting NMR data until the solution is allowed to go to dryness (similar to the desolvation process in ESI) and resolubilized. The resolubilized residue produces the two methyl singlets (NMR) which are characteristic of $Me_2As(S)SAsMe_2$ and provides preliminary evidence that indicates the m/z 275 ion is produced by the ESI desolvation process. Third, the DMDTA and $Me_2As(S)SAsMe_2$ species cannot be rapidly interconverting in solution because the conversion between the two species involves a redox reaction. Fourth, the dissolution of $Me_2As(S)SAsMe_2$ in water is characterized by the formation of four aqueous species with DMA^{III} representing the major product. This is contrary to the trace amount of DMA^{III} production associated with the formation of Product 2 and the dissolution of NaS_2AsMe_2.

The assignment of the second product species as DMDTA and the protonated molecule as m/z 171 requires that the other observed ions (m/z 137 and 275) are not characteristic of the solution phase. The ion at m/z 137 (Me_2AsS^+) is easily explained as it is the major product ion formed in CAD analysis of the major ions at m/z 275 and 171 and is therefore a likely fragmentation product. ESI−MS conversion of m/z 171 to produce m/z 275 is more difficult to describe because any mechanism must involve a bimolecular reaction followed by a chemical

reduction (see Figure 2) and occur on the time scale of the ESI source. Nonetheless, the desolvation process in electrospray may be responsible for this conversion as indicated on a macroscale when the solution of DMDTA was taken to dryness and shown to convert to $Me_2As(S)SAsMe_2$. The four observations described above are consistent with the identification of Product 2 as DMDTA, but the authors remain tentative in the identification because the desolvation experiment was conducted on a macroscale and may not truly replicate the actual desolvation process in ESI−MS.

From these results, the NaS_2AsMe_2 standard is suitable for use in determination of the presence of DMDTA (Product 2). An aqueous standard of DMDTA made up from NaS_2AsMe_2 was stable for at least several days. For this reason, DMDTA is believed to be a stable species under physiological conditions. Although $Me_2As(S)SAsMe_2$ does not chromatograph intact, it may be suitable as a standard for evaluating chromatographic separation of dimethylthioarsenicals because it produces quantities of four species of interest (DMA^V, $DMTA^V$, DMDTA, and DMA^{III}) when dissolved in aqueous mobile phase. Although $Me_2As(S)SAsMe_2$ is the "classic product" of the reaction of DMA^V and H_2S, this species may not be biologically relevant at cellular arsenic concentrations. The toxicological significance of the reaction of DMA^V with H_2S may be the direct reduction to DMA^{III} or in the production of the novel species $DMTA^V$ and DMDTA.

Disclaimer

This research was supported in part by appointment to the Postgraduate Research Participation Program administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. DOE and the U.S. EPA. The U.S. Environmental Protection Agency, through its Office of Research and Development, and the U.S. Food and Drug Administration funded and managed the research described in this paper. This manuscript has been reviewed in accordance with EPA peer and administrative review policies and approved for publication. No official support or endorsement by FDA of this article is intended or should be inferred. The mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Acknowledgment. Matthias Zeller thanks Allen D. Hunter of Youngstown State University for granting access to the X-ray diffractometer, which was funded by NSF Grant 0087210, by Ohio Board of Regents Grant CAP-491, and by YSU.

Supporting Information Available: Figures showing the LC−ICP−MS of the reaction of DMA with H_2S in water and aqueous ethanol (SI-1), the FT-IR and dispersive Raman spectra of $MeAs(S)SAsMe_2$ (SI-2) and NaS_2AsMe_2 (SI-3), and a graph of the effect of capillary temperature and the ESI−MS production of major ions found for NaS_2AsMe_2 (SI-4). This material is available free of charge via the Internet at http://pubs.acs.org.

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