Methylated and thiolated arsenic species for environmental and health research — A review on synthesis and characterization

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ABSTRACT

Hundreds of millions of people around the world are exposed to elevated concentrations of inorganic and organic arsenic compounds, increasing the risk of a wide range of health effects. Studies of the environmental fate and human health effects of arsenic require authentic arsenic compounds. We summarize here the synthesis and characterization of more than a dozen methylated and thiolated arsenic compounds that are not commercially available. We discuss the methods of synthesis for the following 14 trivalent (III) and pentavalent (V) arsenic compounds: monomethylarsonous acid (MMAIII), dicysteinylmethyldithioarsenite (MMAIII(Cys)2), monomethylarsonic acid (MMAV), monomethylmonothioarsonic acid (MMMTAV) or monothio-MMAV, monomethyldithioarsonic acid (MMDTAV) or dithio-MMAV, monomethyltrithioarsonate (MMTTAV) or trithio-MMAV, dimethylarsinous acid (DMAIII), dimethylarsino-glutathione (DMAIII(SG)), dimethylarsinic acid (DMAV), dimethylmonothioarsinic acid (DMMTAV) or monothio-DMAV, dimethyldithioarsinic acid (DMDTAV) or dithio-DMAV, trimethylarsine oxide (TMAOV), arsenobetaine (AsB), and an arsenicin-A model compound. We have reviewed and compared the available methods, synthesized the arsenic compounds in our laboratories, and provided characterization information. On the basis of reaction yield, ease of synthesis and purification of product, safety considerations, and our experience, we recommend a method for the synthesis of each of these arsenic compounds.

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Introduction

The Agency for Toxic Substances and Disease Registry (ATSDR, 2015) has, for many years, consistently ranked arsenic at the top of its Priority List of Hazardous Substances, on the basis of its occurrence, toxicity, and potential for human exposure. Arsenic is commonly associated with oxygen, sulphur, carbon, and hydrogen, forming a variety of arsenic compounds (species). A variety of arsenic compounds have been made in the laboratory primarily for medical research (Cullen, 2008; Chen et al., 2015). Nearly one hundred arsenic species have been detected in the environment (Cullen and Reimer, 1989; Edmonds and Francesconi, 1977; Foster and Maher, 2016; Francesconi and Edmonds, 1997; Francesconi, 2010; Goessler et al., 1997; Jackson et al., 2012; Mandal and Suzuki, 2002; Nearing et al., 2016; Oremland and Stolz, 2003; Popowich et al., 2016; Reimer et al., 2010; Wallschläger and Stadey, 2007; Zhao et al., 2010) and as human metabolites (Abedin et al., 2002; Jeffers et al., 2013; Rubin et al., 2014; Thomas et al., 2001, 2007; Vahter, 2002; Wang et al., 2015). These arsenic species have very different toxicities, ranging by several orders of magnitude in median lethal dose (LD₅₀) and median lethal concentration (LC₅₀) values (Charoenasuk et al., 2009; Ebert et al., 2014; Kaise and Fukui, 1992; Moe et al., 2016; Molin et al., 2015; Naranmandura et al., 2011; Petrick et al., 2000; Styblo et al., 2000). Therefore, it is crucial to characterize all arsenic species encountered in studies of arsenic biotransformations, human exposure, and health effects (Carlin et al., 2016; Feldmann and Krupp, 2011; Le et al., 2004; Liu et al., 2016). The identification and quantification of arsenic species are greatly facilitated by the availability of appropriate arsenic standards.

Synthetic arsenic chemistry has a long history dating back to the late 1700s. The French chemist Louis Claude Cadet de Gassicourt prepared a noxious inflammable mixture of two liquids; one became known as cacodyl, As₂(CH₃)₄, and the other as cacodyl oxide, (As₂(O)(OH)₂) (MMAV). Bunsen was a major contributor to these early studies, and subsequent extensive research by others has provided an understanding of the chemistry of these organoarsenicals. However, some newly discovered arsenic metabolites have presented synthetic challenges. There is no review on the preparation and characterization of some important newly discovered arsenic metabolites, such as some methyl arsenicals and their sulphur-containing analogues: monomethylarsonous acid (MMAIII), dimethylarsinous acid (DMAII), monomethylmonothioarsinous acid (MMMTAII), dimethylarsinous acid (MMTAIII), dimethyldithioarsinous acid (DMDTAIII), and dimethyldithioarsenic acid (DMDTAIV) (Chen et al., 2013, 2016; Kubachka et al., 2009; Le et al., 2000a, 2000b; Naranmandura et al., 2007; Thomas et al., 2007; Ochi et al., 2008; Rubin et al., 2014; Sun et al., 2016; Suzuki et al., 2004; Wallschläger and Stadey, 2007; Wang et al., 2015; Yoshida et al., 2003). The separation, identification, and detection of these arsenic species often rely on high performance liquid chromatography (HPLC), inductively coupled plasma mass spectrometry (ICP-MS), and electrospray ionization mass spectrometry (ESI-MS) (Beauchemin et al., 1989; Heitkemper et al., 1989; Gong et al., 2002; Larsen et al., 1993; McSheehy et al., 2002; Hansen et al., 2003; Nischwitz and Pergantis, 2006; Peng et al., 2014; Yang et al., 2016). The use of respective arsenic standards in speciation analysis is vital to confirm the identity of the suspected arsenic species.

A lack of authentic arsenic standards or the use of improperly characterized synthetic products can lead to incorrect identification of arsenic species. For example, a dimethylthioarsenical has been mistaken for DMAIII, because of the use of an uncharacterized synthetic product serving as arsenic standard (Hansen et al., 2004; Suzuki et al., 2004). In this case the method used for synthesizing DMAIII was based on the method that Reay and Asher (1977) used to reduce the pentavalent inorganic arsenate to the trivalent arsenite. However, when this method is used to reduce the pentavalent dimethylarsenical, the product is not the trivalent dimethylarsenical as assumed but the thiolated dimethylarsenicals, dimethylmonothioarsinate (Me₂As(S)OH) and dimethylthioarsionate (Me₂As(S)SH) (Hansen et al., 2004; Fricke et al., 2005). Thus, relying on this uncharacterized product led to the incorrect identification of arsenic species (Hansen et al., 2004; Suzuki et al., 2004; Naranmandura et al., 2007).

The primary objectives of this paper are (1) to review and compare the methods of synthesis reported in the literature, and (2) to recommend detailed procedures for the synthesis and characterization of the methylated and thiolated arsenicals. These new arsenicals are not commercially available but are in high demand for research purposes. This review of the various methods of synthesis provides perspectives on the diverse chemistry of arsenic. The detailed procedures for the synthesis of specific arsenic species will help guide other researchers to synthesize arsenic compounds for use in studies of the transformations/metabolism, environmental fate and behaviour, human exposure, and toxicological effects of arsenicals.

1. Monomethylarsonic acid or monomethylarsonate ([CH₃As(O)(OH)₂]) (MMAV)

MMAV is a common metabolite of inorganic arsenic formed intracellularly in the majority of organisms: animals, plants, and microorganisms. MMAV, e.g., the commercially available monosodium methanearsonate, has been used as an active ingredient in human medicines and in pesticides and herbicides for weed control (Moore and Ehman, 1977). MMAV can be prepared on an industrial scale.

1.1. Methods for the synthesis of MMAV

MMAV is usually prepared as sodium methylarsonate (NaCH₃AsO₄·H₂O·6H₂O) or disodium methylarsonate (Na₂CH₃AsO₄·6H₂O). Several methods are available to synthesize MMAV. The Meyer reaction (Meyer, 1883) produces sodium...
methylarsonate by reacting sodium arsenite with an alkyl halide, such as methyl iodide (CH₃I). Quick and Adams (1922) investigated the synthesis of arsonic (RAsO₃H₂) and arsine (R₃AsO₃H) acids by using different alkyl halides. Miller and Seaton (1948) developed and patented a method for the industrial manufacturing of sodium methylarsonate. This method employs a closed system, high temperature (60°C), and high pressure (60 psi), and the less expensive methyl chloride as the methylating agent.

### 1.2. Comparison of the methods for the synthesis of MMAV

The Meyer reaction is an effective method for preparing sodium methylarsonate. When methyl iodide is used in the Meyer reaction, practically pure sodium methylarsonate can be obtained with mere cooling of the reaction mixture (Miller and Seaton, 1948). The patented Miller reaction uses conditions under which the less costly methyl chloride is able to act as the methylating agent (Miller and Seaton, 1948; Morgan, 1918). Furthermore, the addition of ammonia and calcium chloride to the reaction mixture allows for the precipitation of the arsenic compound as a calcium salt, which is easily isolated. Because the Miller reaction requires a closed system, high pressure, and elevated temperature, it is not always practical for use in a research laboratory. It is an efficient method for larger scale manufacturing of sodium methylarsonate.

