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Characterization of Arsenic Species in Kidney of the Clam *Tridacna derasa* by Multidimensional Liquid Chromatography-ICPMS and Electrospray Time-of-Flight Tandem Mass Spectrometry

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The identification of water-soluble arsenic species in a kidney of the *Tridacna* clam by electrospray quadrupole/time-of-flight mass spectrometry (ES Q-TOF MS) was investigated. The species were isolated by three-dimensional LC (size exclusion−anion exchange−cation exchange); the elution of arsenic was monitored by ICPMS. The average accuracy and precision of the molecular mass measurements, studied for a number of organoarsenic standards, were 22 (negative bias) and 15 ppm, respectively. Structural information was obtained in the tandem Q-TOF mode. A total of 15 organoarsenic species were identified, and 13 of these possessed the dimethylarsinoyl group (8 ribofuranosides, 4 acyclic compounds, and 1 dihydroxyfuran). Four of these species were previously unreported. Arsenobetaine and dimethylarsinic acid were also detected. The major species (accounting for up to 50% water-soluble arsenic) was 5-dimethylarsinoyl-2,3,4-trihydroxypentanoic acid. The metabolic interrelationships of these compounds and their significance are briefly discussed.

Biotransformations of arsenic, present in marine biota at unusually high (1−10 mg g^{-1} of wet weight) concentration levels, are known to lead to a wide range of organoarsenic compounds.[1] Since the discovery of arsenobetaine[2] and arsinoylribosides by Edmonds and Francesconi[3−5] the identification, quantification. and monitoring of these species have been the major challenges of the analytical chemistry of arsenic, aimed at the elucidatation of the environmental pathways of this element.

The most widely used analytical techniques for arsenic speciation analysis in marine organisms have been based on the coupling of high-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (HPLC−ICPMS).[6−11] Whereas single-mechanism chromatographic techniques, such as anion exchange (AE),[6,7] cation exchange (CE),[8] size exclusion (SE),[9] and ion-pairing reversed phase (RP),[10,11] were initially used, the complexity of arsenic speciation soon required the parallel use of two (AE, CE)[12] or three (AE, CE, RP)[13] complementary separation mechanisms. These studies allowed the detection of 10 arsenic compounds in shellfish, but unidentified peaks were still present.

Despite its undeniable merits for the rapid monitoring of organoarsenic compounds, HPLC−ICPMS requires the availability of standards for the identification of new species, and even then, the complexity of arsenic speciation may lead to errors due to the coelution of species and uncontrolled ion-pairing with matrix constituents leading to a shift in retention time.[14] The identification of new species can only be achieved by the optimization of molecule-specific techniques. The original approach was based on the use of nuclear magnetic resonance (NMR) spectroscopy, but the subject has now been advanced by the use of electrospray tandem MS (ES MS/MS) which requires far less sample and less preliminary purification.

As early as 1996, Corr and Larsen[15] obtained tandem mass spectra of four arsenosugars and demonstrated the possibility of

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identification of one of them in oyster by CE HPLC−ES MS/MS, but it is only during the past two years that the number of applications of this technique has dramatically increased.[14,16−20] All these studies focused on the identification of the four main (dimethylarsinoylribosides) arsenosugars in extracts of macroalgae. In addition, Pergantis et al.[17] reported tandem mass spectra of the four synthetic trimethylated analogues of these sugars but could not demonstrate the presence of any of them in the sample analyzed.

A successful application of ES MS/MS for the identification of organoarsenic compounds in a biological matrix in the absence of standards remains a challenging task. To date, the only two successful examples refer to the identification of a new analogue of arsenobetaine[21] and 5-dimethylarsinoyl-β-ribofuranose in oyster.[22] The major difficulties are associated with the purification of target analytes, which is required for the simplification of the interpretation of mass spectra, especially since arsenic is monoisotopic. Hence, there is a need for the optimization of multidimensional chromatography,[14,20] for the use of more accurate MS techniques, and for the use of fast tandem MS techniques allowing the acquisition of collision-induced dissociation (CID) data for all the molecular ions seen in the mass spectrum. Of mass spectrometric techniques, quadrupole time-of-flight (Q-TOF) mass spectrometry represents a good compromise in terms of attainable figures of merit and cost and ease of use.

The work reported here was carried out to evaluate the potential of ES Q-TOF MS for the characterization of arsenic species in a kidney of the giant clam, *Tridacna derasa*. Clams of the genus *Tridacna* support, within their tissues, symbiotic unicellular algae and contain what are presumably products of algal metabolism in their large and accumulatory kidneys. Speciation of arsenic in kidneys of *Tridacna maxima*, studied earlier by NMR, was found to be complex; several arsenic species not reported in other organisms were observed.[23−25] In this study, speciation of *T. derasa* could be comprehensively characterized owing to (i) the development of a multidimensional chromatographic purification protocol employing volatile buffers, (ii) reaching the precision and accuracy of mass measurement allowing the determination of empiric formula of isolated species, and (iii) acquiring structural information with tandem MS using a linearly accelerating high-pressure collision cell.

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EXPERIMENTAL SECTION

Apparatus. Ultrasonic extraction was performed using a Branson 1210 ultrasonic cleaner (Danbury, CT). The supernatant was separated by centrifugation using model Hettich Universal 16 (Tuttlingen, Germany). Lyophilization was carried out using a model LP3 lyophilizer (Jouan, France).

For preparative low-pressure chromatography, sample and mobile phase were circulated with a peristaltic pump (Minipuls3, Gilson, France). Fractions were collected using an automatic fraction collector (Dynamax FC-2). HPLC separations were carried out using a Hewlett-Packard series 1100 pump as the sample delivery system. Injections were done using a model 7725 injection valve with a 100-μL injection loop (Rheodyne). All the connections were made of PEEK tubing (i.d. 0.17 mm).

The ICPMS instrument used in this work was the ELAN 6000 (PE-Sciex, Thornhill, ON, Canada) equipped with a Ryton spray chamber fitted with a cross-flow nebulizer. Chromatographic signals were processed using the Turbochrom4 software (Perkin-Elmer). The electrospray MS instrument used was a Perkin-Elmer Sciex API (ion spray) QSTAR pulsar hybrid quadrupole-time-of-flight mass spectrometer.

