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Photochemical alkylation of inorganic arsenic

Part 2.[†] Identification of aqueous phase organoarsenic species using multidimensional liquid chromatography and electrospray mass spectrometry[‡]

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The alkylation of arsenic via a new photochemical pathway was investigated. Photochemical alkylation was achieved by irradiating a solution of 5 mg l^{-1} As(III) containing 1.4 M (20% v/v) acetic acid with UV light. Water-soluble arsenic species formed during the alkylation process were separated using multidimensional LC (anion- and cation-exchange chromatography) and isolated for off-line identification by electrospray mass spectrometry (ES-MS) or on-line reverse phase LC-MS. The molecular ions of potential organoarsenic species were subjected to collision induced dissociation and identification was based on structural information obtained from fragments or by comparison of fragmentation spectra of known species. Mass Frontier software was employed to help elucidate the structures of unknown compounds. Thirteen organoarsenic species were identified, six of which are previously unreported organoarsenic species.

Introduction

The phenomenon of metal alkylation is a hot topic in current environmental impact studies, as the 'transformation' of metal from an inorganic to an organo-bound species not only has a direct influence on the toxicity of the metal, but also on its mobility and availability in ecosystems. Metal alkylation has led to accumulation and biomagnification in ecosystems (especially the marine environment), which could lead to profound consequences. Biomethylation, the most referenced form of alkylation, is commonly known to occur for a number of metal species (e.g., Hg, As, Se) via enzymatic processes within algae, bacteria and other microorganisms.¹ Abiotic processes also contribute to methylation via natural methyl donors such as methylcobalamin and S-adenosylmethionine. Pure chemical pathways leading to methylation are less common, but have been reported for Sn in the marine environment² and for Hg³ and Se⁴ under simulated conditions in laboratories.

There is intense interest in the biotransformation and lability of arsenic as there is evidence that it can act as a carcinogen in inorganic forms' and recent findings suggest that trivalent methylated forms could be even more toxic than previously thought.⁶ In the developing world, acute and chronic arsenic exposure via drinking water has been reported in many countries, especially Argentina, Bangladesh, India, etc., where a large proportion of the ground water is contaminated with arsenic at levels from 0.1 to over 2 mg l^{-1} .

The biological transformation of arsenic has been considered to be the only principal pathway responsible for synthesis of alkylated (primarily methylated) arsenic species. The ability to produce the volatile trimethylarsine is widespread amongst bacteria, yeasts and fungi, plants, algae, animals and even humans. The first part of this two part article⁷ demonstrated that inorganic arsenic can be transformed, via a purely photochemical process, into various volatile, fully saturated trialkylorganoarsenic compounds (including the infamous "Gosio gas", trimethylarsine). It is hypothesized that a number of water-soluble compounds may also be formed during the photochemical alkylation process (partial alkylation, monoand di-alkylarsenic species) and the prime objective of this study is to investigate this hypothesis using spectrometric techniques.

The use of mass spectrometry for the identification of unknown arsenic species in natural samples has been reported⁸ but it is not particularly common. High-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) is the most popular modern technique for speciation of arsenic. However, this technique relies on species identification by time retention matching with commercially available standards using an analytical column. The limited availability of standards and the unlikely separation of all arsenic species on one analytical column render this approach problematic. ES-MS offers molecular and structural information which aids in structure elucidation. However, this approach also has drawbacks due to lower sensitivity relative to the ICP-MS technique, the incompatibility of the ionization process with many matrices and the monoisotopic nature of arsenic. To overcome these problems, the approach adopted here uses both ICP- and ES-MS techniques and multidimensional LC to separate, isolate and preconcentrate the arsenic species.

Experimental

Apparatus

The ICP-MS instrument used was an ELAN 6000 (PE-SCIEX, Concord, ON, Canada) equipped with a Ryton spray chamber and cross-flow nebulizer. ICP-MS parameters (nebulizer gas flow, rf power and lens voltage) were optimized daily using a standard procedure recommended by the manufacturer. A Thermo Finnigan TSQ quantum AM triple quadrupole instrument (San Jose, CA, USA) was used for ES-MS analysis. ES-MS conditions (e.g., capillary voltage, lens voltage, multipole

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offset and entrance voltage) were optimized for arsenobetaine (AsB) using the standard tune procedure.