### 1.3. Our procedures for the synthesis of MMAV

According to the method of Quick and Adams (1922), As₂O₃ (9.9 g or 50 mmol) was added to a 10 mol/L NaOH solution, forming tri-sodium arsenite (Na₃AsO₃). CH₃I (15 g, 106 mmol) was added to the solution. The mixture was heated to 50°C for 3 hr, forming a white solid. Ethanol (30 mL) was then added, and the mixture was allowed to stand overnight. A white crystalline solid was isolated by filtration and air dried, producing 25 g of MMAV, corresponding to a yield of 86%.

### 1.4. Characterization of MMAV

Previous reports have shown that MMAV has a melting point of 161°C (Morgan, 1918). Proton nuclear magnetic resonance (¹H NMR) of MMAV dissolved in D₂O plus D₂SO₄ detected the methyl protons at 1.99 ppm (Naranmandura et al., 2007). ESI-MS analysis of Na₂CH₃AsO₃ in negative ionization mode detected fragment ions at m/z 91 ([AsO⁺]), 107 ([AsO₂⁻]), 121 ([CH₂AsO₂⁻]), and 124 ([AsO₂OH⁻]).

### 2. MMAV [CH₃As(OH)₂]

MMAV is a trivalent methylarsenical metabolite of inorganic arsenic. It is formed during arsenic biomethylation that involves the stepwise reduction of the pentavalent arsenicals to the trivalent arsenicals followed by the oxidative addition of a methyl group (Challenger, 1945; Cullen et al., 1989; Cullen, 2014; Le et al., 2004; Thomas et al., 2001, 2004, 2007; Vahter, 2002). MMAV is more toxic than its pentavalent counterpart MMAV (Charoensuk et al., 2009; Naranmandura et al., 2011; Petrick et al., 2000; Styblo et al., 1997, 2000; Vega et al., 2001). MMAV has been detected in biological and environmental samples (Aposhian et al., 2000; Currier et al., 2016; Del Razo et al., 2001; Le et al., 2000a, 2000b; McKnight-Whitford et al., 2010; Naranmandura et al., 2013).

#### 2.1. Methods for the synthesis of MMAV

Both diiodomethylarsine (CH₃AsI₃) and methylarsine oxide (CH₃AsO) have been synthesized. The main process for the synthesis of diiodomethylarsine (CH₃AsI₃) involves the treatment of sodium methylarsonate solution with iodide, hydrochloric acid, and sulphur dioxide (Auger, 1906) (Scheme 1).

Two methods are available for the synthesis of methylarsonic oxide. The first method, developed by Cullen et al. (1989), uses the commercially available sodium methylarsonate as the starting material, and SO₂ gas for the reduction of MMAV to MMAV as shown in Scheme 2.

The second method, which is depicted in Scheme 3, is that of Auger (1903, 1906). The starting material, diiodomethylarsine, is dissolved in benzene and then treated with an excess of sodium carbonate and cooled until the solution becomes discoloured. After the benzene is decanted, the remaining solvent is evaporated and methylarsine oxide crystallizes.

It was previously thought that the Reay and Asher (1977) method produced methylarsine oxide through the reduction of MMAV using a reducing solution containing sodium metabisulphite, sodium thiosulphate, and sulphuric acid. Hansen et al. (2004) and Naranmandura et al. (2007) demonstrated that the Reay and Asher method produced mostly thioarsenicals instead of methylarsine oxide.

#### 2.2. Comparison of the methods for the synthesis of MMAV

Diodomethylarsine (CH₃AsI₃) is more stable than methylarsine oxide (CH₃AsO) during storage, although the trivalent methylarsenicals are prone to oxidation in general. Both methylarsonic oxide and diiodomethylarsine dissolve in dilute aqueous solutions as MMAV. Both methods for the synthesis of CH₃AsO are simple and produce (CH₃AsO), in high yields. Cullen’s method (Cullen et al., 1989) uses a commercially available starting material, whereas Auger’s method requires CH₃AsI₃, which is not commercially available and must be synthesized prior to the production of CH₃AsO.

#### 2.3. Our procedures for the synthesis of MMAV

We used the following procedures (Auger, 1906; Millar et al., 1960; Zingaro, 1996a) for the synthesis of CH₃AsI₃. Na₂CH₃AsO₃ (25.1 g, 86 mmol) and KI (31.3 g, 188 mmol) were dissolved in a mixture of 16 mL of concentrated hydrochloric acid and 40 mL of water. SO₂ gas was bubbled into the solution for 20–30 min. A yellow solid, with an oily liquid at the bottom, formed. The solid was collected by filtration and recrystallized in diethyl ether, affording 20 g of CH₃AsI₃ (68% yield and 98.1% purity).

We synthesized methylarsine oxide using two methods. Method A followed the report of Cullen et al. (1989). Na₂CH₃AsO₃ (10 g, 34 mmol) was dissolved in 20 mL of warm water. SO₃(g) was bubbled into the solution for 20 min. The solution was heated to boiling for 10 min and then allowed to cool. The solution was...
neutralized with Na₂CO₃ and evaporated to dryness. The resulting powder was extracted with hot benzene and the extracts were evaporated to dryness, giving an oily substance that solidified later. After crystallization in ether, white crystals were obtained, giving 3.5 g (75% yield) of methylarsine oxide.

Method B for the synthesis of CH₃AsO was adopted from Auger (1903, 1906). CH₃AsI₂ (20 g, 58 mmol) was dissolved in benzene. Na₂CO₃ (6 g) and 10 mL of H₂O were added. The solution was refluxed for 2 hr and the benzene layer was then removed. The aqueous layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The resulting white powder was recrystallized from ether, giving 5.1 g (84% yield) of methylarsine oxide (98.2% purity).

2.4. Characterization of MMAⅢ

Analysis of our synthesized CH₃AsI₂ showed a melting point of 30°C, which is consistent with that reported by Millar et al. (1960). ¹H NMR gave signals at 3.11 ppm in CDCl₃ and 1.37 ppm in D₂O (DHO residual at 4.72 ppm). CH₃AsI₂ undergoes slow decomposition and darkening even when stored under an inert atmosphere. For this reason, CH₃AsI₂ should be stored in the dark, but does not need to be stored under nitrogen (Matuska et al., 2010).

Analysis of our methylarsine oxide showed a melting point of 95°C and ¹H NMR signals in CDCl₃ at 1.406 ppm, 1.451 ppm (Major), 1.476 ppm and 1.484 ppm. ESI-MS analysis of MMAⅢ is difficult because MMAⅢ is not easily ionizable by electrospray. We introduced on-line derivatization with dimercaptosuccinic acid (DMSA), by mixing the chromatographic effluent with DMSA, to form a DMSA–MMAⅢ complex. DMSA introduces negative charges, making the DMSA–MMAⅢ complex amenable for ESI-MS analysis (McKnight-Whitford et al., 2010; McKnight-Whitford and Le, 2011).

When dissolved and diluted in aqueous solutions, both methylarsine oxide and diiodomethylarsine are present as CH₃As(OH)₂ (MMAⅢ). In dilution aqueous solutions, MMAⅢ is easily oxidized to the pentavalent MMAⅤ (Gong et al., 2001), and therefore MMAⅢ solutions need to be prepared/diluted fresh before use.

3. Dimethylarsinic acid [(CH₃)₂As(O)OH] (DMAⅤ)

DMAⅤ is a major metabolite of inorganic arsenic in many organisms including humans (Vahter, 2002). DMAⅤ has been produced in large quantities for industry (agricultural) uses, and it is also known as cacodylic acid.

3.1. Methods for the synthesis of DMAⅤ

The first reported method of industrial preparation of DMAⅤ dates back to 1923 (Inverni, 1923). The methylation of As₂O₃ was achieved by heating a mixture of As₂O₃ and crude fused potassium acetate. Cacodyl oxide ((CH₂As)₂O), which was produced and distilled during the heating, was further air-oxidized and converted to cacodylic acid. The general reaction is described in Scheme 4. The use of this method was continued into the 1940s (Treffler, 1944). The air oxidation of cacodyl oxide was further optimized (Fioretti and Portelli, 1963).

Another method, using alkyl halides and dimethyl sulphate as alkylating agents, is more efficient and applicable to research laboratory settings. The synthesis reaction is described in Scheme 5, as summarized by Moore and Ehman (1977).

