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Arsenic speciation in whelks, *Buccinum undatum*†

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The arsenic species present in the foot muscle of whelks, *Buccinum undatum*, collected from Newfoundland, Canada, were characterized by using high-performance liquid chromatography–inductively coupled plasma mass spectrometry. All samples contain high amounts of arsenic, mostly over 100 μg g^{-1} (as arsenic, dry weight basis), and one sample contained up to 1360 μg g^{-1}. These values are considerably higher than those reported in other gastropods. Speciation studies of representative samples revealed arsenobetaine as the major water-soluble arsenic compound, together with trace amounts of an arsenosugar. No inorganic arsenic species were detected in the sample extracts, indicating that consumption of the whelks poses little human risk. Copyright © 2002 John Wiley & Sons, Ltd.

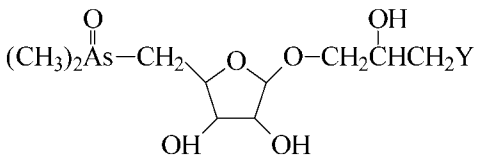
KEYWORDS: arsenic; speciation; whelks; arsenobetaine; arsenosugar; HPLC; ICP-MS

INTRODUCTION

The US Environmental Protection Agency (EPA) has been investigating the need to lower the allowable arsenic level in drinking water to below 50 ppb.[1,2] A new maximum concentration level (MCL) of 10 ppb has recently been declared, meaning that a person who drinks 2 l of water per day could consume up to 20 μg of arsenic from this source. However, the arsenic content of food can be significant, and this also contributes to the daily intake. For example, it is estimated that a 62 kg female takes in about 27 μg of arsenic per day from food, with most of the arsenic coming from products of marine origin.[1] Clearly the nature of this arsenic is important.

The distribution of arsenicals in the marine environment has been studied for over 80 years.[3–9] The chemical forms of arsenic in marine organisms remained unknown until 1977, when the first major advance was made: arsenobetaine, $(CH_3)_3As^+CH_2COO^-$, was identified from the Western rock lobster (*Panulirus cygnus*) by Edmonds *et al.*[6] Since then, arsenobetaine, a zwitterion, has been found as a major arsenic compound in most marine animals investigated.[5,7,8] Arsenosugars, such as those shown in Scheme 1, have been found in marine algae and some marine bivalves.[9–11] The tetramethylarsonium ion, $(CH_3)_4As^+$, has been found in clams.[12,13] The new zwitterionic species $(CH_3)_3As^+CH_2CH_2COO^-$ was recently isolated from a tropical fish.[14]

The detection limit for arsenic speciation has been significantly improved by the use of advanced methodology, such as high performance liquid chromatography–induc-



	Y
1a	-OH
1b	$-OPO_3HCH_2CH(OH)CH_2OH$
1c	$-SO_3H$
1d	$-OSO_3H$

Scheme 1. The major arsenosugar derivatives found in biological systems.

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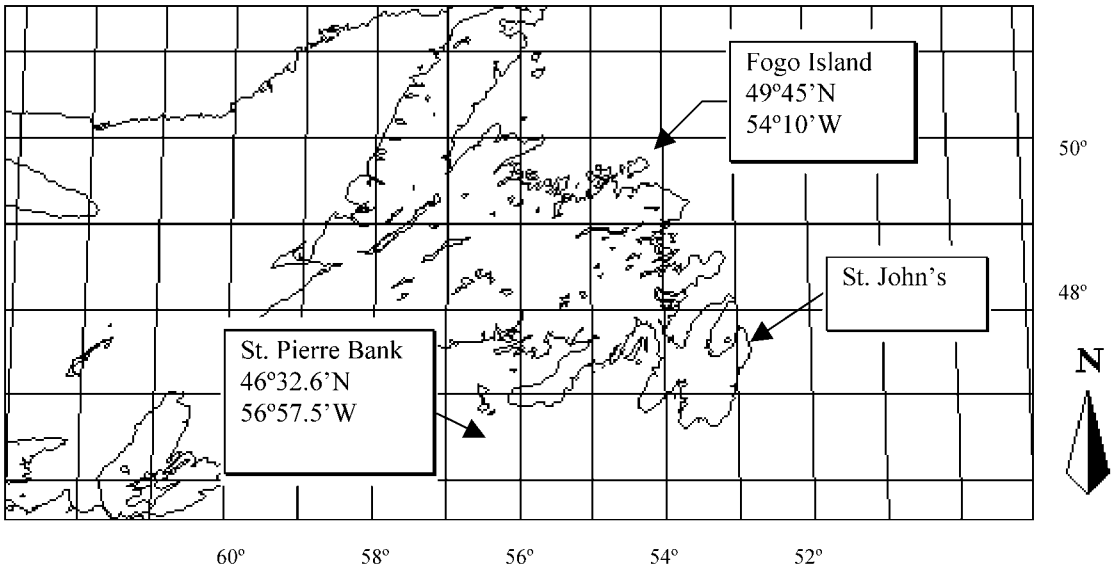