For preparative anion exchange chromatography, a 700 \times 16 mm column (Pharmacia, Uppsala, Sweden) was filled with Sephadex DEAE-A25 Gel (Pharmacia) according to the manufacturer's protocol. The sample and mobile phases were circulated with a peristaltic pump (Minipuls 2, Gilson, France). Semi-preparative anion-exchange HPLC separations were achieved using a Hamilton PRPx-100 (250 \times 4.6 mm \times 5 µm) column (Hamilton, Reno, NV, USA) with a PRPx-100 guard column (Hamilton). Fractions were collected using an automatic fraction collector (RediFrac, Pharmacia). Cationexchange separations were undertaken using a Supelcosil SCX $(250 \times 4.6 \text{ mm} \times 5 \mu\text{m})$ column (Supelco, Bellefonte, PA) with a Supelguard SCX guard column (Supelco). A Dionex BioLC, model LCM (Dionex Corp., Sunnyvale, CA, USA), fitted with a 100 µL injection loop, was employed for the HPLC separations.

For on-line LC-MS, reversed phase separations were undertaken using a Prevail C18 (150 \times 2.1 mm \times 5 μ m) column (Alltech, Deerfield, IL) and cation exchange separations were undertaken using a Supelcosil SCX (250 \times 2.1 mm \times 5 μ m) column (Supelco, Bellefonte, PA). A Hewlett-Packard HP 1100 pump with autosampler was used for the separations.

A bench top freeze dryer (Labconco, Kansas City, MO, USA) was used to remove water from LC fractions.

Photoreduction was accomplished using a 100 ml septumsealed glass batch reactor (20 cm diameter \times 30 cm depth) described earlier.^{4,7} Sample solutions containing 0.05 mg l⁻¹ arsenite were irradiated with a UVC pen lamp (Analamp, Claremont, CA, 79 μ W cm⁻², λ_{max} 253.7 nm) inserted into a quartz finger (12 mm od, 10 mm id, 11.5 cm depth) positioned at the center of the reactor such that it was effectively immersed in the analyte solutions but isolated from direct contact with the liquid medium. The photoreactor was enclosed in aluminium metal foil for safety of operation. A scanning monochromator, used to record the relative energy distribution of the UV light source before and after passage through the quartz finger used in the photoreactor, showed that most output occurs at 253.7 nm or longer (296.6 nm, 313.0 nm and 365.1 nm), the primary emission lines of mercury.

Standards and reagents

Standard stock solutions at a concentration of 1 mg ml⁻¹ were prepared by dissolving the target compound in water. Arsenic (III) and arsenic(v) standards were prepared from As₂O₃ and As₂O₅, respectively (Aldrich, Oakville, ON, Canada) in 0.2 M HCl. Dimethylarsinic acid was prepared from cacodylic acid, sodium salt (Sigma, Oakville, ON, Canada) and arsenobetaine was obtained from Fluka. Monomethylarsonic acid, arsenocholine and tetramethylarsonium ion were obtained from Tri Chemical Laboratory Inc. Trimethylarsonium propionate was isolated from an aqueous extraction of TORT-2 by 2-D LC, as described elsewhere.⁹ The chromatographic purity was verified by LC-ICP-MS and ES-MSⁿ was employed to confirm the identity of the species. Working solutions were prepared on the day of analysis by appropriate dilution of the stock solutions with water. The stock solutions were kept at 4 °C in the dark.