3.2. Comparison of different methods for the synthesis of DMAⅤ

Cost and efficiency are two major considerations in choosing the preferred method for the industrial preparation of cacodylic acid. The method described in Scheme 4 predominated in the early 20th century because crude fused potassium acetate is much cheaper than methyl iodide (CH₃I). However, the method using crude fused potassium acetate has a very limited yield. Only 5 kg of cacodylic acid was obtained from 60 kg of the mixed As₂O₃ and potassium acetate (Inverni, 1923).
The second method uses an alkyl halide as the methylating agent. CH₃I has a higher reactivity but is more expensive than methyl chloride (CH₃Cl). In 1965, a patent on the synthesis of DMAV using methylarsine oxide and CH₃Cl was approved (Moyerman and Ehman, 1965). The cost of manufacturing DMAV was reduced using CH₃Cl as the methylating agent in Scheme 5.

3.3. Characterization of DMAV

DMAV is commercially available from most chemical reagent distributors. The melting point of the commercially available DMAV is 192–198°C. ESI-MS analysis in negative ionization showed parent ion [M − H]⁻ at an m/z of 137. MS/MS analyses detected characteristic fragment ions at m/z of 107 ([AsO₂]⁻) and 122 ([CH₃AsO₂]⁻).

4. DMAIII [(CH₃)₂AsOH]

Similar to MMAIII, DMAIII is another trivalent methylarsenical metabolite of inorganic arsenic in animals including humans (Cohen et al., 2016; Currier et al., 2016; Del Razo et al., 2001; Le et al., 2000b; Wang et al., 2004). DMAIII ((CH₃)₂AsOH) is more toxic than DMAV, and DMAIII is reactive with available cysteine groups in proteins (Lu et al., 2004). DMAIII in dilute aqueous solutions is unstable, and can easily be oxidized to the pentavalent DMAV (Gong et al., 2001). Therefore, its iodide form, dimethyliodoarsine ((CH₃)₂AsI), is usually prepared and stored. (CH₃)₂AsI can be dissolved in dimethyl sulphoxide (DMSO) and further diluted in water, forming (CH₃)₂AsOH.

4.1. Methods for the synthesis of DMAIII

Cacodyl oxide is first converted into cacodyl chloride in the presence of mercuric chloride, and further converted into cacodyl bromide or cacodyl iodide using KBr or KI. The reaction is described in Scheme 6.

Another method to synthesize (CH₃)₂AsI involves the reduction of cacodylic acid using sulphur dioxide (Burrows and Turner, 1920; Millar, 1960). Burrows and Turner (1920) developed this method, borrowing Auger’s method (Auger, 1906) for the reduction of methylarsinic acid to diiodomethylarsine. The main reaction is shown in Scheme 7.

4.2. Comparison of different methods for the synthesis of DMAIII

The method that uses cacodyl oxide as the starting material is a two-step reaction (Scheme 6). The second conversion is nearly quantitative. The conditions for the reduction of cacodylic acid using SO₂ are mild (Scheme 7). Burrows and Turner (1920) obtained a yield of 90%.

Because cacodylic acid could not be cost-effectively synthesized from MMAIII and CH₃Cl until the late 1960s, cacodyl oxide was produced in a large quantity as a starting material for the syntheses of other organoarsine compounds in the early 1920s. This is probably the main reason why cacodyl oxide was directly used to prepare (CH₃)₂AsI at that time. Now that cacodylic acid can be cost-effectively synthesized, the method shown in Scheme 7 is widely used.

After cacodylic acid is reduced to (CH₃)₂AsI, an oily yellow product is formed, which can be easily separated. The oil solution is dried over calcium chloride and distilled. A yellow liquid that boils at 154–157°C is collected. Millar et al. (1960) synthesized (CH₃)₂AsI from CH₃AsI₂, which involved methylation to form cacodylic acid and reduction to produce (CH₃)₂AsI. Improvements were made to the purification procedures. The oil solution separated from the reaction system was first extracted with ether, and then the ether solution was dried and distilled under nitrogen. Fractional distillation was used to collect the fraction boiling at 154–166°C. The material collected was re-distilled and the fraction boiling at 154–157°C was collected. The overall yield of (CH₃)₂AsI starting from CH₃AsI₂ was 50%.

4.3. Our procedures for the synthesis of DMAIII

Potassium iodide (56 g, 0.34 mol) and cacodylic acid (28 g, 0.20 mol) were dissolved in 230 mL water mixed with 18 mL H₂SO₄. SO₂ gas was bubbled into the solution for 30 min. The colour of the solution changed from orange to colourless, then to yellow. The yellow oil was separated and dried over anhydrous Na₂SO₄. After distillation under a nitrogen atmosphere and collecting the fraction boiling at 155–157°C, 40 g (85% yield) of (CH₃)₂AsI (pure yellow liquid) was obtained.

4.4. Characterization of DMAIII

Dimethylarsine hydroxide [(CH₃)₂AsOH] was obtained after dissolving a small amount of (CH₃)₂AsI in DMSO and further
diluting it in water. The purity of (CH₃)₂AsI was determined using HPLC separation followed by ICP-MS detection. We found that the freshly prepared dilute DMAIII solution contained 88% ± 1% DMAIII and 12% ± 1% DMAV. The presence of DMAV is likely due to oxidation of DMAIII during sample preparation and HPLC analysis. ¹H NMR spectra showed signals at δ 2.02 ppm in CDCl₃ and 1.3 ppm in D₂O. The boiling point of (CH₃)₂AsI was 154–160°C and the melting point was ca. –35°C.

Because DMAIII can be easily oxidized to DMAV in solution under normal laboratory atmosphere, and DMAIII is poorly ionized by electrospray, it is difficult to obtain a mass spectrum of DMAIII. We used post-column derivatization with DMSA to form the DMSA–DMAIII complex (McKnight-Whitford et al., 2010; McKnight-Whitford and Le, 2011). The presence of DMSA contributed to the ionization of the complex. HPLC separation of DMAIII, followed by DMSA derivatization and ESI-MS detection of the DMSA–DMAIII complex gave a molecular ion (M⁺) at m/z 285. MS/MS analysis of this molecular ion detected characteristic fragment peaks at m/z 103 ([C₃H₃O₂S]⁻), 122 ([CH₃AsS]⁻), and 137 ([(CH₃)₂AsS]⁻).

5. Trimethylarsine oxide [(CH₃)₃AsO] (TMAO⁻)

TMAO⁻ has been detected in marine microorganisms (Cullen et al., 1979; Kaise et al., 1987). As a metabolite of inorganic arsenic, it has also been shown to be produced in rodents (Chen et al., 2011, 2013; Lu et al., 2003; Yamauchi et al., 1990; Yoshida et al., 2001).

5.1. Methods for the synthesis of TMAO⁻

Two methods have been reported to synthesize TMAO⁻ from trimethylarsine [(CH₃)₃As]. The main difference between these methods lies in the choice of oxidizing agent.

Razuvaev et al. (1935) used iodine to oxidize (CH₃)₃As in an aqueous solution. The di-hydroxyl derivative, [(CH₃)₃As(OH)₂], was obtained instead of (CH₃)₃AsO. The product was then dried over P₂O₅ under vacuum, giving the oxide form. The overall reaction is described in Scheme 8. Razuvaev et al. also mentioned the use of bromine to oxidize (CH₃)₃As in diethyl ether, producing (CH₃)₃AsO. The yield varied from 70% to 80%.

Another method is to use hydrogen peroxide (H₂O₂) to oxidize (CH₃)₃As to (CH₃)₃AsO (Merijani and Zingaro, 1966; Zingaro, 1996). The reaction is described in Scheme 9. However, because of safety concerns over using hydrogen peroxide to oxidize (CH₃)₃As, we recommend that this method be avoided.

5.2. Comparison of different methods for the synthesis of TMAO⁻

Both methods described above use (CH₃)₃As as the starting material. (CH₃)₃As was synthesized by methylating dimethylarsine halide using Grignard reagents. Other reagents, such as I₂ and Br₂, can be used to oxidize (CH₃)₃As. The solvent can affect the chemical form of the product. Generally, the di-hydroxyl form is obtained in an aqueous solution, and the oxide form is obtained in ether.

When H₂O₂ is used to oxidize (CH₃)₃As in ether, extreme precautions should be taken. This reaction is exothermic and (CH₃)₃As is volatile and flammable. Peroxides could be formed. An efficient fume hood and effective personal protective equipment should be used. Secondly, exposure of (CH₃)₃As to normal laboratory atmosphere or to an excessive amount of H₂O₂ solution will result in the formation of cacodylic acid instead of (CH₃)₃AsO. Therefore, the reaction should take place in an atmosphere of nitrogen and only equivalent molar amounts of H₂O₂ (30%, aq.), not excessive amounts, should be added dropwise into the ether solution of (CH₃)₃As.
The oxidation of (CH₃)₃As using H₂O₂ described by Zingaro and coauthors (Merijani and Zingaro, 1966; Zingaro, 1996) has been the primary synthetic route for preparing (CH₃)₃AsO for 40 years. The procedures can be scaled up to 25 g. However, note that in one case a damaging explosion occurred during this reaction, causing personal injury. This accident prompted the re-evaluation of the method (Fricke, 2004).

