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Arsenic speciation in clams of British Columbia

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The water-soluble arsenic compounds in five species of clams — Butter clam (*Saxidomus giganteus*), Horse clam (*Schizothoerus nuttalli*), Soft-shelled clam (*Mya arenaria*), Native littleneck clam (*Protothaca staminea*), and Manila clam (*Venerupis japonica*) — are described. Varying amounts of arsenobetaine and tetramethylarsonium ion are the major arsenicals found in all species. Butter clams show the presence of a third compound which appears to be trimethylarsine oxide. Small amounts of as-yet-unidentified arsenicals can also be isolated.

Keywords: Arsenic compounds, clams, speciation, tetramethylarsonium ion, arsenobetaine.

INTRODUCTION

Edmonds *et al.*[1] isolated and characterized an organoarsenic compound, arsenobetaine [$(CH_3)_3As^+CH_2COO^-$], from the Western rock lobster. Since then the virtual ubiquity of this arsenical in marine animals has been established.[2–4] The related ion arsenocholine [$(CH_3)_3AsCH_2CH_2OH^+$] has been reported in shrimp[5,6] although there are some problems with the indentification.[4] Trimethylarsine oxide [$(CH_3)_3AsO$], has been identified as a minor component in a number of fish species[7–9] and the tetramethylarsonium ion [$(CH_3)_4As^+$] has been found in the clam *Meretrix lusoria*[10] and in some gastropods.[11,12]

The present paper reports the results of some speciation studies on clams collected from the coast of British Columbia*

EXPERIMENTAL

Instrumentation employed in this work has been described previously.[13]

Determination of total arsenic

Two independent methods were used for sample decomposition.

(a) Wet digestion with nitric acid, sulfuric acid, and hydrogen peroxide

Wet digestion was carried out in a 250 cm^3 round-bottom flask fitted with a specially designed stopper and an air condenser containing a diffusion funnel. The stopper, funnel and plugs were made of Teflon.[14] The dried sample (0.25 g) or wet sample (1.0 g) was transferred into the flask. Nitric acid (3 cm^3, 69%), sulfuric acid (1 cm^3, 98%), and hydrogen peroxide (3 cm^3, 30%) were added. The apparatus was placed in a 250 cm^3 heating mantle and heated for 3 h at 250°C in a fume hood. The heat was switched off and the flask allowed to cool. The digestate was transferred to a 100 cm^3 volumetric flask and made to the mark with deionized water. Arsenic was determined in the digestate by hydride generation atomic absorption spectroscopy (HG AA) by using the conditions previously described.[13] Where the sample size was greater than 1.0 g, the solutions added were in the ratio 1:3:3 cm^3 H_2SO_4:HNO_3:H_2O_2 g^{-1} wet tissue (total volume 7 cm^3 g^{-1}).

(b) Microwave digestion

The dried material (0.25 g) or wet sample (1.0 g) was placed in the Teflon pressure decomposition vessel (Parr Instrument Company, 45 cm^3). Nitric acid (6 cm^3, 69%) was added. The vessel was closed with its screw cap and heated for 2 min at full power (500 W) in the microwave oven (Toshiba model no. ERX 5610C). After cooling to ambient temperature,

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*This paper describes some of the material presented at the 3rd Chemical Congress of the North American Continent in Toronto, June 1988. Brief mention was made then of the UV–HG AA technique, which has been published as Ref. 13 of this paper. The remainder of the material will be published later in this Journal.

the solution was made up to 100 cm^3 in a volumetric flask and arsenic determined by graphite furnace atomic absorption spectroscopy (GF AA) and HG AA. Standard arsenic solutions were made in nitric acid blanks prepared by heating 6 cm^3 concentrated nitric acid in the bomb for 2 min and diluting to 100 cm^3. For GF AA, 20 μL was injected into the furnace. Normal calibrations and standard additions were used.

Extraction of organoarsenic compounds in the clams

Frozen clam specimens of known origin were thawed and their shells were removed. The soft tissues were bulked and homogenized in a blender. A 10 g portion of the homogenate was reserved for the determination of total arsenic by acid digestion. The homogenate was weighed and transferred into an Erlenmeyer flask. Methanol (2.5 $cm^3\ g^{-1}$ of tissue) was added. The flask was stoppered with a rubber plug and left on a mechanical shaker for 2 days. The extracts were combined and arsenic was determined by HG AA and GF AA. The residue was air-dried and arsenic was determined by HG AA after acid digestion.