Analytical reagent grade chemicals were used throughout. Water was purified to 18.2 M Ω cm resistivity using a NANOpure mixed bed ion exchange system fed with reverse osmosis domestic feed water (Barnstead/Thermolyne, Dubuque, Iowa, USA). Acetic acid was purified in-house by sub-boiling distillation of reagent grade feedstock. Methanesulfonic acid (98% purity) was purchased from Fluka (Oakville, ON, Canada). OmniSolv[®] acetonitrile and formic acid (98% purity) were purchased from EM Science (Gibbstown, NJ, USA). Ammonium acetate was obtained from Sigma–Aldrich (Oakville, ON, Canada). Eluent B for anion exchange chromatography was prepared with 200 ml acetic acid (1 M) and 200 ml ammonium acetate (1 M) diluted to 1 l with water. Eluent A was prepared by diluting eluent B 10-fold. Eluents A and B for reversed phase chromatography were prepared by adding 200 μ l of formic acid to 1 l of water and 1 l of acetonitrile, respectively. The gradient elution programs employed for anion exchange and reversed phase chromatography are outlined in Table 1. For cation exchange chromatography, isocratic elution with 20 mM pyridine buffer adjusted to pH 3 with formic acid was employed.

Sample preparation procedures

UV photoirradiation of As. Volatile and water-soluble arsenic compounds were generated by UV photolysis of 5 mg l^{-1} As(III) solutions containing 1.4 M (20% v/v) acetic acid. The irradiated solution (100 ml) was freeze-dried to remove all liquid and the residue was re-dissolved in 1 ml water.

 Table 1
 Experimental conditions and LC sample preparation parameters

ICP MS operating conditions	
Nebulizer gas flow (argon)	$0.71 \mathrm{min}^{-1}$
ICP-MS plasma gas	Argon at 15 1 min ⁻¹
ICP rf power	1100 W
Lens voltage	10 V
Elements/isotopes monitored	⁷⁵ As, ⁵³ Cr, ⁷⁷ Se
ES MS operating conditions	
Spray voltage	3000 V
Capilliary temperature	350 °C
Sheath gas flow	40
Auxilliary gas flow	10
Tube lens	100
Scan range	50–600 u
Preparative anion exchange	
Eluent	Ammonium acetate–acetic acid, pH 4.7
Gradient	Stepwise increase
Elaw rata	(20, 30, 100, 150, 200 mW)
Flow rate	1.0 ml min 250 fractions, ranging from
Tractions conected	250 fractions, ranging from
	every 2–10 mm
Anion exchange	
Eluent A	20 mM ammonium acetate-acetic
	acid, pH 4.7
Eluent B	200 mM ammonium acetate-acetic
	acid, pH 4.7
0–5 min	100% eluent A
5–30 min	100–0% eluent A, 0–100% eluent B
30–40 min	100% eluent B
Flow rate	1.5 ml min^{-1}
Fractions collection for	60 fractions, every 30 s
semi-prep	
Cation exchange	
Eluent	20 mM pyridine-formate, pH 3
Flow rate for ICP-MS	1.0 ml min^{-1}
Flow rate for ES-MS	0.2 ml min^{-1}
Reversed phase	
Eluent A	0.02% formic acid, 99.98% water
Eluent B	0.02% formic acid, 99.98%
	acetonitrile
0–5 min	100% eluent A
5–30 min	100–50% eluent A, 0–50% eluent B
30–40 min	50% eluent B
Flow rate for ES-MS	0.2 ml min ⁻¹

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Fig. 1 The multidimensional LC isolation of alkylated As-containing species in a UV irradiated solution of As(m) in the presence of acetic acid. (a) Preparative anion exchange chromatography with fraction envelopes highlighted and numbered i through to ix; (b) separation of cationic arsenic standards by analytical cation exchange; (c) fraction i; (d) fraction ii; and (e) fraction iii by analytical cation exchange. Numbering of cationic As-containing fractions is from 1 through to 11.