5.3. Our procedures for the synthesis of TMAOV

Because of the challenges associated with handling (CH₃)₃As and the danger of the H₂O₂ oxidation, we developed a new method to synthesize (CH₃)₃AsO. The synthesis starts from (CH₃)₂AsI instead of (CH₃)₃As. (CH₃)₂AsI is methylated using dimethyl sulphate (method A) or methyl iodide (method B). During the methylation, the trivalent arsenic is oxidized to the pentavalent species, thus producing (CH₃)₃AsO. This process mimics the biotransformation of DMAIII to TMAOV in microorganisms (Cullen et al., 1979). The general reaction is described in Scheme 10.

Method A: (CH₃)₂AsI (1.15 g, 5 mmol) was dissolved in 5 mL of water containing NaOH (0.8 g, 20 mmol). (CH₃)₂SO₄ (0.75 g, 6 mmol) was added slowly. The reaction mixture was stirred overnight and then heated to 80°C for 1 hr. The solution was neutralized with concentrated H₂SO₄. Ten grams of pre-treated Amberlite IRA-402 resin (Sigma, St. Louis, MO, USA) was added and the mixture was shaken for 10 min. The purpose of using this anion exchange resin was to remove the excess iodine ion; otherwise, TMAOV could not be isolated and only hydroxytrimethylarsonium iodide could be obtained (Patrick et al., 2005). The resin was filtered out and washed with water and the washings were combined with the filtrate. The combined filtrate and washings were evaporated to dryness, leaving a solid which was sublimed to give 0.6 g of (CH₃)₃AsO. The yield was 88%.

Method B: (CH₃)₂AsI (2.0 g, 8.6 mmol) was dissolved in 10 mL of water containing NaOH (1.38 g, 34.5 mmol) in a Carius tube. CH₃I (1.2 g, 8.6 mmol) was added slowly into the reaction solution. The tube was sealed and left in an oven at 60°C overnight. The cleanup procedure of Method A was used to obtain (CH₃)₃AsO in a similar yield.

5.4. Characterization of TMAOV

As reported by Kaise et al. (1987), the signal of ¹H NMR spectrum was at δ 1.778 ppm (singlet) and the signal of ¹³C NMR spectrum was at δ 17.27 ppm (CH₃). Infrared analysis of (CH₃)₃AsO showed 921 cm⁻¹ (As–O stretch), 590 cm⁻¹ (As–C stretch), 275 cm⁻¹ (As–O bend), and 215 cm⁻¹ (C–As–C bend) (Jensen and Jensen, 2004).

Our ESI-MS analysis in the positive mode showed a characteristic [M + H]+ peak at m/z 137. Fragments of the parent ion [M + H]+ obtained from MS/MS analysis included peaks at m/z 89 ([AsCH₂]+), 91 ([AsOH]+), 107 ([CH₃AsOH]+), and 122 ([(CH₃)₂AsOH]+). We also observe a peak at m/z 117, as did by McSheehy et al. (2002); however, we could not assign the identity of this fragment. Similar results have also been reported in previous studies (McSheehy et al., 2002; Nischwitz and Pergantis, 2005, 2006; Schaeffer et al., 2006).

6. Arsenobetaine

In 1925, Cox (1925) found elevated levels of arsenic in human urine from those who consumed shellfish as part of their diet. One year later, Chapman (1926) showed that crustaceans contained particularly high concentrations of arsenic. Arsenic was eliminated rapidly in the urine of people who had eaten shellfish (Crecelius, 1977; Freeman et al., 1979; Le et al., 1994a, 2004; Popowich et al., 2016).

Arsenobetaine was referred to as “hidden arsenic” because it could not be detected using the then available hydride generation methods (Cullen and Reimer, 1989; Le et al., 1993). The identification of arsenobetaine was reported in 1977 by...
Edmonds et al. (1977). The structure was confirmed by using X-ray crystallography (Cannon et al., 1981).

Before the structure of arsenobetaine became known, it had been isolated from commercially important seafood, such as the liver of cod, the tail muscle of the western rock lobster, and from the flesh of the dusky shark (Cannon et al., 1981; Lunde, 1975). Methanol was used for extraction. After evaporation of the organic solvent, the residue was dissolved in water. The aqueous solution was then mixed and shaken with phenol. The impurities remained in the aqueous layer. The phenol layer was diluted with ether and shaken with water, and the aqueous solution passed through an ionic exchange column to remove acids and bases. The final product was purified by using chromatography and crystallized.

### 6.1. Methods for the synthesis of arsenobetaine

Edmonds et al. (1977) synthesized arsenobetaine from DMA\(^V\) (Scheme 11). Cacodylic acid was converted to dimethyldioarsine, based on the method of Burrows and Turner (1920). The resulting dimethyldioarsine was treated with methylmagnesium iodide, as recommended by Challenger and Ellis (1935), to generate trimethylarsine, which was then condensed with ethyl bromoacetate to afford the quaternary arsonium bromide. According to the method of Kosower and Patton (1961), hydrolysis of this substance produced arsenobetaine. The total yield from dimethyldioarsine was 10.8%.

Goetz and Norin (1983) used the reaction depicted in Scheme 12 to synthesize \(^{73}\text{As}\)-labelled trimethylarsine. The synthesis of trimethylarsine is a key step in the preparation of \(^{73}\text{As}\)-labelled arsenobetaine. \(^{73}\text{As}\)(OH)\(_3\) was added to a solution of HCl, forming AsCl\(_3\), which was then extracted by n-pentane. LiCH\(_3\) in di-n-butyroether was used as a methyl donor in the preparation of trimethylarsine. After boiling at 190°C, 95% \(^{73}\text{As}\) was distilled out as \(^{73}\text{As}\)(CH\(_3\))\(_3\). The rest procedure of using \(^{73}\text{As}\)(CH\(_3\))\(_3\) to synthesize arsenobetaine followed the method of Edmonds et al. (Scheme 11). The total yield of this method was 72%.

To improve the yield of arsenobetaine from trimethylarsine, Ismail and Toia (1988) used bromoacetic acid to react with trimethylarsine; their procedures are illustrated in Scheme 13 (bottom). A yield of 81% was obtained in this reaction. The reaction of trimethylarsine with bromoacetic acid leads to the formation of the hydrobromide of arsenobetaine, which crystallizes directly from the reaction mixtures.

Minhas et al. (1998) modified Ismail and Toia’s method to synthesize arsenobetaine bromide. (CH\(_3\))\(_3\)As was reacted with bromoacetic acid, and the final product was collected and gave a yield of 96%.

Although the yields for the synthesis of arsenobetaine gradually improved, trimethylarsine remained difficult to isolate due to its low boiling point (52°C) and flammability. Fricke (2004) developed a synthesis method for arsenobetaine that avoided the use of volatile trimethylarsine (Scheme 14). Sodium dimethylarsenide, instead of trimethylarsine, was condensed with ethyl bromoacrylate. The product was then converted to quaternary arsonium salts by the addition of methyl iodide, and the quaternary arsonium ion could be hydrolyzed to the arsenobetaine.

Bernardo et al. (2004) also developed a “one-pot” synthesis method to produce \(^{14}\text{C}\)-labelled arsenobetaine, as shown in Scheme 15. Dimethylarsinylacetic acid was the reduction product of the pentavalent 2-(dimethylarsinyl)acetic acid. The trivalent arsinylic acid was methylated by \(^{14}\text{C}\)methyl...
iodide, which resulted in 14C-labelled arsenobetaine. The chemical yield of 14C-labelled arsenobetaine was only 19%. Starting from trimethylarsine, Lischka et al. (2011) followed the methods of Irgolic et al. (1987) and Lagarde et al. (1999) to synthesize a 13C-labelled arsenobetaine by using ethyl bromoacetate-13C2. The yield of 13C-labelled product was 88%.

6.2. Our procedures for the synthesis of arsenobetaine

DMAV (50 g, 362 mmol) was dissolved in 200 mL of water. Potassium iodide (165 g, 994 mmol) and sodium bisulphite (5 g, 48 mmol) were added, followed by rapid addition (2 min) of 250 mL of concentrated hydrochloric acid. This mixture was stirred for 24 hr at room temperature with occasional addition of sodium bisulphite (total of 23 g, 221 mmol). The resulting yellow oil was separated and dried over calcium chloride. Distillation gave 75 g of dimethyliodoarsine as yellow oil.