Materials and Reagents. Preparative size exclusion chromatography was performed with a column (700 × 16 mm) (Pharmacia, Uppsala, Sweden) filled with Sephadex G-15 gel (Pharmacia). Preparative anion exchange was performed with a column of the same dimensions (Pharmacia) filled with DEAE -A25 gel (Pharmacia). Anion-exchange HPLC separations were carried out using a Hamilton PRP X-100 (250 × 4.6 mm × 5μm) column (Hamilton) with a Supelguard SAX1 guard column (Supelco, Bellefonte, PA). Cation-exchange separations were carried out using a Supelcosil SCX (250 × 4.6 mm × 5 μm) column (Supelco) with a Supelguard SCX guard column (Supelco).

Analytical reagent grade chemicals were used throughout unless otherwise stated. Water was purified to 18.2 MΩ cm resistance using a Milli-Q water purification system (Millipore, Milsheim, France). The eluent for anion-exchange chromatography was prepared with 200 mM acetic acid and 200 mM ammonium acetate. The eluent at 20 mM was prepared by diluting the 200 mM eluent 10 times. For cation-exchange chromatography, a 20 mM pyridine buffer was prepared and adjusted to pH 3 using formic acid. For size exclusion chromatography, a 1% (v/v) solution of acetic acid solution was used. The buffers were degassed by sonication for 20 min.

Standards and Samples. Standard stock solutions at a concentration of 1 mg mL^{-1} were prepared by dissolving the respective compound in water. Arsenic(III) and arsenic(V) standards were prepared from sodium arsenate and sodium arsenite (Sigma Aldrich, (St. Quentin Fallavier, France) in water. Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were prepared from MMA (Sigma) and DMA (Fisher) disodium salts. Arsenobetaine was a gift of Prof. W. Cullen (UBC, Vancouver). A secondary stock solution at 1 mg mL^{-1} of each of the compound was prepared for HPLC−ICPMS analyses. Working solutions were prepared on the day of analysis by the appropriate dilution of the stock solutions with water. The stock solutions were kept at 4 °C in the dark.

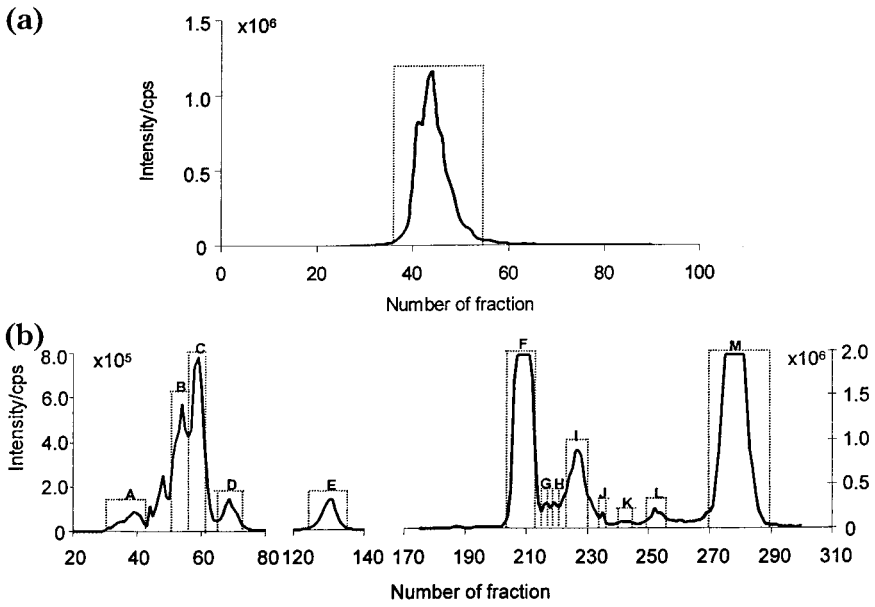

Figure 1. Fractionation of organoarsenic species in the kidney of *T. derasa* by (a) preparative size exclusion and (b) preparative anion-exchange chromatography (monitored *off-line* by ICPMS).

The kidney was removed from a specimen of *T. derasa* cultured commercially in the Solomon Islands and was air-freighted frozen to Europe.

PROCEDURES

Extraction of Arsenic Species from *Tridacna* Kidney. The kidney was thawed and the fresh tissue was weighed, freeze-dried, and reweighed to calculate water content. The dried kidney was then ground with a pestle and mortar. Arsenic compounds were extracted with water. A 0.2-g portion of homogenized sample was weighed into eight individual extraction/centrifugation tubes and suspended in 20 mL of water. The tubes were sonicated for 2 h at 50 °C and centrifuged for 20 min at 2500 rpm. The supernatant was removed by means of a Pasteur pipet, and the residue was re-extracted. The residue was then washed with a 10-mL portion of water by sonicating for 20 min. The extracts and washings were combined and freeze-dried. The residue was redissolved in 5 mL of water and ultracentrifuged at 50 000 rpm (4 °C).

Fractionation of Organoarsenic Compounds by Size Exclusion and Anion-Exchange Chromatography. The Sephadex G-15 preparative column was conditioned by washing with water and flushing the column with the eluent. The mobile phase was 1% (*v*/*v*) acetic acid (pH 2.97) pumped at 0.85 mL min^{-1}. A 3.5-mL portion of the prepared aqueous extract was introduced to the column, and fractions (1.7 mL) were collected every 2 min for 4 h. A 150-μL aliquot of each fraction was analyzed by ICPMS, and an elution profile was drawn using Excel software (Figure 1a). Fractions giving more than 50 000 counts/s (F37−53) at the *m*/*z* 75 (^{75}As) channel were combined, freeze-dried, and redissolved in 4.0 mL of water. A 3.5-mL aliquot of the redissolved extract was introduced on the preparative anion-exchange column. Elution was carried out with an increasing (20−200 mM) concentration of 1:1 ammonium acetate/acetic acid at pH 4.7. Fractions were collected during 24 h and then analyzed by ICPMS for As, and the elution profile was reconstructed (Figure 1b). Fractions producing peaks were combined as shown in Figure 1b), freeze-dried, and dissolved in 200−500 μL of water (the exact amount of water was dependent on the As concentration and the apparent matrix content). A total of 13 As-containing fractions were finally obtained (A−M).