LC protocols. LC protocols parameters used to isolate the arsenic species in the irradiated arsenic solution are summarized in Table 1. Chromatographic off-line elution profiles were obtained by analyzing an aliquot of the chromatographic eluate by ICP-MS for As content and plotting the intensity of the signal *versus* the fraction number. Fractions for each eluting peak were combined, freeze-dried and dissolved in 100–500 μ l water for preparative fractions (the exact amount of water was dependent on the As concentration and the apparent matrix content) and 30–50 μ l water for semi-preparative fractions, and set aside for analysis by LC-ICP-MS and LC-MS.

Results and discussion

Multidimensional LC protocol 1

Preparative anion-exchange chromatography has previously been described for processing large volumes of extract from natural samples, with the goal of isolating enough species for identification by ES-MS/MS.⁹ The elution protocol employed allowed separation of nine peaks, which were numbered as shown in Fig. 1a. The fractions contributing to the respective peaks were combined (as highlighted) and concentrated by freeze-drying. The early eluting fractions, i through to iii, which are cationic in nature, were subsequently analyzed by cation-exchange ICP-MS (Fig. 1c-1e, respectively) and the later eluting fractions, iv through to ix, were analyzed by anion-exchange ICP-MS (not shown).

Fraction i (Fig. 1c) is the most cationic in nature and yields 8 eluting peaks, excluding the As peak detected in the void volume. A comparison with standards eluting on the cation exchange column (Fig. 1b) shows that arsenobetaine (AsB), trimethylarsonium propionate (TMAP), arsenocholine (AsC⁺) and tretramethylarsonium ion (TMAs⁺) could all be present based on matches in retention time. One major peak, which does not match the retention times of the available standards, is apparent in fraction ii (Fig. 1d). In fraction iii, two peaks elute after the void volume (Fig. 1e). Peak 10 does not match the retention time of peak 11 is similar to that of AsB. These three cationic fractions (i, ii and iii) were analyzed in parallel by cation-exchange ES-MS. The results are presented in the section 'Identification of cationic species by LC-MS'.

Fractions iv through to ix were analyzed by anion-exchange ICP-MS (not shown). Fraction iv matched the retention time of As(III) but this is relatively close to the void volume of the column and does not ultimately confirm identification. The identification of this species as As(III) is, however, supported by FI-ES-MS and RP-MS analyses, which show no apparent signal (inorganic As species do not yield a signal in ES-MS in the positive ion mode). Fractions v through to ix all gave peaks corresponding to the retention time of As(v) and subsequent analyses by FI-ES-MS and RP-MS show no detectable signal for any arsenic species. It is assumed that the process of concentrating the fractions after collection was too long (one sample was freeze-dried at any given time) and the species degraded to inorganic As(v). Rather than repeat the preparative chromatography, a second protocol based on semi-preparative anion exchange with an analytical column was employed, and is outlined in the following section. The advantages of this approach include a smaller elution volume for each fraction (meaning all fractions could be simultaneously freeze-dried and the freeze-dry period is greatly reduced), a shorter chromatographic run, and higher resolution of the anionic species.

Multidimensional LC protocol 2

An analytical anion-exchange column was used in a semipreparative fashion for the collection of alkylated arsenic species generated during the UV irradiation. A portion of the concentrated sample was diluted to give approximately the same total concentration of arsenic as in the original UV treated solution and analysed by anion-exchange ICP-MS (Fig. 2b). A zoom of the elution spectrum is shown in Fig. 2c.

There are some differences apparent between this elution profile of the sample and the elution profile obtained with preparative anion exchange chromatography illustrated in Fig. 1a. The cationic species, which elute in fractions i, ii and iii using the preparative column (Fig. 1a), have co-eluted with As(III), evident as peak 12 on the semi-preparative column (Fig. 2a). The major peak in the preparative anion-exchange elution profile, peak ix, which has eluted over 50 fractions, is assumed to be As(v) and the intermediate fractions, v through to viii, are thought to correspond to the minor fractions observed in semi-preparative anion-exchange chromatography. The discrepancies in relative peak area probably result from slightly different sample treatments between the sample used for preparative chromatography and the sample used for semi-preparative chromatography. (The UV irradiated sample was not the same in each LC protocol. Each time the sample was prepared, minor differences in the quantity and ratio of the species formed were evident.)