Sodium (9.0 g, 390 mmol) in 150 mL of tetrahydrofuran (THF) was prepared and the flask was cooled in a dry ice/acetone bath. Dimethyliodoarsine (26.8 g, 116 mmol) dissolved in 65 mL of THF was added with stirring in 1 hr. The reaction was observed to produce a dark brownish-green mixture. The solution of sodium dimethylarsenide was separated from excess sodium and the sodium iodide byproduct under a positive argon pressure. Ethyl bromoacetate (19.3 g, 115 mmol) was slowly added (30 min) to the above green product, as the reaction was stirred and cooled in an ice bath. This reaction was allowed to stir for two days, under argon, at room temperature. This arsine (9.8 g, 51 mmol) was purified as a clear oil by vacuum distillation and dissolved in 20 mL of toluene. An excess of methyl iodide (9.1 g, 64 mmol) was slowly added with stirring under argon. Immediately, a white precipitate began to form. After 24 hr, 20 mL of pentane was added and the precipitate was collected by filtration and washed with pentane. The resulting white solid was dried and recrystallized from methanol to yield 15.5 g of ethoxycarbonylmethyl(trimethyl)arsenium iodide as a white microcrystalline precipitate.

The ethoxycarbonylmethyl(trimethyl)arsenium iodide (15 g, 50 mmol) in water was applied to 250 g of Dowex 2 (OH−) resin. Upon elution with 400 mL of water, evaporation, and drying, the resulting white crystals were dissolved in a minimum of hot ethanol (15 mL), and 200 mL of acetone was added to initiate recrystallization. Argon was bubbled into the solution and the container sealed. After 48 hr, the white crystals were removed by filtration and dried under vacuum to afford 5.58 g (64% yield) of arsenobetaine (Scheme 14).

6.3. Characterization of arsenobetaine

The synthetic arsenobetaine melted at 204–210°C, consistent with the report of Edmonds et al. (1977). Lischka et al. (2011) reported the melting point of the synthesized 13C-labelled arsenobetaine as 203°C. In the 1H NMR spectra of arsenobetaine bromide (Minhas et al., 1998), the singlet at 1.997 ppm was attributed to nine equivalent protons of the (CH3)3As group. The second singlet at 3.577 ppm was assigned to the two protons of the AsCH2 group. The proton ratio as calculated from the integration of peaks was 8.98:2 (9:2). The signal at 4.726 ppm was from the DHO impurity in the D2O solvent. The singlets at 33.28 and 172.46 ppm corresponded to AsCH2 and COOH groups, respectively. These results are similar to those reported by Fricke (2004) and Cannon et al. (1981). In the 13C NMR spectra of arsenobetaine (Fricke, 2004), the three singlets at δ 9.87, 32.81,
and 172.17 ppm corresponded to the carbon(s) of the (CH₃)₃As group, the AsCH₂ group, and the COOH group, respectively. Mass spectrometry analysis of arsenobetaine, a zwitterion, is usually carried out in positive ionization mode. The major product ions from the mass spectrum (200°C/70 eV) of Edmonds et al. (1977) were: m/z 134 (37% — intensity normalized against m/z 91), 120 (20%), 117 (18%), 105 (28%), 103 (50%), 101 (20%), 91 (100%), 90 (18%), and 89 (55%). The accurate precursor ion and product ions were also obtained by high resolution MS (Fricke, 2004). The detected molecular ion [M + H]⁺ at m/z 179.0040 was consistent with the theoretical value (179.0053) for arsenobetaine (C₅H₁₂As+O₂). The major product ions were similar to the results of Edmonds et al. (1977).

7. Arsenicin-A model compound

Arsenicin-A, the only naturally occurring polyarsenic compound, has been isolated from extracts of the poecilosclerid sponge Echinochalina bargibanti, collected in New Caledonia (Mancini et al., 2006). NMR, MS, and Fourier transform infrared spectroscopy (FT-IR) analyses were used to characterize arsenicin-A. Crystals of the arsenical suitable for X-ray studies were not available but an initial quantum chemical calculation of the IR spectral data suggested that the structure was an adamantane-type (Scheme 16) with C₂ symmetry. A model as a structural analogue of arsenicin-A was synthesized and characterized (Marx et al., 1996; Mancini et al., 2006). Lu et al. (2010, 2012) subsequently synthesized arsenicin-A and confirmed its adamantane-type structure with NMR and crystallography.

7.1. Methods for the synthesis of the arsenicin-A model compound

Marx et al. (1996) and Mancini et al. (2006) synthesized the arsenicin-A model compound according to Scheme 17. It involved heating a mixture of arsenic trioxide, propionic acid, propionic anhydride, and potassium carbonate at 165°C for 2 hr, followed by separation and purification.

7.2. Our procedures for the synthesis of the arsenicin-A model compound

The method of Marx et al. (1996) and Mancini et al. (2006), with minor modifications (Chen, 2013), was used for the synthesis of the arsenicin-A model compound. As₂O₃ (0.40 g, 2.02 mmol), K₂CO₃ (0.29 g, 2.10 mmol), propionic acid (0.5 ml, 6.68 mmol), and propionic anhydride (2.0 mL, 17.16 mmol) were mixed together in a 50 mL round-bottom flask, and refluxed at 165°C for 2 hr. After the mixture was cooled to room temperature, 0.8 mL of water was added, followed by heating to 88°C for 1 hr. Additional water (20 mL) was added to quench the reaction. The reaction product was extracted with CH₂Cl₂ (3 × 30 mL). The combined organic extracts were treated with Na₂SO₄, filtered, and the liquid phase evaporated from the filtrate. The resultant oily solid was dissolved in CH₂Cl₂ (20 mL) and fractionated by silica gel chromatography. CH₂Cl₂ was used as the eluent. The solvent was evaporated.

Scheme 14 – Synthesis of arsenobetaine from dimethylarsinic acid (DMAV). The final product is arsenobetaine where n = 1.

Scheme 15 – Synthesis of ¹⁴C-labelled arsenobetaine.
from the fractions of interest, yielding the arsenic-A model compound (0.11 g, 0.26 mmol, 26% yield). Our yield was lower than those reported by Marx et al. (1996) (87%) and Mancini et al. (2006) (80%). The use of too much water for quenching the reaction is likely the cause of our lower yield (we used 20 mL water for one tenth of the starting materials compared to Marx et al. who used 25 mL water with a starting material of 4.0 g As2O3).

7.3. Characterization of the arsenic-A model compound

The melting point of the compound is 132°C (Marx et al., 1996). Infrared spectra showed strong signals at 726 and 794 cm\(^{-1}\) (As\(-\)O stretching) and a weaker signal at 1112 cm\(^{-1}\) (CH bending) (Mancini et al., 2006). Mass spectrometry analyses in positive ionization mode showed the parent ion at \(m/z\) 421 \([\text{M} + \text{H}]^+\) and the fragments at \(m/z\) 377 \([\text{M} + \text{H} - \text{CH}_3\text{CHO}]^+\), 333 \([\text{M} + \text{H} - 2\text{CH}_3\text{CHO}]^+\) and 225 \([\text{As}]^+\) (Mancini et al., 2006).

\(^1\text{H}\) and \(^3\text{C}\) NMR results of the compound have also been reported (Mancini et al., 2006; Marx et al., 1996; Tähtinen et al., 2008).

8. Dicysteinylmethyldithioarsenite (MMA\(^{\text{III}}\)(Cys)\(_2\))

Styblo et al. (1997) prepared MMA\(^{\text{III}}\)(Cys)\(_2\) based on the reduction of TMAO\(^{\text{V}}\) by thiols (Cullen et al., 1984a, 1984b). Suzuki et al. (2004) synthesized MMA\(^{\text{III}}\)(Cys)\(_2\) by reducing MMA\(^{\text{V}}\) with cysteine (Scheme 18). While MMA\(^{\text{III}}\) is readily oxidized, MMA\(^{\text{III}}\)(Cys)\(_2\) could be more stable than MeAsI\(_2\) as a trivalent MMA\(^{\text{III}}\) species. A higher stability of MMA\(^{\text{III}}\)(Cys)\(_2\) was achieved probably because of the excess unreacted cysteine present in the solution, minimizing the oxidation of the trivalent methyl arsenical back to MMA\(^{\text{V}}\). \(^1\text{H}\) NMR analysis of MMA\(^{\text{V}}\)(Cys)\(_2\) dissolved in D\(_2\)O plus D\(_2\)SO\(_4\) showed expected signals corresponding to the methyl protons at 1.34 ppm (Naranmandura et al., 2007).