Determination of arsenic in extracts

The concentration of arsenic in the extracts was determined by (a) GF AA and (b) HG AA after UV decomposition.

(a) Determination of arsenic in extracts by GR AA

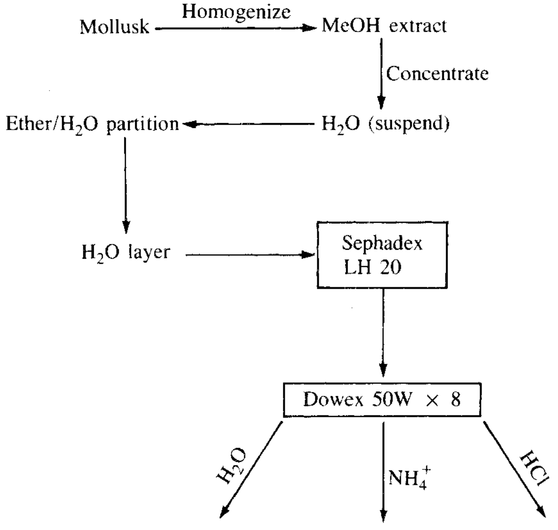
Arsenic was determined by injecting 20 μL of the extract into the graphite tube directly without any sample retreatment. However, in most cases, it was found necessary to dilute aliquots of the extracts with deionized water to give a working concentration range of 10–100 ng cm^{-3} As. Aqueous arsenic standards were used with normal or standard addition calibration. Determinations were carried out in triplicate and peak area absorbance was used in most cases.

(b) Determination of arsenic in extracts by hydride-generation AA after UV decomposition (UV–HG AA)

Aliquots of the extracts were made up to 50 cm^3 with water to give working concentrations between 10–100 ppb As [ppb, parts per billion (10^9)]. The solutions were placed in quartz containers, 2.5 cm o.d. × 28 cm, and irradiated with a 1200 W Hanovia lamp. The solution was subsequently analyzed by HG AA.[13]

Purification procedures for arsenic compounds in extracts

The procedures used for the extraction of the soft tissues of the organisms and the purification of the extracts are summarized in Scheme 1.



Scheme 1 Schematic representation of the procedures used for the extraction of arsenic compounds from organisms and for purification of the extracts.

The combined methanol extract was evaporated to dryness by using a rotary evaporator to remove methanol. The residue was suspended in a small volume of water. Repeated extractions with diethyl ether were performed until the ether phase was colorless. The aqueous phase, which was regarded as the water-soluble arsenic fraction, was evaporated to dryness and dissolved in a small volume of water (10–20 cm^3). This solution was triturated with methanol and then filtered; the insoluble residue was discarded, and the filtrate was evaporated to about 10 cm^3. The solution was subjected to gel-permeation chromatography on Sephadex LH-20 in a 2.5 cm × 60 cm column, with methanol as eluant. Fractions (12.5 cm^3 volume) were collected and arsenic was determined in each by GF AA. The arsenic compounds eluted within one band and retention volumes of the specific extracts are given under Results and Discussion. The arsenic-containing fractions were combined and evaporated to dryness. The residue was dissolved in 10 cm^3 of water and put onto a Dowex 50W × 8 (H$^+$) 200–400 mesh column (1.5 cm × 20 cm, or 2.0 cm × 30 cm). (The resin was prepared by first extracting it with acetone until the filtrate was colorless. It was washed with 2 mmol dm^{-3} sodium hydroxide (three times), followed by distilled water until neutral, then with 2 mmol dm^{-3} hydrochloric acid (three times) and finally with distilled water until neutral.) For the elution of the arsenic compounds, the column was first washed with 100–200 cm^3 water (unadsorbed fraction) and then with 200–300 cm^3 5% ammonium hydroxide solution (NH_4OH fraction), 50 cm^3 water (water fraction) and 2 mol dm^{-3} hydrochloric acid (HCl fraction). Fractions (12.5 cm^3) were collected and each fraction analyzed for arsenic. The arsenic containing fractions in the unadsorbed, NH_4OH and HCl fractions were bulked separately and concentrated on the rotary evaporator.