The elution profile was compared with that from a set of standards using this analytical column. Two major peaks can



Fig. 2 Semi-preparative anion-exchange of (a) anionic arsenic standards, (b) a UV irradiated solution of As(III) in the presence of acetic acid and (c) a zoom of the chromatogram in Fig. 2(b) highlighting the minor As-containing species. Numbering of anionic As-containing fractions is from 12 through to 18.

be observed in Fig. 2b: peak 12 in the void volume, which matches the retention time of As(III), and peak 15, which is similar in retention time to that of As(v). In the zoomed chromatographic profile, five minor peaks can be seen. Peaks 13 and 14 have similar retention times to those of monomethyl arsenic acid (MMA) and peaks 16 through to 18 are more anionic in nature than any of the standards.

The cationic species eluting in the void volume were not analyzed further. Further analysis of fraction 15 by FI-ES-MS and RP-MS yielded no signal, which supports the retention time match information. This peak was thus identified as As(v). The five minor fractions were collected and concentrated as soon as possible after collection and analyzed by RP-MS within one working day. The results of these analyses are described in the section 'Identification of anionic species by LC-MS'.

Identification of cationic species by LC-MS

Fractions i through to iii, isolated using preparative anion exchange chromatography, were concentrated and analyzed by cation-exchange ES-MS. The approach adopted to identify the compounds is outlined below, as arsenic is monoisotopic and this complicates identification of unknowns by ES-MS (theoretically, any molecular ion observed in the LC-MS spectra could belong to an As-containing species). As only analytical grade reagents have been used in the processing of the sample, problems arising from matrix interferences that are encountered with the analysis of biological extracts are avoided. Nevertheless, the chromatograms obtained by cation-exchange ICP-MS analyses of the respective fractions were used to assist in finding the molecular ions of the species of interest in LC-MS. Additionally, a list of analogous species to those already known, and which could be candidate species in our irradiated solution, was created. This list, with respective molecular ion information, is shown in Table 2. To confirm or elucidate the identity of possible As-containing species, the corresponding molecular ions were subjected to collision-induced dissociation (CID). This could be triggered on-line using a data-dependent acquisition (DDA) option in the method, where the most intense eluting ion from a list of molecular ions would induce fragmentation of that molecular ion. Fragmentation spectra of known compounds could be compared to characteristic spectra of the respective standards. In the case of unknowns, Mass Frontier software was employed as an aid to comparing theoretical fragments of a compound with those actually observed.

Fig. 3, which shows the analysis of fraction i eluted from the preparative anion-exchange column, illustrates how this approach works. Retention time based information from the chromatographic elution of this fraction on ICP-MS (Fig. 3a) suggests that the fraction may contain AsB, TMAP, AsC⁺ or TMAs⁺. Thus, in LC-MS, the molecular ions of interest for these compounds were extracted from the chromatogram. In the case of AsB, which has a molecular ion of m/z179, the SIM chromatogram, shown in Fig. 3e, does indeed show a peak, but not at the retention time of AsB. The peak has a similar retention time to peak 6 in Fig. 3a and so it was deduced that AsB was not present, but a species having the same molecular ion eluting at a later time was. The molecular ion of TMAP was extracted and no peak was apparent in the SIM chromatogram (not shown). In Fig. 3d and 3f, the SIM chromatograms of the molecular ions of AsC⁺ and TMAs⁺ at m/z 165 and 135, respectively, are evident. In each case, peaks corresponding to the retention time of these two compounds (numbered 5 and 7 in Fig. 3a) were detected. From the identification of AsC^+ and $TMAs^+$, it was possible to relate the retention time of these species with LC-MS to those from LC-ICP-MS (the chromatographic duration is longer for LC-MS due to the smaller id of the analytical column) and estimate retention times for the other species of interest yet to be identified. From the molecular ions extracted so far, which appear to correspond to As peaks in the ICP-MS chromatogram, it is evident that there should be 4 peaks eluting prior to AsC⁺ and one peak eluting after TMAs⁺. By manually scanning the eluting spectra occurring within the expected elution time of these peaks, it was possible to identify molecular ions giving peaks with similar retention times for peaks 2, 3 and 8 at m/z 137, 195 and 191, respectively. No molecular ions were detected for peaks 1 or 4.