9. Dimethylarsino-glutathione (DMA\(^{\text{III}}\)(SG))

DMA\(^{\text{III}}\)(SG) has been suggested as an intermediate in the metabolism of inorganic arsenic (Hirano and Kobayashi, 2006), although it has not been consistently detected in urine or bile samples of test animals exposed to inorganic arsenic. DMA\(^{\text{III}}\)(SG), under the trade names of Darinaparsin, ZIO-101, and SGLU-1, has been shown to have antitumor activity on an As\(_2\)O\(_3\)-resistant myeloma cell line (Matulis et al., 2009) and on an MRP1/ABCC1-overexpressed cell line (Diaz et al., 2008).

9.1. Methods for the synthesis of DMA\(^{\text{III}}\)(SG)

Two methods for the synthesis of DMA\(^{\text{III}}\)(SG) have been reported. In the method reported by Cullen et al. (1984a, 1984b), cacodylic acid and glutathione (GSH) were mixed in a 1:3 molar ratio in water under nitrogen atmosphere. GSH reduced the pentavalent arsenic to trivalent arsenic, which formed the complex with GSH (Scheme 19).

The second method (Scheme 20) uses cacodyl chloride as the starting material (Amedio and Waligora, 2009). GSH reacts with cacodyl chloride in the presence of trimethylamine at temperatures no higher than 5°C, under nitrogen atmosphere.

9.2. Comparison of the methods for the synthesis of DMA\(^{\text{III}}\)(SG)

GSH acts as both the reducing agent and the coordinating agent in the method developed by Cullen et al. (1984a, 1984b). Cacodylic acid is stable in a normal laboratory atmosphere making handling the starting material and the reaction simple to execute. However the reaction is slow. Cullen et al. (1984a, 1984b) kept the incubation for 12 hr and obtained a yield of 38%.

\[\text{As}_2\text{O}_3 + (\text{CH}_3\text{CH}_2\text{COOH})_2\text{O} \rightarrow \text{H}_3\text{C}-\text{As} + 5 \text{Cys} \rightarrow \text{H}_3\text{C}-\text{As} + 5 \text{Cys}\]
Cacodyl chloride oxidizes easily under a normal atmosphere, but it is more reactive and can bind with GSH quickly. The reaction duration was 4 hr. Amedio and Waligora (2009) reported a yield of 75% and product purity of 99.5%. The reaction can be scaled up at least to 100 g of the product DMAIII(SG).

9.3. Our procedures for the synthesis

We followed the method of Cullen et al. (1984a, 1984b) for the synthesis of DMAIII(SG). DMAV (0.899 g, 6.5 mmol) and GSH (6 g, 19.5 mmol) were dissolved in 50 mL of water and the solution was stirred for 24 hr under a nitrogen atmosphere. After drying with a vacuum rotary evaporator at room temperature, the residue was extracted three times with 100 mL of cold methanol. The methanol solution was evaporated to dryness and a white solid was recrystallized from water/methanol (50:50).

9.4. Characterization of DMAIII(SG)

As reported by Cullen et al. (1984a, 1984b), the 1H NMR spectrum of DMAIII(SG) in D2O showed signals at δ 1.73 (doublet, 6H), 2.53 (quartet, 2H), 3.38 (doublet, 1H), 4.14 (triplet, 1H), and 4.94 (triplet, 1H). The 13C NMR spectrum in D2O/HCl showed signals at δ 15.19 (CH3), 27.22 (CH2), 32.67 (CH2), 33.79 (CH2), 42.85 (CH2), 54.28 (CH), and 56.68 (CH).

ESI-MS analysis of DMAIII(SG) observed parent ions at m/z 412 ([{(CH3)2As(SG) + H}+]). Park and Butcher (2010) reported a negative parent ion at m/z 410 ([(CH3)2As(SG) + H]+) and a product ion at m/z 306 (GSH). Kala et al. (2000) stated that it was difficult to scale up this reaction to produce concentrated DMMTA V solution for use in vivo experiments.

10. DMMDTA V [Me2As(S)(OH)], or monothio-DMAV

Yoshida et al. (2001) found several unidentified metabolites of DMAV in the liver of rats administered DMAV. Kuroda et al. (2004) and Yoshida et al. (2003) later identified these metabolites as monothio-DMAV and dithio-DMAV, also known as DMMDTA V and DMMDTA V. DMMTA V was also found as a common metabolite in the urine of Bangladesh women who were chronically exposed to inorganic arsenic (Raml et al., 2007). DMMDTA V has also been detected in rice samples (Ackerman et al., 2005). DMMDTA V is more toxic than DMAV (Moe et al., 2016; Naranmandura et al., 2009, 2011).

10.1. Methods for the synthesis of DMMTA V (monothio-DMAV)

Three methods have been reported for the synthesis of DMMTA V. The first method was developed by Reay and Asher (1977). The main reaction is shown in Scheme 21.

Suzuki et al. (2004) modified the method of Reay and Asher (1977), attempting to obtain DMAIII, but the reaction actually produced DMMTA V (Scheme 22). The identity of the DMMTA V product was determined by Hansen et al. (2004), Fricke et al. (2005), and Naranmandura et al. (2006).

The third method for the synthesis of DMMTA V is to bubble H2S directly into a DMAV solution (Scheme 23). This reaction is successful under either acidic (HCl solution) (Wallschläger and London, 2008) or alkaline (NaOH solution) conditions (Fricke et al., 2007).

10.2. Comparison of the methods for the synthesis of DMMTA V

All three methods involve the replacement of an oxygen on DMAV with a sulphur to form DMMTA V. The difference between them lies in how the sulphur-containing reducing reagents are supplied. Although the method of Reay and Asher (1977) produced some DMMTA V (no yield was reported), a portion of the starting DMAV remained, resulting in a mixture of arsenic compounds. Suzuki et al. (2004) stated that it was difficult to scale up this reaction to produce concentrated DMMTA V solution for use in vivo experiments. The method of Suzuki et al. (2004) uses Na2S and stepwise addition of H2SO4 to form H2S, and thus the stoichiometry ratio of the reducing reagent H2S and DMAV can be controlled. When the ratio of Na2S:H2SO4:DMAV was 1.6:1:6:1, the predominant species in the product mixture was DMMTA V. During this process, the H2SO4 solution was added stepwise under a nitrogen atmosphere, and the reaction solution was allowed to stand for...
The DMMDTA\textsuperscript{V} was extracted using an organic solvent, such as chloroform or ethyl ether, while DMA\textsuperscript{V} remained in the aqueous solution. DMMDTA\textsuperscript{V} was recrystallized from ethyl ether/methanol under nitrogen atmosphere. In the third method, H\textsubscript{2}S was bubbled directly into an organic solution containing DMA\textsuperscript{V} and NaOH. The use of ethanol facilitated the direct extraction of DMMDTA\textsuperscript{V}. Raml et al. (2007) used HCl instead of NaOH, preventing the formation of undesired products.

### 10.3. Our procedures for the synthesis of DMMDTA\textsuperscript{V}

Our procedures for the synthesis of DMMDTA\textsuperscript{V} were slightly modified from those of Suzuki et al. (2004). DMA\textsuperscript{V} (2.76 g, 20 mmol) and sodium sulphide nonahydrate (7.6 g, 32 mmol) were dissolved in 30 mL of water. Concentrated H\textsubscript{2}SO\textsubscript{4} (1.7 mL, 32 mmol) was added dropwise to the solution. The reaction mixture was extracted with ether and dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. The ether was evaporated under N\textsubscript{2} and colourless crystals were formed. We obtained 2.7 g of DMMDTA\textsuperscript{V} corresponding to a yield of 88%.

### 10.4. Characterization of DMMDTA\textsuperscript{V}

The melting point of our synthesized DMMDTA\textsuperscript{V} was 68.5°C and the \textsuperscript{1}H NMR signals in CDCl\textsubscript{3} were at 1.54 (s, 3H) and 2.15 (s, 3H). Further characterization of DMMDTA\textsuperscript{V} has been carried out using HPLC separation with mass spectrometry detection, including ICP-MS, high resolution quadrupole time-of-flight (qTOF) MS, and triple quadrupole MS/MS. The arsenic compounds were separated using ion pairing chromatography on an ODS-3 column (Prodigy 3 \textmu{}m) with a mobile phase containing 3 mmol/L malonic acid, 5% methanol, and 0.15% tetrabutylammonium hydroxide (pH 5.7). Negative ionization ESI with high resolution MS analysis produced a main peak at m/z 153.9505 and characteristic fragment ions at m/z 122.8885 and 137.9114.