Determination of the Number of Arsenic Compounds in Each Fraction (A−M) from Preparative AE Chromatography by Anion-Exchange and Cation-Exchange HPLC−ICPMS. A 20-μL aliquot of each of the 13 fractions was diluted with water to 200 μL. The first five fractions (A−E) were analyzed by CE-HPLC−ICPMS, and the remaining eight (F−M) by AE-HPLC−ICPMS. The optimum chromatographic conditions are summarized in Table 1.

Purification and Isolation of Organoarsenic Compounds prior to Electrospray MS/MS. Fractions after preparative AEC (undiluted) were fractionated by CE-HPLC (fractions A−E) and AE HPLC (fractions F−M) under the conditions summarized in Table 1. Depending on the length of the chromatographic run and the number of peaks eluted, fractions were collected from every 15 s to every 1 min. Arsenic was determined in each fraction, and the elution profiles were constructed. Fractions corresponding to the peaks were pooled, lyophilized, and redissolved in 20−200 μL of 0.1 M formic acid in 50% methanol and analyzed by ESMS.

ICPMS Conditions. ICPMS measurement conditions (nebulizer gas flow, rf power, lens voltage) were optimized daily using a standard built-in software procedure. Typical examples are a nebulizer gas flow of 1.0 L/min, ICP rf power of 1000 W, and a lens voltage of 9 V. The chromatographic off-line elution profiles were obtained by introducing an aliquot of the chromatographic eluate into the ICP until a steady-state signal was established for 3−5 s. The chromatogram was reconstituted by plotting the intensity of this signal versus the fraction number (corresponding to the elution time or volume). For the on-line acquisition, the dwell time for each isotope was 60 ms and the number of replicates allowed for continuous scanning for the duration of the chromatogram. Typically, 1000 replicates were applied to give a scan

Table 1. HPLC−ICPMS Separation Conditions

	cation exchange	anion exchange
column	Supelcosil LC-SCX	Hamilton PRPx-100
dimensions	250 × 4.6 mm × 5 μm	250 × 4.6 mm × 5 μm
eluent	pyridine/formate, pH 3	ammonium acetate/acetic acid, pH 4
elution program	2 mM eluent from 0 to 5 min, increase to 20 mM eluent from 5 to 20 min; 20 min and over 20 mM	20 mM eluent from 0 to 5 min, increase to 200 mM eluent from 5 to 30 min; 30 min and over 200 mM
flow rate/ mL min^{-1}	1.0	1.0
operating pressure/bar	95−100	50−60
injection volume/μL	100	100
run time/min	35	55

Table 2. ESI Q TOF Operating Conditions

parameter	positive TOF MS	positive product ion
ion source gas	20 mL min^{-1}	20 mL min^{-1}
curtain gas	25 mL min^{-1}	25 mL min^{-1}
ion spray voltage	5000 V	5000 V
declustering potential	50 V	50 V
focusing potential	265 V	225 V
declustering potential 2	15 V	15 V
collision energy	0	20 eV
collision gas	0	5
resolution ino energy	2.3 eV	2.3 eV
MCP (CEM)	2200 V	2200 V
duration	1.0 min	1.0 min
cycle time	1.0 s	1.0 s
no. of cycles	60	60

duration of 945 s. All signal quantification was done in the peak area mode.

Electrospray MS/MS Conditions. ES MS/MS conditions were optimized using a mixture of arsenobetaine, arsenocholine, and tetramethylarsonium cation, a mixture of As(V), MMA, and DMA (10 μg mL^{-1} each), and for each of the individual arsenosugars (10 μg mL^{-1} each) in a solution of water/methanol (50:50, v/v) containing 30% 0.1 M formic acid. The optimum operating conditions are given in Table 2. An m/z range of 70−500 u was scanned. Acquisition of spectra was typically done using 60 scans, each scan having a duration of 1 s giving an acquisition time of 1 min. MS/MS spectra were acquired in the product ion mode for the molecular ion of interest. The collision energy was optimized daily for each species and varied between 15 and 35 eV. The collision gas used was nitrogen.

RESULTS AND DISCUSSION

Extraction of Arsenic Species from *Tridacna* Kidney. The kidney sample analyzed weighed 4.435 g freshly thawed and 1.606 g after freeze-drying, which corresponds to a 63.8% water content. Water-soluble arsenic accounted for 181 ± 5 μg g^{-1} in the dry tissue.

Fractionation of Organoarsenic Componds by Preparative Size Exclusion and Anion-Exchange Chromatography. Preliminary experiments aimed at the optimization of a one-dimensional HPLC using either of CE, AE, RP, or SE separation mechanisms proved unsuccessful in obtaining a baseline separation of the organoarsenic compounds present in the extract. Moreover, when the peaks were collected for ES MS/MS, almost no signal could be observed because of suppression by the matrix. Consequently, multidimensional chromatography was optimized.

To be successful, ES MS/MS requires the absence of nonvolatile salts and organic matrix components in the solution analyzed. Dilute (1% v/v) acetic acid (which is readily removed during the lyophilization step) proved successful in our former work.[14,20] Using the acetic acid mobile phase, 94% of the arsenic injected onto the size exclusion column was recovered. A single large peak eluted close to the total volume of the column is observed (Figure 1a). The presence of a small front shoulder was not judged sufficient to divide this fraction into two.

Initial investigations into the removal of a carbonate buffer prior to ESI MS was promising, but the resolution of the organoarsenic compounds was not satisfactory. Our attempt to substitute it by a buffer based on triethylamine and acetic acid, marketed as volatile for ES MS, was also unsuccessful due to insufficient removal of the mobile phase during freeze-drying. When fractions were collected in this buffer, signal intensity was reduced by more than 10−20 times when compared to direct injection. The third of the buffers tested, ammonium acetate/acetic acid, proved to be much more volatile and was nearly completely removed by freeze-drying even when the buffer concentration was 200 mM. Organoarsenic standards eluted with this buffer were measured with only a 2−5-fold loss of sensitivity.