A repeat of the LC-MS analysis with DDA of fragmentation spectra of the above ions resulted in the fragmentation of each molecular ion of interest. These are shown in the insets of the respective SIM chromatograms for each molecular ion in Fig. 3. For each molecular ion, confirmed or possible structures, a list of fragment ions and fragment structures associated with those ions is presented in Table 3.

The As-containing species in fraction i, peak 2, is believed to have a molecular ion of m/z 137 based on the comparison of retention time with cation exchange ICP-MS. The fragmentation of this ion yields fragment ions at m/z 107 and 122, characteristic of trimethylarsine oxide (TMAO). The fragments could be compared with those obtained in a similar study by Larsen *et al.*, who investigated the fragmentation sequences of this ion using an ion-trap instrument.¹⁰ Minor differences between the spectra (*i.e.*, fewer fragment ions in this study) can be explained by the fact that radial energy used to fragment the ions in a trap will yield several fragmentation pathways, whereas with a triple quad, the fragmentation will often follow a single sequence of loss events.

The appearance of the characteristic ions at m/z 105 and 121 (with the exception of the fragment ions of TMAs⁺, which are seen at m/z 105 and 120 in the inset of Fig. 3f) in all of the other fragmentation spectra acquired for each molecular ion suggests that a common base structure is present in each of these species. That base structure is presumed to be trimethylarsenic, as the fragment ions at m/z 105 and 121 are believed to



correspond to the dimethyl and protonated trimethyl arsenic moieties, respectively. The fragmentation spectra of the molecular ions at m/z 165 (inset Fig. 3d) and 135 (inset Fig. 3f) confirm identities as AsC⁺ and TMAs⁺, respectively. The three other species fragmented in fraction i, *i.e.*, peaks 3, 6 and 8, are all unknowns. Possible structures for these species, shown in Table 3, were conceived based on the m/z, which gives information on the molecular mass and the fragments observed in the CID spectra. For the species with m/z 195, a loss of 18 u from the molecular ion (195 \rightarrow 177), similar to a loss observed for AsC⁺ (165 \rightarrow 147), suggests the loss of a hydroxyl group (as water) from the extremity of the alkyl side chain. A subsequent loss of 30 u (177 \rightarrow 147) suggests the further loss of CHOH. The structure proposed is therefore (CH₃)₃As $(CH_2)_2CH(OH)_2$. For the species constituting fraction i, peak 6, with m/z 179, a similar loss of 18 u is observed (179 \rightarrow 161), suggesting a terminal hydroxyl group. An analogue of AsC⁺, with an extra methyl group in the alkyl chain (much like TMAP, the recently identified analogue of AsB¹¹), is proposed as a possible structure. The most cationic peak, 8, is believed to have an m/z of 191. Collision induced dissociation of this molecular ion yields characteristic fragment ions at m/z 105 and 121 (Fig. 4f, inset), but the lack of other fragments in the CID spectra does not aid in the identification of this species.

Relying mostly on molecular mass evidence, a structure with an isopentane side chain is tentatively proposed.

Following the same analytical protocol, the major species in fraction ii (peak 9) was found to have an m/z of 137 and the same fragmentation pattern as fraction i, peak 2. It was therefore identified as TMAO. In fraction iii, the two peaks, 10 and 11, were found to have molecular ions at m/z 139 and 179, respectively. Fragmentation of these two molecular ions gave the characteristic fragmentation patterns (Table 3) of dimethylarsenous acid (DMA) and AsB, respectively.