Suzuki et al. (2004) and Naranmandura et al. (2006) used similar gel filtration and anion exchange chromatography for separation and both ICP-MS and ESI-MS for detection of arsenic compounds. The anion exchange chromatography (column ES-502 7N) could not resolve DMMDTA\textsuperscript{V} from DMDTA\textsuperscript{V}, although it separated the two sulphur-containing compounds from other arsenicals. The gel filtration chromatography (column GS-220 HQ) successfully separated DMMDTA\textsuperscript{V} from DMDTA\textsuperscript{V} and other arsenicals including MMA\textsuperscript{V}, DMA\textsuperscript{V}, and inorganic arsenicals.

Hansen et al. (2004) used anion exchange chromatography and slightly different conditions. The effluent from HPLC was split between ICP-MS and ESI-MS for simultaneous detection. The simultaneous detection of m/z 34(\textsuperscript{64}S), m/z 48 (\textsuperscript{32}S\textsuperscript{16}O) and m/z 75 (\textsuperscript{75}As) by ICP-MS, and the molecular ion at m/z 155 ([\text{C(CH\textsubscript{3})\textsubscript{2}AsSO\textsubscript{2}H\textsubscript{2}]+) together with the fragment ions at m/z 137 ([\text{C(CH\textsubscript{3})\textsubscript{2}AsS}\textsubscript{2}]+) and m/z 107 ([AsS\textsubscript{2}]\textsuperscript{+}) by ESI-MS indicated that the detected arsenic compound contained sulphur. ESI-qTOF-MS analysis showed a species at m/z 153.941, consistent with the theoretical value (153.950) of DMMDTA\textsuperscript{V}. To determine whether the structure of DMMDTA\textsuperscript{V} is Me\textsubscript{2}As(S)OH or Me\textsubscript{2}As(=O)SH, the authors used tautomeric energies' models to calculate the energy difference and Boltzmann population ratio at 300 K. Their results suggest that Me\textsubscript{2}As(=O)SH is more favoured.

In the work by Fricke et al. (2005), the reaction of DMA\textsuperscript{V} and H\textsubscript{2}S was monitored using reversed phase chromatography with ICP-MS and ESI-MS detection. Positive ionization ESI-MS analysis produced a main peak at m/z 155 ([C\textsubscript{2}H\textsubscript{4}As\textsubscript{2}S\textsubscript{2}]+), corresponding to DMMDTA\textsuperscript{V}. DMMDTA\textsuperscript{V} became the predominant species in the reaction mixture after 1 hr of reaction.

Wallischläger and London (2008) used anion exchange chromatography and ICP-MS with the dynamic reaction cell (DRC) function to monitor simultaneously arsenic (m/z 91, AsO) and sulphur (m/z 48, 32SO and m/z 50, 34SO). ESI-MS/MS in negative mode detected ions at m/z 152.9, 137.8, and 122.8, corresponding to DMMDTA\textsuperscript{V}.

### 11. DMDTA\textsuperscript{V} [Me\textsubscript{2}As(S)OH], or dithio-DMA\textsuperscript{V}

DMDTA\textsuperscript{V}, also known as dithio-DMA\textsuperscript{V}, was observed by Yoshida et al. (2001) and identified by Yoshida et al. (2003) and Kuroda et al. (2001). Yoshida et al. (2001) and identified by Yoshida et al. (2003) and Kuroda et al. (2001). Yoshida et al. (2003) isolated Escherichia coli AS-36 from the intestinal tract of rats orally administered DMA\textsuperscript{V} and detected DMDTA\textsuperscript{V} as one of the microbial metabolites of DMA\textsuperscript{V}. DMDTA\textsuperscript{V} has also been detected in a groundwater aquifer that was severely impacted by methylated arsenic pesticides.

\[ \text{Scheme 21 – An earlier method for the synthesis of dimethylmonothioarsinic acid (DMMDTA\textsuperscript{V}) from dimethylarsinic acid (DMA\textsuperscript{V})}. \]

\[ \text{Scheme 22 – A second method for the synthesis of dimethylmonothioarsinic acid (DMMDTA\textsuperscript{V}) from dimethylarsinic acid (DMA\textsuperscript{V})}. \]

\[ \text{Scheme 23 – A third method for the synthesis of dimethylmonothioarsinic acid (DMMDTA\textsuperscript{V}) from dimethylarsinic acid (DMA\textsuperscript{V})}. \]
11.1. Methods for the synthesis of DMDTA\textsuperscript{V} (dithio-DMA\textsuperscript{V})

Two methods have been reported for the synthesis of DMDTA\textsuperscript{V}. One method is similar to that of Suzuki et al. (2004) for the synthesis of DMMTA\textsuperscript{V}, except that the molar ratio of Na\textsubscript{2}S and H\textsubscript{2}SO\textsubscript{4} to DMA\textsubscript{V} was increased (Na\textsubscript{2}S:H\textsubscript{2}SO\textsubscript{4}:DMA\textsubscript{V} ratio 7.5:7.5:1), and the reaction time was extended (to 1 day). The increased amount of H\textsubscript{2}S formation resulted in replacement of oxygen by sulphur, forming DMDTA\textsuperscript{V} (Scheme 24).

Fricke et al. (2005) provided an alternative method for the synthesis of DMDTA\textsuperscript{V}. H\textsubscript{2}S was directly bubbled into an alkaline solution containing DMA\textsubscript{V} and NaOH (Scheme 25). A H\textsubscript{2}S gas cylinder was used to supply H\textsubscript{2}S. The reaction vessel was sealed to allow residual H\textsubscript{2}S to react. The reaction rate is likely due to the enhanced solubilisation of water, could be enhanced if ethanol or a mixture of ethanol and water was used as the solvent, instead of water. The increased reaction rate is likely due to the enhanced solubilisation of H\textsubscript{2}S in ethanol.

11.2. Comparison of the methods for the synthesis of DMDTA\textsuperscript{V}

The two methods differ by how H\textsubscript{2}S is supplied to the reaction mixture. Suzuki et al. (2004) used Na\textsubscript{2}S and H\textsubscript{2}SO\textsubscript{4} to produce H\textsubscript{2}S in the reaction, while Fricke et al. (2005) introduced H\textsubscript{2}S from a gas cylinder. Wallschläger and Stadey (2007) stated that the method of Fricke et al. (2005) gave a higher purity (>95%). In both methods, DMDTA\textsuperscript{V} can be further self-conjugated to form Me\textsubscript{2}As(S)SMe\textsubscript{2} (Fricke et al., 2005).

11.3. Our procedures for the synthesis of DMDTA\textsuperscript{V}

We synthesized sodium dimethyldithioarsinate [Me\textsubscript{2}As(S)SNa] using the method of Fricke et al. (2005, 2007). Cacodylic acid (2.02 g, 14.6 mmol) and NaOH (0.58 g, 14.5 mmol) were dissolved in 25 mL of boiling ethanol. Hydrogen sulphide was bubbled into the boiling solution for 30 min, and a white solid precipitated. After cooling, colourless crystals were isolated by filtration and air dried, giving a yield of 2.61 g (93%).

11.4. Characterization of DMDTA\textsuperscript{V}

DMDTA\textsuperscript{V} cannot be purified as the free acid, and can only be isolated as a salt, which requires the presence of appropriate metal ions. Förster et al. (1970) listed several metals as appropriate counter ions, including Na(I), Cr(II), Ni(II), Co(II), Zn(II), and Cd(II).

The sodium salt of DMDTA\textsuperscript{V} is a stable colourless crystal with a melting point of 181 – 182°C. The melting point may vary according to the degree of hydration and purity. Other salts of DMDTA\textsuperscript{V} have different colours and varying melting points. \textsuperscript{1}H NMR signals were found at 1.53 ppm in CDCl\textsubscript{3} and 1.90 ppm in D\textsubscript{2}O (DHO residual at 4.63 ppm). Slow evaporation of the ethanol solution afforded a quality single crystal for X-ray crystal structure determination.

Chromatography and mass spectrometry methods for the characterization of DMDTA\textsuperscript{V} were similar to those for DMMTA\textsuperscript{V}. Our results from HPLC–ICP–MS analyses showed that the product contained 98.5% ± 0.3% DMDTA\textsuperscript{V}, 1.2% ± 0.1% DMMTA\textsuperscript{V}, and 0.22% ± 0.03% DMA\textsuperscript{V}. ESI-MS analysis in negative mode revealed a characteristic molecular ion of DMDTA\textsuperscript{V} at m/z 168.9184 and characteristic fragment ions at m/z 153.8894 and 138.8663.