Figure 1. Map of Newfoundland showing the two sampling locations: St Pierre bank and Fogo Island.

tively coupled plasma mass spectrometry (HPLC–ICP-MS) and HPLC–electrospray MS, and invaluable structural information can be obtained by using the latter. For example: (a) arsenobetaine and arsenosugars are found in the haemolymph of the Dungeness crab;[15] (b) arsenosugar speciation in algal extracts is improved by using HPLC–MS;[16] (c) arsenic speciation is found to vary with the season in algae,[17] and in scallops the speciation varies with the sampling location and the organs[18] and the sex and the spawning cycle.[19]

The present study is concerned with arsenic speciation in the (foot muscle only of the) gastropod *Buccinum undatum*, fished commercially, and sold and eaten as 'whelks'. Some of the whelk samples were collected from St Pierre Bank, Canada, one of the collection sites for the scallops used in previous studies.[18] Arsenic speciation was carried out to establish if the consump-tion of these animals as food would pose any risk to human health.

EXPERIMENTAL

Sample collection and preparation

Whelks (*B. undatum*) were collected from two locations, St Pierre Bank (46°32.6′N, 56°57.5′W) and Fogo Island (49°45′N, 54°10′W), Newfoundland, shown in Fig. 1. All samples were frozen for transportation to the laboratory and stored at −20 °C until they were dissected. After dissection, foot muscles from individual whelks were quickly, but thoroughly, washed with distilled, deionized water and stored at −20 °C. The tissues were individually homogenized using a Sorvall Omnimix blender. The samples were then freeze-dried and stored at −20 °C.

Reagents and chemicals

All chemicals used were of analytical grade, unless stated otherwise, and include: sodium arsenate heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$, Sigma), arsenic(III) oxide (As_2O_3, Alfa products), methylarsonic acid ($CH_3AsO(OH)_2$, Pfalz & Bauer, Stamford), dimethylarsinic acid ($(CH_3)_2AsO(OH)$ (DMAA), Aldrich), methanol (HPLC grade, Fisher), tetraethylammonium hydroxide (TEAH, 20 wt%, Aldrich), malonic acid (BDH), nitric acid (69%, sub-boiling distilled, Seastar Chemicals) and rhodium solution (ICP standard, 1000 μg ml^{-1} in 20% HCl, Specpure, Alfa). Arsenobetaine, arsenocholine bromide, and tetramethylarsonium iodide were synthesized as described in the literature.[6,20,21] Deionized water with resistivity better than 18 MΩ cm was used for the extractions and to make up the eluent for HPLC.

The glassware and plasticware were cleaned by soaking in 2% Extran solution overnight, rinsing with water and deionized water, followed by a soak in 0.1 M HNO_3 solution overnight. They were then rinsed with deionized water and air-dried.

Analytical procedures

Arsenic speciation analysis (HPLC–ICP-MS)

Freeze-dried samples (0.3 g dry weight) were extracted with a methanol/water mixture (1:1, v/v) by using a procedure similar to that previously described.[9,22] Extracts were stored at −20 °C and transferred to 4 °C on the day of analysis.

The HPLC system consisted of a Waters Model 510 delivery pump, a Reodyne Model 7010 injector valve with a 20 μl sample loop and a reverse-phase C_{18} column (GL Sciences Inertsil ODS, 250 mm × 4.6 mm) equipped with a C_{18} guard column (Supelco, 2 cm). The HPLC system was

Table 1. Operating parameters for ICP-MS

Forward r.f. power (w)	1350
Reflected power (w)	<10
Outer (cooling) gas flow rate (l min^{-1})	13.8
Intermediate (auxiliary) gas flow rate (l min^{-1})	0.65
Nebulizer gas flow rate (l min^{-1})	1.002
Analysis mode	TRA, 1 s time slice
Quadrupole pressure (mbar)	9×10^{-7}
Expansion pressure (mbar)	2.4

connected to the ICP nebulizer via a PTFE tube (20 cm × 0.4 mm) and appropriate fittings.