An LC chromatogram with the off-line detection of arsenic is shown in Figure 1b. It shows two major peaks (denoted F and M) and several minor peaks. Note that in our experimental conditions the chloride interference can be considered negligible since the presence of 1 mol of chloride generates an interference equivalent to 0.2 ppb of arsenic. The fractions were collected accordingly to Figure 1b, and the number of arsenic compounds in each fraction was determined.

Determination of the Number of Arsenic Compounds in Each Fraction (A−M) by CE HPLC−ICPMS and AE HPLC−ICPMS. Cation-exchange HPLC−ICPMS was used to analyze cationic fractions (A−E), and anion-exchange HPLC−ICPMS was used to analyze anionic fractions (F−M). Mobile-phase conditions were chosen according to our previous work.[22] Chromatograms are shown in Figure 2. Retention times of standards were marked in the HPLC−ICPMS chromatograms.

Three of the five cationic fractions appeared to contain a single arsenic compound. The void material from the preparative AE column gave one major strongly cationic species in CE HPLC−ICPMS and ∼20% of an apparently polar species. Fraction B gave two well-separated peaks of similar intensity. The elution order of peaks corresponding to individual fractions is, as expected, inverse to that seen in Figure 1b for anion-exchange chromatography.

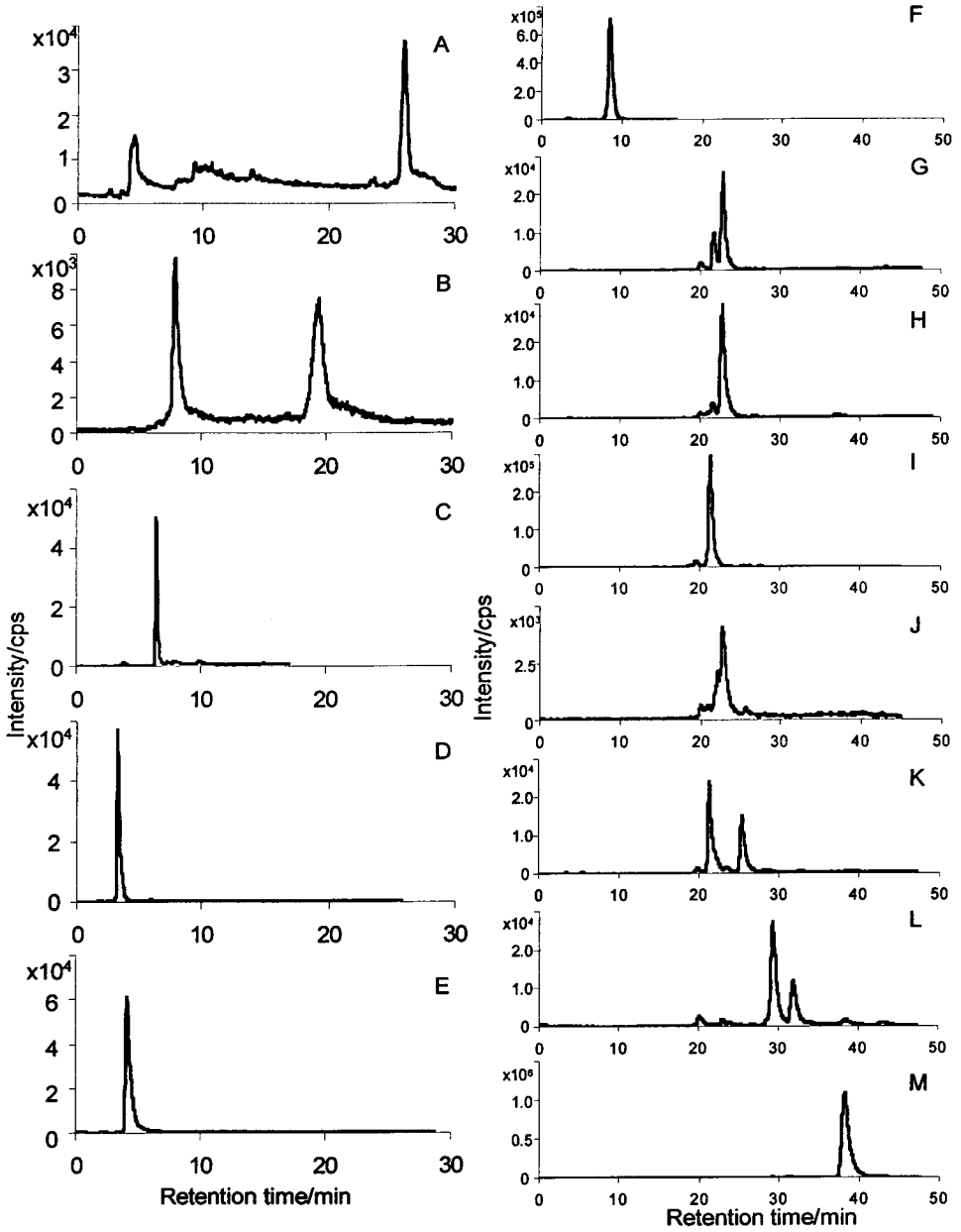

Figure 2. Verification of chromatographic purity of the fractions A−E by cation-exchange chromatography and F−M by anion-exchange chromatography with *on-line* ICPMS detection.

Arsenical material in fractions G−M (chromatograms not shown) all eluted as single unresolved peaks with retention times between 5 and 10 min. Therefore, it was decided to determine the number of arsenic compounds in these fractions by anion-exchange HPLC−ICPMS using a higher resolution column. Contrary to the findings for the cationic fractions, the anionic fractions mostly contain more than one arsenic compound. However, the fractions containing the most arsenic (F, I, M) contained only one arsenic compound each. The minor fractions G, H, and J contain a major peak and smaller peaks due to the coelution of neighboring compounds. In the preparative anion-exchange chromatogram, fractions K and L showed broad peaks of low intensity. The more efficient HPLC−ICPMS revealed at least two compounds accompanied by a number of less intense ones for these two fractions.