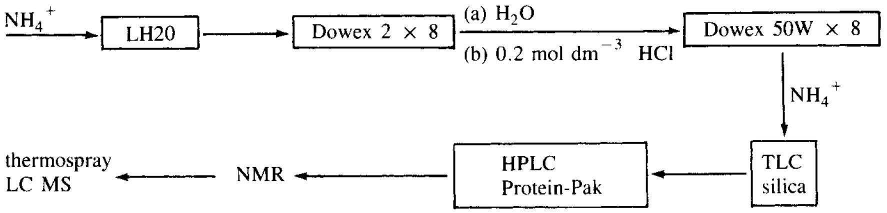
(a) Isolation of arsenic compounds in the NH_4OH fraction

A schematic representation of the purification and identification of the arsenic compounds in the NH_4OH fraction is shown in Scheme 2.

The solid obtained from the NH_4OH fraction was dissolved in a minimum amount of water and subjected to gel-permeation chromatography on a Sephadex LH-20 column (2.5 cm × 30 cm). Elution was carried out with 500 cm^3 water and fractions of 12.5 cm^3 were collected. The arsenic-containing fractions (specific retention volumes for the different organisms are given below) were combined, evaporated to dryness, dissolved in water and chromatographed on a Dowex 2 × 8 column (1.5 cm × 50 cm, OH$^-$ form). The column was first washed with water (100 cm^3) and then with 200 cm^3 0.2 mol dm^{-3} hydrochloric acid. Fractions of 12.5 cm^3 were collected. The arsenic-containing fractions were concentrated to 10 cm^3 and chromatographed on Dowex 50W × 8 (1.5 cm × 20 cm, 200–400 mesh, H$^+$ form) with 0.2 mol dm^{-3} aqueous ammonium hydroxide, 200 cm^3, as the eluant. Fractions of 5 cm^3 were collected. The arsenic-containing fractions were combined and evaporated to dryness. The residue obtained was dissolved in a minimum amount of water. A small aliquot of this solution was examined by TLC on silica gel with ethanol–acetic acid–water (65:1:34, by volume) as eluant against standards of arsenobetaine, arsenocholine and tetramethylarsonium iodide. The spots were visualized by exposure to iodine vapor. The R_f values of the arsenic-containing spots were obtained — arsenobetaine, 0.52; arsenocholine, 0.32; tetramethylarsonium iodide, 0.38. The remaining solution was then similarly processed by TLC. The area with R_f corresponding to the arsenic compound identified in the preliminary TLC experiment was scraped, homogenized in 10 cm^3 methanol, and centrifuged. The methanolic extract from the scrapings was concentrated. Final purification was achieved by HPLC on a Protein-Pak DEAE column (7.5 mm × 7.5 cm) with 5 mmol dm^{-3} ammonium acetate solution, pH 6.65. The flow was maintained at 1 cm^3 min^{-1} and 0.5 cm^3 fractions were collected. The arsenic-containing fractions (fraction numbers 5–7) were combined and evaporated to dryness. The residue obtained was analyzed further by NMR and MS.

(b) Isolation of arsenic compounds in the HCl fraction

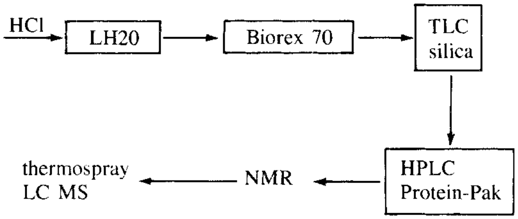
The solid obtained from the HCl fraction was dissolved in 0.2 mol dm^{-3} ammonium hydroxide (20 cm^3) and subjected to gel-permeation chromatography on a Sephadex LH-20 column (2.5 cm × 30 cm); water



Scheme 2 Purification and identification of arsenic compounds in the NH_4OH fraction.

(500 cm^3) was the eluant. Fractions (12.5 cm^3) were collected and analyzed for arsenic. The arsenic-containing fractions (retention volumes between 150 and 200 cm^3) were combined and concentrated to about 10 cm^3. The concentrate was chromatographed on a Biorex 70 column (1.5 cm × 50 cm), which was eluted with water (100 cm^3) followed by 200 cm^3 0.1 mol dm^{-3} hydrochloric acid. Fractions (12.5 cm^3) were collected and analyzed. The arsenic-containing fractions were bulked, concentrated and rechromatographed on Dowex 50W × 8, which was eluted with 200 cm^3 of 0.2 mol dm^{-3} ammonium hydroxide solution; 5 cm^3 fractions were collected. The arsenic-containing fractions were concentrated and purified by TLC and HPLC by using the same procedures as for the NH_4OH fraction. The final purified residue was analyzed by NMR and MS.

A summary of the experimental scheme is shown in Scheme 3.



Scheme 3 Purification and identification of arsenic compounds in the HCl fraction.