Fricke et al. (2005) reported a main mass spectral peak at m/z 171 ([C\textsubscript{2}H\textsubscript{8}AsS\textsubscript{2}])\textsuperscript{+} and fragment ion at m/z 137 ([C\textsubscript{2}H\textsubscript{4}AsS\textsuperscript{2}])\textsuperscript{+} (positive mode). Suzuki et al. (2004) observed an m/z of 169 (in negative mode) ([C\textsubscript{2}H\textsubscript{2}As(S)S\textsuperscript{2}])\textsuperscript{−}. Wallschläger and London (2008) detected m/z 166.8, 153.8 and 138.8 by ESI-MS/MS (in negative mode).

Wallschläger and London (2008) suggested that an oxidation half-life of all methylated As – S species in unpreserved samples at 4°C was on the order of one month. During storage, the thiolated arsenic compounds converted slowly into their oxy-analogues following: DMDTA\textsuperscript{V} → DMMTA\textsuperscript{V} → DMA\textsuperscript{V}. Cryofreezing or acidification did not improve stability.

12. MMMTA\textsuperscript{V}[CH\textsubscript{3}As(−S)(OH)\textsubscript{2}], or monothio-MMA\textsuperscript{V}

MMMTA\textsuperscript{V}, also known as monothio-MMA\textsuperscript{V}, was detected in the urine (Naranmandura et al., 2007; Chen et al., 2016) and blood plasma (Chen et al., 2013) of experimental animals. The mechanism underlying the biotransformation of MMMTA\textsuperscript{V} is not yet well understood. Emerging evidence suggests that gastrointestinal microbiota with the ability to produce H\textsubscript{2}S are responsible for the thiolation of MMA\textsuperscript{V}, leading to the formation of MMMTA\textsuperscript{V} (Van de Wiele et al., 2010; Bu et al., 2011; Alava et al., 2012; Rubin et al., 2014).

12.1. Methods for the synthesis of MMMTA\textsuperscript{V}

Methods for the synthesis of MMMTA\textsuperscript{V} are similar to those for the synthesis of DMMTA\textsuperscript{V}, such as the method of Naranmandura et al. (2007) (Scheme 26). MMMTA\textsuperscript{V} was prepared by the dropwise addition of H\textsubscript{2}SO\textsubscript{4} to an aqueous solution of MMA\textsuperscript{V} and Na\textsubscript{2}S to give a final molar ratio of MMA\textsuperscript{V}:Na\textsubscript{2}S:H\textsubscript{2}SO\textsubscript{4} = 1:3:4. The reaction solution was allowed to stand for 1 hr. MMMTA\textsuperscript{V} in the reaction solution was purified...
by chromatography (on a Wako Gel 100 C18 column by elution with 10 mmol/L ammonium acetate buffer, pH 6.5, 25°C).

Alava et al. (2012) used a mixture of MMA V and H₂S solutions to prepare MMMTA V (Scheme 27). They mixed 900 μL MMAV solution (equivalent 40 μg As/mL) with 100 μL saturated H₂S solution in a 1-mL glass vial, and placed the mixture on a mechanical shaker overnight. The 40 μg As/mL MMAV solution was prepared in 10% V/V formic acid. The saturated H₂S solution was prepared using a reaction between iron(II) sulphide and hydrochloric acid, which generated H₂S.

12.2. Comparison of the methods for the synthesis of MMMTA V

Although the two methods used different reaction conditions, the principle of the reaction is the same. However, neither yield nor purity information was provided for either method. The method described by Naranmandura et al. (2007) is more convenient, since it is easier to use solid Na₂S than to prepare a saturated H₂S solution as described by Alava et al. (2012).

12.3. Our procedures for the synthesis of MMMTA V

We prepared MMMTA V according to the method of Naranmandura et al. (2007) with slight modifications. First, sodium methylarsonate (2.9 g, 10 mmol) and sodium sulphide nonahydrate (7.2 g, 30 mmol) were dissolved in 100 mL of water and kept under nitrogen atmosphere. Then, H₂SO₄ (60 mmol) diluted in 20 mL of water was added dropwise to the solution. The reaction mixture was stirred at room temperature for 3 hr. The molar ratio of MMAV/Na₂S/H₂SO₄ was 1:3:6.

12.4. Characterization

A molecular ion detected with HPLC–ESI-MS in negative mode was at m/z 155 [M – H]⁻, corresponding to the theoretical value for MMMTA V. The ratio of sulphur to arsenic (S:As) was determined to be 1:1 by HPLC-ICP-MS. The chemical shift of methyl protons measured by ¹H NMR was 1.235 ppm in D₂O plus D₂SO₄, which is similar to that of pentavalent MMA V (1.317 ppm) and different from that of trivalent MMAIII (0.731 ppm). The results of characterization were consistent with those reported by Naranmandura et al. (2007) for MMMTA V.

MMMTA V is air sensitive and rapidly converts to MMA V when exposed to an air atmosphere. MMMTA V in solution is stable for a few days in a sealed flask at room temperature.

13. Sodium monomethyltrithioarsonate (MMTTA V), MeAs(S(SNa)₂)

There has been no report on the occurrence of MMTTA V in the natural environment or in biological systems. It is possible that MMTTA V is not stable or that its environmental concentration is very low.

13.1. A method for the synthesis of MMTTA V

There has been no report on a method for the synthesis of MMTTA V. We synthesized MMTTA V (Scheme 28) using a method similar to that for the synthesis of DMDTAV. H₂S gas was bubbled into the solution containing MMA V and NaOH. A H₂S gas cylinder provided a stable source of H₂S to enable the thiolation of MMA V to MMTTA V.

13.2. Characterization

Ion pairing and weak anion exchange HPLC separation with ICP-MS detection indicated several arsenic species in the reaction products, including: 22% ± 1% MMTTA V, 55% ± 2% MMDTAV, 8% ± 2% MMMTA V, 9.1% ± 0.3% MMA V, 4.2% ± 0.2% MMA III, and 0.17% ± 0.06% AsV. HPLC–ESI-TOF-MS analysis showed a molecular ion [M − 1]⁻ at m/z 186.898 and a fragment ion at m/z 152.8817, consistent with the theoretical value of MMTTA V. Additional fragment ions at m/z 138.8662 and m/z 106.8942, corresponding to AsS⁻ and As⁻, were also observed. Likewise, MMDTAV and its fragment ions were also detected using HPLC-ESI-TOF-MS. A molecular ion at m/z 170.8916 and fragment ions at m/z 155.9695, 136.9044, 122.8890, 106.8940, and 90.9167 are consistent with the expected spectrum of MMDTAV.

¹H NMR analysis showed a peak located at 1.99 ppm (D₂O, 4.744 ppm). An attempt to crystallize the compound from the crude white solid failed due to the decomposition of the products in the air. Consequently, X-ray crystallographic analysis could not be performed. No other physical and chemical information has been reported.
14. Concluding remarks

We have focused this review on 14 trivalent (III) and pentavalent (V) organic arsenic compounds, MMA\textsuperscript{III}, MMA\textsuperscript{III}(Cy), DMA\textsuperscript{V}, MMMA\textsuperscript{III}, MMDTA\textsuperscript{V}, MMTTA\textsuperscript{V}, DMA\textsuperscript{III}, DMA\textsuperscript{III}(SG), DMA\textsuperscript{V}, DMMTA\textsuperscript{V}, DMDETA\textsuperscript{V}, TMAO\textsuperscript{V}, arsenobetaine, and an arsenic in-A model compound. Although these 14 arsenic compounds are very important because of their frequent occurrence and diverse toxicities, they represent only a small fraction of arsenic compounds reported.

Other major groups of naturally occurring organic arsenic compounds, such as arsenosugars (Feldmann and Krupp, 2011; Francesconi, 2010; Le et al., 1994b), arsenolipids (Khan and Francesconi, 2016), and arsenoproteins/peptides, are worthy of similar review. Arsenosugars in particular occur widely in marine bivalves and some terrestrial organisms. The potential health effects of species. Arsenosugars are also present in marine bivalves and some terrestrial organisms. The potential health effects of arsenosugars have not been elucidated (Carlin et al., 2016), although DMA\textsuperscript{V} is one of the major metabolites excreted into the urine following human ingestion of arsenosugars in food (Le et al., 1994a; Ma and Le, 1998; Thomas and Bradham, 2016).

Another class of arsenic-containing compounds deserving future attention were synthesized for the purposes of analytical detection, biosensing, and cellular imaging (Adams et al., 2002; Griffin et al., 1998; Shen et al., 2013; Yan et al., 2016). Most of these arsenic compounds were designed to alter the fluorescence properties of test molecules, enabling fluorescence detection/imaging. FlAsH is a good example (Adams et al., 2002); it is widely used for imaging cellular production of specific target proteins.

This review provides a summary of the methods available for the synthesis of key arsenic compounds encountered during environmental, biological, and health research. The availability of these arsenic compounds should facilitate further studies of the role of arsenic in these human endeavours.

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