Purification and Isolation of Organoarsenic Compounds for Electrospray MS/MS. Based on the above results, the fractions showing cationic character (A−E) were fractionated by CE HPLC prior to ES MS/MS. To avoid missing any eluting arsenic, the eluent was collected continuously throughout the duration of the chromatogram. The reconstruction of the chromatograms shows that the arsenic species were eluted with slightly reduced retention times compared to the on-line CE HPLC−ICPMS chromatogram, due to the increase in sample concentration and therefore sample matrix effects.

The fractions containing more anionic species (F−M) were performed with AE HPLC, which offered a much improved resolution in comparison with the preparative anion-exchange column. Unfortunately, the elution of species from the AE column seems to be influenced to a greater extent by increased matrix

Table 3. MS Data of Daughter Fragments of (a) Low Molecular Weight and (b) Arsenosugar Standards for the Evaluation of Accuracy and Precision in MS Mode

	mass, m/z		difference, ppm
	calcd	exptl	
(a) Low Molecular Weight Standards			
TMAs+	135.0152	135.014	−10.4
DMA	138.9738	138.971	−18.0
MMA	140.9531	140.954	−0.7
AsV	142.9324	142.927	−35.0
AsC	165.0257	165.020	−36.4
AsB	179.005	178.999	−35.8
mean			−22.7
st dev			15.3
(b) Arsenosugar Standards			
As-sugar A	393.0194	393.019	0.3
As-sugar B	329.0575	329.045	−37.7
As-sugar C	409.0143	409.015	0.7
As-sugar D	483.0804	483.054	−55.5
mean			−23.0
st dev			28.1

effects compared to the CE column and retention times are significantly reduced. Thus, the close elution of the species in fractions G−J and L created a problem when the undiluted sample was introduced, and baseline resolution of the major and the minor peaks was not achieved. For fraction K, the resolution of two major peaks was achieved. Fraction M eluted as one major peak, the effect of sample concentration reducing the elution time by 15 min.

Evaluation of the Performance of the Q-TOF Mass Spectrometry for the Analysis of Organoarsenic Standards. In comparison with quadrupole MS, the higher mass resolution and accuracy of time-of-flight MS should allow the elucidation of the exact empirical formula of the analyte and the distinguishing of matrix components with the same molecular mass overlapping the arsenic peaks. CID spectra with similar highly resolved daughter fragments could allow for more detailed structural information on the fragmentation. With the improved resolution (compared to the triple quadrupole instrument API 300), the molecular ions due to the natural distribution of ^{13}C can also be investigated.

Preliminary experiments, aimed at the evaluation of the accuracy and precision of the molecular mass measurement using the Q-TOF mass spectrometer, were run for a series of organoarsenic standards. Table 3 shows the precision and accuracy of the data obtained.

The molecular mass measurement values (Table 3) show, in terms of accuracy, an average negative bias of 0.0023. What is more important, however, is the precision of 15 ppm of the mass measurement, which is random. Therefore, the true value with a confidence limit is between 8 and 38 ppm higher than the measured value.

In the same way, accuracy and precision were evaluated in the collision dissociation mode for the fragments. The fragmentation pattern was similar as that described elsewhere for the triple quadrupole instrument.[22] The mass data are summarized in Table 4. Again a negative bias of 75 ppm can be observed. The precision of the molecular mass measurement in the Q-TOF mode was evaluated at ±33 ppm.

Table 4. MS/MS Data of Daughter Fragments of (a) Low Molecular Weight and (b) Arsenosugar Standards for the Evaluation of Accuracy and Precision in the Product Mode

fragment	mass, m/z		difference, ppm
	calcd	exptl	
(a) Low Molecular Weight Standards			
TMAs+			
Me_3AS	119.9918	119.984	−60.8
Me_2As	104.9684	104.959	−87.7
MeAsCH	102.9528	102.947	−56.3
CHAsCH	100.9372	100.932	−51.5
DMA			
Me2AsO	120.9633	120.953	−87.6
As(OH)2H	110.9426	110.920	−202.8
As(OH)O	108.9270	108.921	−55.1
MeAsO	106.9477	106.940	−71.1
MeAsH	90.9528	90.943	−110.0
MeAs	88.9372	88.928	−108.0
MMA			
MeAsOHO	122.9426	122.935	−59.4
AsO	90.9165	90.905	−130.9
AsV			
As(OH)2O	124.9219	124.914	−63.2
As(OH)2	108.9270	108.921	−55.1
AsO	90.9165	90.909	−84.7
AsC+			
$Me_2AsCH2CH$	147.0152	147.009	−40.8
Me_3AsC	131.9918	131.982	−74.3
Me_3As	120.9996	120.992	−63.6
MeAsO	106.9477	106.940	−71.1
Me_2As	104.9684	104.959	−87.7
MeAsCH	102.9528	102.947	−56.3
CHAsCH	100.9372	100.932	−51.5
AsB			
$Me_3AsCHCO$	160.9945	160.987	−46.0
Me_3AsOH	136.9945	136.988	−47.4
Me_3As	119.9918	119.984	−60.8
Me_2As	104.9684	104.959	−87.7
MeAsCH	102.9528	102.947	−56.3
MeAsH	90.9528	90.947	−62.7
mean			−74.7
st dev			33.0
(b) Arsenosugar Standards			
Arsenosugar A			
237	237.0104	237.003	−29.5
195	195.986	194.986	−69.2
97	97.104	97.104	0.0
Arsenosugar B			
237	237.0104	236.996	−58.6
195	195.986	194.986	−69.2
97	97.104	97.019	45.4
Arsenosugar C			
329	329.0575	329.046	−34.9
237	237.0104	236.989	−87.8
195	194.9999	194.980	−101,5
97	97.0142	97.014	0.0
Arsenosugar D			
465	465.0699	465.052	−39.1
409	409.0438	409.006	−93.4
391	391.0333	391.008	−63.4
329	329.0575	329.062	14.3
237	237.0104	236.996	−58.6
195	194.9999	194.986	−69.2
97	97.0142	97.019	45.4
mean			−39.4
st dev			45.8