A VG Plasma Quad 2 Turbo Plus ICP mass spectrometer (VG Elemental, Fisons Instrument), equipped with an SX 300 quadrupole mass analyser, a standard ICP torch, and a de Galan V-groove nebulizer, was used as the detector. Samples were analysed in the 'peak jump' mode. The mass analyser was set to monitor the $m/z = 75$ signal peak corresponding to As^+ and $m/z = 77$ corresponding to the interference possibly caused by chloride in the samples ($ArCl^+$). Since $m/z = 77$ also corresponds to $^{77}Se^+$, $^{82}Se^+$ was also monitored to correct for the selenium portion of the counts collected under $m/z = 77$ signal peaks. The time-resolved analysis (TRA) mode was used and it allowed simultaneous monitoring of more than one m/z value over the time course for the chromatography. A summary of the operating parameters for the ICP-MS is given in Table 1.

The eluent used contained the following: 10 mM TEAH, 4.5 mM malonic acid, 0.1% CH_3OH, pH 6.8 (by HNO_3) at 0.8 ml min^{-1}. All samples were filtered (0.45 μm) prior to injection onto the column. The injection volume was 20 μl. Arsenic compounds in the samples were identified by matching the retention times of the peaks in the chromatograms with those of standards and the standard reference materials. Quantification was done by comparing peaks with those of separately injected matching standards. DMAA was used as the standard for arsenosugars.

Analysis for total arsenic

Freeze-dried tissue samples, standard reference material, freeze-dried residue samples after extraction and extracts were weighed or pipetted into glass test tubes (outer diameter 16 mm). Solid samples (0.05 to 0.2 g) and extracts (200 μl from extraction) were used. Nitric acid (2 ml) and three Teflon boiling chips were added to each tube. The samples were heated in a test tube block heater (VWR Canlab) at temperatures increasing stepwise from 70 to 150 °C until they were evaporated to dryness. The residue was redissolved in 3 ml of an aqueous solution containing 1% (v/v) nitric acid and 5 ppb rhodium. The samples were mixed thoroughly by using a vortex mixer and filtered (0.45 μm). The samples were frozen until analysis.[15]

Samples after digestion were diluted appropriately with rhodium–nitric acid solution and analysed by using FIA–ICP-MS. The injection volume was 100 μl and the flow rate was 0.8 ml min^{-1}. The settings for the ICP-MS for the total arsenic analysis were the same as those for the HPLC–ICP-MS, with the exception that 'single ion monitoring' mode was used instead of TRA. Signals were corrected according to the signal of the internal rhodium standard.

Quality assurance was maintained by analyses of the standard reference material SRM 1566a (Oyster tissue) from the National Institute of Standards and Technology, US Department of Commerce (total arsenic: 13 ± 1.3 μg g^{-1};

Table 2. Arsenic species (μg of arsenic per gram dry weight) in some whelks collected at St Pierre Bank and Fogo Island

Tissue ID	[Arsenic] by acid digestion[a]	[Extract] by acid digestion	[Residue] by acid digestion	[Extract] + [Residue] by acid digestion	[AsB] from HPLC[b]	[**1a**] from HPLC[b]	[AsB] + [**1a**] from HPLC[b]
St Pierre Bank							
SP1	7.7	8.4	0.9	9.3	8.2	0.5	8.7
SP2	11	9.7	3.9	13.6	9	0.5	9.5
SP3	56	55.2	3.1	58.3	52	2.9	54.9
SP4	76.7	58	9.1	67.1	62	–	62
Fogo Island							
FG1	133	78.3	55.9	134	82	–	82
FG2	142	124	3.3	127	128	–	128
FG3	405	361	20.5	382	389	–	389
FG4	1360	1200	94.9	1290	1610	–	1610

[a] Representative samples from each site. There were six samples with total arsenic concentration ranging from 7.7 to 76.7 μg g^{-1} from St Pierre Bank and six samples with total arsenic concentration ranging from 133 to 1360 μg g^{-1}. Speciation was not performed on these samples.
[b] Concentrations from HPLC can be compared with [Extract] by acid digestion (extractable or water-soluble arsenic). AsB: arsenobetaine.