Identification of anionic species by LC-MS

A different approach to that described above was adopted for identification of the anionic species; a reversed phase separation mechanism was used on-line with ES-MS rather that cation-exchange, and only one species was expected in each anionic fraction. The elution of each fraction was compared with the elution of a blank injection and any molecular ions of significance were subjected to CID. Table 2 was used to select the most probable ions for fragmentation. Fragmentation ions with the same m/z as characteristic fragments of As-containing moieties suggested the possibility of an As-containing species and Mass Frontier software was employed to help in the





Fig. 3 Cation-exchange separation of fraction i with: (a) ICP-MS detection and (b–g) ES-MS detection. Panels b–g show SIM chromatograms with fragmentation spectra in the insets of the molecular ions (b) 137, (c) 195, (d) 165, (e) 179, (f) 135 and (g) 191 m/z.

elucidation of the structure. The two major peaks, numbered 12 and 15, eluting from the semi-preparative anion-exchange column were collected in fractions and analyzed immediately by RP-MS. The absence of a peak in the RP chromatograms (resulting in chromatograms similar to a blank solution of anion exchange eluent) supported evidence that the arsenic species contained in the LC fractions are As(III) and As(v), respectively.

The minor peaks observed in the semi-preparative anionexchange chromatogram, numbered 13, 14, 16, 17 and 18 in Fig. 1c, were isolated and concentrated prior to analysis by RP-MS. For each fraction, very little retention of species was seen and the most important molecular ions eluted just after the void volume of the column (chromatograms not shown).



Fig. 4 Fragmentation spectra of molecular ions detected in the RP-MS chromatograms of the anionic LC: (a) fraction 13—molecular ion at m/z 181; (b) fraction 14—molecular ion at m/z 223; (c) fraction 16—molecular ion at m/z 225; (d) fraction 17—molecular ion at m/z 269; (e) fraction 18—molecular ion at m/z 267.

In fraction 13, a molecular ion at m/z 181 was fragmented and yielded the characteristic fragments of dimethylarsenic acetate (DMAsAc) (Fig. 4a). Although the standard was not available for comparison, the fragmentation spectrum of this species has been reported¹² and was identified from a comparison of CID spectra.

On the basis of the acetate side chain found in DMAsAc, it was speculated that As species containing additional acetate side chains could exist and thus analogous species with acetate side chains and either a quaternary arsonium or dimethylarsinoyl moiety were added to Table 2. These hypothetical species provided m/z information for molecular ions, if indeed they proved to exist in the LC fractions. The acetate side chain, as is observed in both AsB and DMAsAc, is characterized by an initial loss of 18 u (loss of H₂O) followed by a loss of 24 u (loss of the acetate chain and hydration at the acetate side chain site).

The analysis of fraction 14 by RP-MS yielded one major molecular ion at m/z 223. The fragmentation of this molecular ion (Fig. 4b) gave fragments at m/z 205, 181, 163 and 139, corresponding to a loss of 18 and 24 u followed by a further loss of 18 and 24 u. This is interpreted as losses from two acetate side chains. Considering the species contains two acetate side chains and has a molecular ion of m/z 223, the species was identified as dimethylarsenic diacetate.

In fraction 16, a molecular ion at m/z 225 was observed, which gave similar losses as those described for the dimethyl-