Analysis of Purified *Tridacna* Extract Fractions by ES QTOF MS/MS. *Fraction A.* The retention time of the arsenic compound in this fraction is similar to the previously found

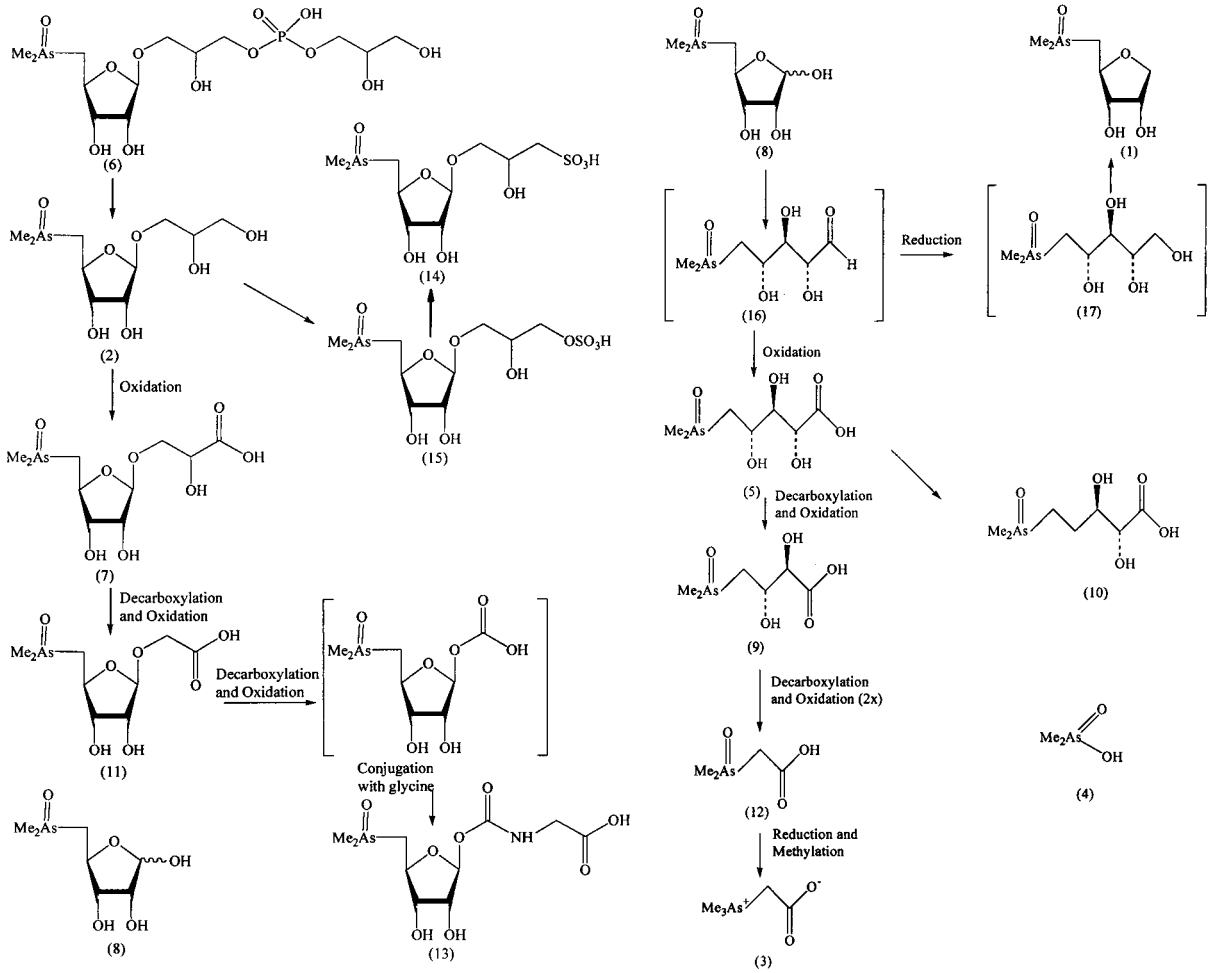

Figure 3. Structures of organoarsenic compounds identified in *T. derasa* and suggested biochemical relationships for some of these compounds: (a) ribofuranosides; (b) acyclic and other compounds.

dimethylarsinoyldihydroxyfuran (**1**; see Figure 3). The protonated molecule signal at m/z 239 (Figure A, Supporting Information) is very weak, but CID fragmentation clearly contains a number of fragments (m/z 122, 133, 148, 166, 178, and 193) characteristic of this compound. The calculated exact mass of this species protonated is m/z 239.026, which is 0.074 u lower than the observed mass: m/z 239.0998. The difference $\delta = 309$ ppm is bigger than expected, but it may be due at the low intensity of this species, the high concentration of matrix components (these fractions were in the preparative AEC void volume), and the likely presence of another compound with the nominal mass of the protonated ion at m/z 239.

Fraction B. Two peaks were observed in CE HPLC−ICPMS (cf. Figure 2). The MS spectrum of the early (8 min)-eluting peak contains a peak at m/z 329, which is the most intense molecular ion above m/z 200 (Figure B, Supporting Information). This resulted from dimethylarsinoylriboside (**2**). The calculated mass was m/z 329.0575, and the measured mass was m/z 329.0460, $\delta = -35$ ppm. The CID fragmentation pattern confirmed the presence of **2**, which is also corroborated by the retention time of the compound.

The mass spectrum contained another molecular ion at m/z 422 (Figure B, Supporting Information) which gave a CID fragmentation pattern characteristic of dimethylarsinoylriboside (presence of fragments at m/z 97, 195, 237, and 329). Since the presence of two arsenocompounds in this fraction is rather improbable, the species may be an artifact or conversely, the earlier seen **2** is a source dissociation product of a previously unreported compound.

The fraction corresponding to the second peak (at 20 min) was also analyzed, but no signal potentially corresponding to an arsenic species could be found.