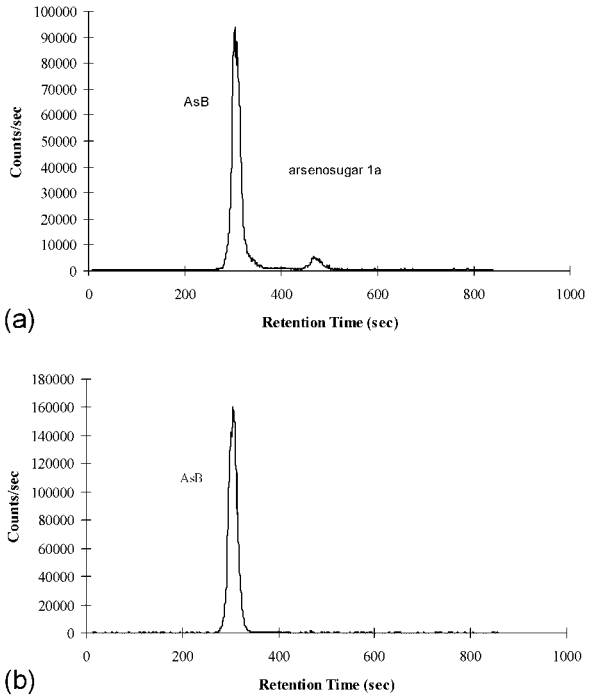

Figure 2. HPLC–ICP-MS trace of a whelk foot muscle from: (a) St Pierre Bank; (b) Fogo Island.

certified value: 14.0 ± 1.2 μg g^{-1}) and an in-house standard, kelp powder.[22]

RESULTS

Whelk samples were collected from Newfoundland, Canada (Fig. 1). Whelks are commonly found in shallow coastal waters of the North Atlantic. In Newfoundland there is a commercial fishery at the two sites where the samples were collected, St Pierre Bank and Fogo Island. Ten individual whelk samples were collected from each site.

The arsenic speciation results of some whelk samples collected from the two sites are shown in Table 2. Examples of chromatograms of the samples are shown in Fig. 2a and b.

St Pierre Bank

Samples ($n = 10$) from St Pierre Bank contained total arsenic in the range 7.7 to 76.7 μg g^{-1}. Detailed speciation work was done on four selected samples, two in the low range of arsenic and two in the high range. The total amount of arsenic extracted as determined by acid digestion of the extracts was similar to the sum of the arsenic compounds in the extracts as determined by integration of the HPLC traces. The extraction efficiency, determined by the ratio between the total arsenic in the extract and the total arsenic in the solid, was found to range from 76 to 110%. The major extractable arsenic compound in the samples was arsenobetaine, and some samples contain low concentrations of arsenosugar **1a** (Table 2).

Fogo Island

Samples ($n = 10$) collected from Fogo Island contained significantly higher amounts of arsenic than those collected at St Pierre Bank. The lowest concentration found in this site (133 μg g^{-1}) is higher than the highest value found in St Pierre Bank whelks. The upper limit of arsenic found in Fogo Island was 1360 μg g^{-1}. This high value is discussed below.

Four samples were selected again for speciation studies. As was the case for samples collected from St Pierre Bank, samples from Fogo Island also contained arsenobetaine as the major water-soluble arsenic compound, although arsenosugar **1a** was not detected (detection limit: ~10 μg As g^{-1}). The extraction efficiency was lower, being in the range 59–89%.

DISCUSSIONS

Arsenobetaine is assumed to be non-toxic to humans.[1] A number of studies suggest that arsenobetaine is excreted unchanged in urine after ingesting seafood, e.g. see Ref. 23. Though trace amounts of arsenosugar **1a** were found in some whelk samples, no other arsenic compounds, especially the more acutely toxic inorganic species, were found in any significant amount. Arsenosugars are also assumed to be non-toxic, although they are metabolized mainly to dimethylarsinic acid.[23]

Francesconi and Edmonds[8] reported in 1997 that the highest arsenic concentration in gastropods was 38 μg g^{-1} dry weight. Goessler et al.[24] found that 95% of the arsenic in the carnivorous gastropod *Morula marginalba* (whole animal: [As] = 233 μg g^{-1}) was present as arsenobetaine. Whelk samples (foot only) from Fogo Island contain far higher arsenic levels, up to 1360 μg g^{-1}. Benson and Summons[25] list arsenic concentrations in a range of marine invertebrates, and the highest numbers seen are 1025 μg g^{-1} in the kidney of the giant clam *Hippopus hippopus* and 1004 μg g^{-1} in the kidney of another clam species, *Tridacna maxima*. A wide variety of arsenicals, mainly arsenosugars, have been identified from the clam kidneys, and it has been suggested[25] that the build up of these species is a result of the activity of symbiotic algae. The exceptionally high arsenic concentration, mainly arsenobetaine, in the muscle of the whelks is unlikely to result from a similar phenomenon, especially since arsenobetaine is yet to be identified in algae. In the present study the arsenic levels found in whelks from one site are ten times higher than those from the other site, which seems to suggest a dependency on an external source of arsenic. However, there is no obvious source of this arsenic other than food. It is possible that the food sources in the two environments are very different, but, even if this is correct, it does not account for the remarkable accumulation for arsenobetaine.

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