Table 3	Suggested species and	corresponding fragmentation	data for arsenic-containing specie	s detected in multidimensional LC fractions
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Fraction/peak	m/z	Cationic compounds Species chemical formula	Fragments and their assignments
i/2	137		89 As+ 107 As+ OH 122 As+ OH
i/3	195	As ⁺ OH	$105 \xrightarrow{As^{+}} 121 \xrightarrow{I} As^{+} 147 \xrightarrow{I} As^{+} CH$ $177 \xrightarrow{I} As^{+} OH$
i/5	165	As⁺OH	105 As+ 121 As+H 147 As+
i/6	179	, As⁺OH	105 As^+ 121 As^+H 161 As^+ CH
i/7	135	Ast	$105 \xrightarrow{As^{+}} 120 \xrightarrow{I} As^{+}$
i/8	191		* 105 As ⁺ 121 As ⁺ H 161 As ⁺ CH
ii/9	137		$89 >_{As^+} 107 As^+ OH 122 As^+ OH$
iii/10	139	O II HO	93 $H \xrightarrow{O}_{As^+}$ 107 As^+ 0H 121 As^+ 121 As^+
iii/11	179	As ⁺ OH	$105 As^{+} 120 As^{+} 135 As^{+} 137 As^{+} 0^{+}$ $161 As^{+} 0$
Fraction/peak	m/z	Anionic compounds Species chemical formula	Fragments and their assignments
13	181		91 As^{+} 107 As^{+} CH 121 As^{+} $Iz1 As^{+}$ $I39 As^{+}$ 163 As^{+} $Is3$ As^{+} $Is3$ As^{+}
14	223	O O As ⁺ OH	$107 As^{+} \qquad 0H 119 + As 131 +As 11$ $149 As^{+} \qquad 0H 163 As^{+} \qquad 0H$ $181 As^{+} \qquad 0H 205 0H As^{+} \qquad 0H$
16	225		93 $\stackrel{H}{} \stackrel{O}{\underset{H}{\overset{H}}} 107$ $\stackrel{As^{+}}{} \stackrel{OH}{\underset{H}{\overset{H}}} 121$ $\stackrel{O}{} \stackrel{H}{\underset{As^{+}}{\overset{H}}} 121$ $\stackrel{OH}{} \stackrel{OH}{\underset{Hs^{+}}{\overset{H}}} 135$ $\stackrel{Hs^{+}}{\underset{Hs^{+}}{\overset{H}{\overset{H}}} OH} \stackrel{OH}{\underset{Hs^{+}}{\overset{H}{\overset{H}}} OH} 149$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{H}{\overset{H}}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{H}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{H}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{H}}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}{\overset{Hs^{+}}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH} 165$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH} OH$ $\stackrel{OH}{\underset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}{\overset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}} OH} OH$ $\stackrel{OH}{\underset{Hs^{+}}} OH$ $\stackrel{OH}{\underset{Hs^{+}}} OH$ $\stackrel{OH}{Hs$

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arsenic diacetate in fraction 14 (Fig. 4c). The molecular ion loses 18 u (225 \rightarrow 207), a further 24 u (207 \rightarrow 183), a further 18 u (183 \rightarrow 165) and again 24 u (165 \rightarrow 141), indicating the presence of two acetate groups in the As-containing species. The molecular ion information implies a monomethylarsinoyl diacetate species.

In fraction 17, a molecular ion of low intensity was observed at m/z 269. This immediately suggests a triacetatearsinoyl species, based on molecular mass information. However, the fragmentation spectrum was poor in quality (Fig. 4d) with expected fragments (due to consecutive losses of 18 and 24 u) at m/z 227 and 207 only. Therefore, this identification remains tentative.

A molecular ion at m/z 267 was observed in the RP-MS spectrum of fraction 18. The species was identified as monomethylarsenic triacetate, based on the fragment ions found in the CID spectrum of the molecular ion. Consecutive losses of 18, 24, 18, 24 and 18 u in the CID spectrum (Fig. 4e) lend evidence to the presence of the three acetate groups in the species.

Conclusions

The photoalkylation of arsenic in organic acid by UV irradiation has not only formed trimethylarsine, but a whole range of aqueous soluble species. With multidimensional LC protocols and use of ICP-MS for monitoring the LC eluent, it was possible to isolate these species for identification by electrospray mass spectrometry. It was necessary to use different LC approaches for cationic and anionic species and it was found that rapid analysis of the LC fractions was necessary for identification of species before they degraded to As(v). The acetate chains in the identified anionic species suggest that their formation is probably dependent on the organic acid used. For example, if the acetic acid used in this study was replaced with propionic acid or butyric acid, similar species having propionate or butanoate side chains might be formed. Further speculation would suggest that small quantities of such compounds as were identified in this study could well be formed in the natural environment.

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