Fraction C. The retention time of the peak in CE HPLC−ICPMS corresponded to arsenobetaine. The mass spectrum showed prominent matrix components despite the three-dimensional chromatography (Figure C, Supporting Information). At least two molecular ions at m/z 179 were present. One of them corresponded to protonated arsenobetaine: calculated for $((CH_3)_3AsCH_2COOH)$ m/z 179.0050, measured m/z 178.9986, $\delta = -36$ ppm. The identity was confirmed by the CID fragmentation pattern.

Fraction D. The MS spectrum of the arsenic-containing fraction did not allow the attribution of any of the peaks to a known arsenic compound. Only the fragmentation of a molecular ion at m/z 212 gave a daughter fragment that has been observed in an arsenic species. Fragments observed at m/z 109 and 124 could be As(O)OH and $CH_3As(O)OH$, respectively, but the identification of this species was not possible.

Fraction E. The CE HPLC−ICPMS peak at 4.5 min had the same retention time as DMA. The measured mass at m/z 138.9660

matches the calculated value m/z 138.9738 ($\delta = -56$ ppm; Figure E, Supporting Information). The CID mass spectrum showed the fragments characteristic of DMA at m/z 89, 91, 109, and 121 due to the loss of water.

Fraction F. The AE HPLC−ICPMS contains the major arsenic compound accounting for ∼50% of the total arsenic present. A potential arsenic species could be identified at m/z 271 (a dehydrated ion at m/z 253 was also seen; Figure F, Supporting Information). This could correspond to a formula of $(CH_3)_2OAsCH_2(CHOH)_3COOH$ with M_r 271.0158 calculated, 271.0008 measured ($\delta = -55$ ppm). The CID MS spectrum indicates a dimethylarsinoyl moiety (m/z 105, 121, and 122) with three hydroxyl groups (m/z 165, 195, and 225) present in the molecule. The fact that the molecule contains a carboxylic acid group was supported by the loss of 45 u to give the daughter fragment at m/z 225. This evidence indicated an acyclic compound, a 5-dimethylarsinoyl-2,3,4-trihydroxypentanoic acid, possibly formed by the opening of a ribofuranose ring.

Fraction G. AE HPLC−ICPMS showed three peaks (at 20, 22, and 23 min), but the elution profile obtained for preparative purposes (not shown, 10-fold higher concentration injected) shows the major compounds were coeluted and preceded by a shoulder. The ES MS/MS data of the shoulder were similar to those discussed above and corresponded to the 5-dimethylarsinoyl-2,3,4-trihydroxycarboxylic acid. Its retention time was significantly longer than in fraction F, probably because of a greater matrix effect in the latter. The principal peak fraction contained three signals at m/z 255, 343, and 483 of which the CID contained fragments characteristic of organoarsenic compounds (Figure G, Supporting Information).

The m/z 483 species is likely to correspond to the phosphoric acid diester **6** with M_r calculated 483.0804, observed 483.0438, $\delta = -76$ ppm. The CID MS spectrum contained a number of fragments (at m/z 97, 195, 237, 329, 391, and 465) confirming this identity. The m/z 343 indicated the presence of the carboxylic acid **7** (theroretical M_r 343.0368, observed 343.0181, $\delta = -54$ ppm). The CID mass spectrum showed fragments at m/z 97, 195, and 237 characteristic of a dimethylarsinoylribose. The m/z 255 species is also likely to correspond to be a dimethylarsinoylribose as the CID contained the characteristic fragments at m/z 195 and 237. Major ion fragments at m/z 134 and 152 could also be accounted for as fragments of a species with empirical formula $C_7H_{15}O_5As$ (M_r calculated 255.0209, observed 255.0639, $\delta = 168$). This evidence suggested the presence of the free dimethylarsinoylribofuranoside shown in **8** as the β-anomer.

Fraction H. The AE HPLC−ICPMS elution profile was similar to that of the preceding fraction, which suggested that the separation by preparative AE chromatography was incomplete. Two species, the phosphoric acid diester **6** and carcoxylic acid **7** were identified by ES MS/MS.

Fraction I. This fraction contains at least three new organoarsenic compounds with protonated molecular ions at m/z 241, 255, and 433 (Figure I, Supporting Information). These were present in a single peak in AE HPLC−ICPMS. The compound with m/z 241 has a molecular formula of $C_6H_{13}O_5As$ (calculated M_r 241.0053, observed 240.9910, $\delta = -59$). The CID mass spectrum showed similarities to that of carboxylic acid **5** in fraction F. There were indications of a dimethylarsinoyl moiety (m/z 105 and 121) and two hydroxy groups (m/z 165 and 195). The presence of a carboxylic acid group was supported by the loss of 45 mass to give the daughter fragment m/z 195. Thus, the evidence supported identification of the compound with molecular ion at m/z 241 as 4-dimethylarsinoyl-2,3-dihydroxybutanoic acid (**9**).The stereochemistry of this and other acyclic compounds is discussed below.

The compounds with m/z 255 have a possible molecular formula of: $(CH_3)_2As(O)(CH_2)_2(CHOH)_2COOH$, calculated M_r 255.0209, found 255.0070, $\delta = -55$. The CID spectrum showed the presence of a dimethylarsinoyl moiety (m/z 103, 105, and 123) and two hydroxy groups (m/z 165). The fragment at m/z 149 supported the placement of the hydroxy groups on carbons 2 and 3. The presence of a carboxylic acid group was indicated by the loss of 45 mass units to give the daughter fragment m/z 209. This putative structure (**10**) is possibly an 4-deoxy analogue of the 5-dimethylarsinoyl-2,3,4-trihydroxypentanoic acid (**5**).

The arsenic species with m/z 433 was also shown to be a dimethylarsinoyl riboside on the basis of the characteristic fragmentation pattern yielding daughter fragments at m/z 97, 195, 237, and 329. Lack of further fragments did not allow for the determination of the structure.

Fraction J. This fraction has an arsenic concentration 2 orders of magnitude lower than the former fraction, AE HPLC−ICPMS showed one major peak with a front shoulder. An arsenic-containing ion at m/z 313 with an empirical formula of $C_9H_{17}O_7As$ (calculated M_r 313.0263, observed 313.0112, $\delta = -48$ ppm) and two fragments (at m/z 97 and 237) in the CID mass spectrum characteristic of a dimethylarsinoylriboside indicated the presence of carboxylic acid (**11**) (Figure J, Supporting Information).

Fraction K. AE HPLC−ICPMS showed two major peaks, which were revealed in the mass spectrum as molecular ions containing arsenic at m/z 181 and 356 (Figure K, Supporting Information). The former corresponded to an molecular formula of $(CH_3)_2AsOCH_2COOH$: M_r calculated 180.9843, measured 180.9829, $\delta = -7.7$ ppm. The CID mass spectrum showed the presence of fragments at m/z 103, 107, and 121 characteristic of a dimethylarsinoyl moiety. Thus, this compound was identified as dimethylarsinoylacetic acid.

The second major peak showed an intense molecular ion at m/z 356 corresponding to an empirical formula of: $C_{10}H_{18}NO_8As$ (m_r calculated 356.0321, observed 356.0104, $\delta = -61$ ppm). The CID fragmentation gave a series of ions characteristic of a dimethylarsinoylriboside (m/z 97, 195, and 237). This compound was identified as the carbonylglycine derivative **13** previously isolated from *T. maxima*.

Fraction L. The AE HPLC−ICPMS chromatogram showed two major species and a number of minor ones. An arsenic-containing ion was observed at m/z 393 (Figure L, Supporting Information) corresponding to an empirical formula of $C_{10}H_{21}O_9SAs$ (M_r calculated 393.0194, observed 393.0019, $\delta = -45$ ppm). The CID fragmentation confirmed the this compound as the sulfonic acid **14**. No other arsenic compound could be identified in this fraction.

Fraction M. The AE HPLC−ICPMS chromatogram showed an intense major peak which, analyzed by ES MS/MS, gave two ions corresponding to arsenic species at m/z 329 and 409 (Figure M, Supporting Information). The latter corresponded to an empirical formula $C_{10}H_{21}O_{10}SAs$ (M_r calculated 409.0143, observed

409.0056, $\delta = -21$ ppm). The CID fragmentation pattern was characteristic of the sulfuric acid ester derivative **15**. The species at m/z 329 was identified as compound **2**, but since it is cationic, its presence in this fraction is unlikely and it is probably a hydrolysis product of sulfuric acid ester **15**. In addition, a weak ion at m/z 469 was present. Its CID fragments at m/z 97, 195, 237, and 329 indicated the presence of another dimethylarsinoylriboside with a formula tentatively assigned as shown in Figure M (Supporting Information).

Quantitative Distribution of Arsenic Species in *T. derasa* Kidney. The structures of organoarsenic compounds detected are shown in Figure 3. Table 3 contains quantitative data of the distribution of arsenic species. In agreement with a recent report,[26] two major species were found in the kidney of *T. derasa*, the sulfuric acid ester **15** and the 5-dimethylarsinoyl-2,3,4-trihydroxypentanoic acid (**5**) which together account for over 90% of the arsenic present. Compounds **7** and **13** account for 3% and 1%, respectively.

Biosynthetic Relationships of the Arsenic Compounds in *Tridacna* Kidney. Of the organoarsenic compounds found in *T. derasa* **2**, **6**, **14**, and **15** are well-known to be elaborated by marine algae,[27] are presumably products of the zooxanthellae in the clam mantle, and have not been further metabolized by the clam. On the other hand, as has been recently observed,[26] **7** and **13** have been previously isolated from the kidney of the related *T. maxima*, have not been found in algae, and might well be the result of metabolism by the clam of algal products. The suggestion that the clam is transforming algal products has received much support by compounds identified in this study. It appears that both aglycons and acyclic compounds derived from opening of the ribose ring are degraded in clam tissues by a series of successive oxidations and decarboxylations as shown in Figure 3. In Figure 3b, the assumption has been made that D-ribose stereochemistry has been retained in the acyclic compounds. Another compound of this type, i.e., a dimethylarsinoylribitol derivative, was previously isolated from the red alga *Chondria crassicaulis* and its stereochemistry confirmed by synthesis.[28]

The free dimethylarsinoylribose **8**, formed by hydrolysis of any of the glycosides, would exist, at least to a small extent, as the acyclic form **16**. Reduction and cyclization of this compound would yield the dihydroxyfuran derivative **1** with D-ribose stereochemistry again being retained. Of more interest though are the products flowing from oxidation and decarboxylation of **16**. Carboxylic acid **5**, the product of immediate oxidation of **16** would upon oxidation and decarboxylation yield **10**, which in turn would be transformed to dimethylarsinoylacetic acid **12** by two series of decarboxylations and oxidations. For **12** to be converted to arsenobetaine (**3**) requires only reduction and further methylation at the arsenic atom. Arsenobetaine (**3**) is by far the major arsenic compound encountered in marine animals[27] and has also been found in terrestrial organisms;[29] its biosynthesis has never been fully explained although microbial breakdown of dimethylarsinoylribosides has been implicated.[27] There have been a number of reports of arsenobetaine coexisting with dimethylarsinoylribosides in marine animals, and there has been an uncertainty as to whether they were biosynthetically connected or accumulated independently.[30] The evidence presented here suggests that metabolism of dimethylarsinoylribosides in animal tissues is at least one way by which the ubiquitous arsenobetaine might be biosynthesized.

CONCLUSIONS

Electrospray Q-TOF MS was shown capable of the comprehensive identification of organoarsenic species in a marine sample. Owing to the higher mass accuracy and precision compared to triple quadrupole instruments, the assignment of molecular ions and daughter fragments is more straightforward. Higher resolution allowed for the observation of minor species that were lost in baseline noise in quadrupole MS. The rapidity of TOF scanning allows the fragmentation of virtually all the ions present in the mass spectrum for a small amount of sample, which makes the identification of arsenic-containing ions easy by verifying the CID fragmentation pattern. Multidimensional chromatography is essential for the fractionation and purification prior to ES MS analysis. The development of LC with volatile buffers proved successful to minimize signal suppression in ESMS. Indentification of the wide range of arsenical metabolites in the kidney of *T. derasa* has provided insight into the biochemical transformations of arsenic compounds and indicated a possible origin of the widely distributed arsenobetaine

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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