RESULTS AND DISCUSSION

Determination of total arsenic

The determination of arsenic in 'solid' marine samples by using GF AA and HG AA requires that the sample be present in solution. Many digestion procedures have been described:[15] they include wet digestion of samples with various combinations of acids[1,10,16–18] and base,[19] and dry ashing with magnesium nitrate.[6,20] Wet digestion with a mixture of nitric acid, sulfuric acid, and hydrogen peroxide was carried out in a reflux assembly originally developed for the analysis of selenium.[14] Bomb decomposition with nitric acid in a microwave oven was also utilized.

The direct determination of arsenic in the sulfuric acid–nitric acid–hydrogen peroxide digestate by GF AA does not give reliable values because of interferences which are mainly associated with residual sulfuric acid. The interference from the acid in the HG AA procedure is minimal and HG AA can be used for the determination of arsenic in the solution from decomposition with acid and peroxide.

A variety of standard reference materials were chosen for analysis with a view to establishing conditions for optimum recovery. These samples included National Bureau of Standards orchard leaves SRM 1571 and bovine liver SRM 1577, and the National Research Council of Canada (NRCC) marine analytical standards dogfish muscle DORM-1, dogfish liver DOLT-1, and marine sediment PACS-1.

A summary of the results obtained for the standard reference materials is shown in Table 1.

Table 1 Arsenic determinations in standard reference materials

Sample	Arsenic certified (μg g^{-1})[a]	Arsenic found (μg g^{-1})[b]
Dogfish muscle NRCC–DORM 1	17.7 ± 2.1	17.2 ± 0.9
Dogfish liver NRCC–DOLT 1	10.1 ± 1.4	8.6 ± 0.6
Marine sediment NRCC PACS 1	211 ± 11	203.4 ± 9.6
Orchard leaves NBS SRM 1571	10 ± 2	9.8 ± 0.3
Bovine liver NBS SRM 1577	0.055 ± 0.005	0.053 ± 0.010

[a] Mean and 95% tolerance limits. [b] Mean and standard deviation of mean for six determinations.

The arsenic concentrations found are in agreement with the certified values. The optimum conditions used to obtain these results involved refluxing 0.250 g of the dry sample with 1 cm^3 98% sulfuric acid, 3 cm^3 67% nitric acid and 3 cm^3 30% hydrogen peroxide for 3 h. In the case of wet samples, a 1 g sample was used and the volume of H_2SO_4, HNO_3 and H_2O_2 was maintained at 1, 3 and 3 cm^3, respectively. These volumes were chosen because they gave identical results from a 0.250 g freeze-dried Manila clam sample and a 1.000 g wet sample. In cases where sample sizes larger than 1 g were used, the reagent ratios were adjusted proportionally. Generally, for the marine organisms, the ratio of H_2SO_4:HNO_3:H_2O_2 used was 1:3:3 cm^3 g^{-1} wet tissue.

As a further test of the efficiency of the digestion procedure, the results of the analysis of dried Manila clam tissue could be compared with that obtained by neutron activation analysis. Identical results of 6 $\mu g\ g^{-1}$ were obtained. Neutron activation analyses were performed by Novatrak Analysis Ltd, University of British Columbia, Vancouver, Canada.

In the course of optimizing the digestion procedure, it was found that losses occur when reflux is carried out for longer periods. Refluxing for 5 h gives an arsenic concentration of 9.8 ± 1.6 $\mu g\ g^{-1}$ for DORM-1, representing recovery of only 56%; a recovery of 50% is obtained after refluxing for 6 h. These results are probably due to volatilization of the arsenic on prolonged heating. Shorter refluxing times, for example 1 h give incomplete digestion as evidenced by lower recoveries (less than 40%), and the presence of residue in the digestion flask.

The alternative method of sample decomposition utilized a microwave oven and a Parr pressure vessel. Following the manufacturer's recommendations, a maximum of 0.5 g dry material or 1.0 g wet material was used for all digestion. The recovery of arsenic from 0.5 g samples of DOLT-1 by using HG AA to analyze the solution after the digestion is shown in Table 2.

Table 2 Recovery of arsenic from DOLT-1[a]

Volume of reagent used					
HNO_3	H_2SO_4	HCl	H_2O_2	Time of radiation (s)	Recovery (%)
3.0	—	—	—	60	6.0
2.0	0.5	—	—	60	15.3
2.0	1.0	—	—	60	22.6
1.5	1.5	—	—	60	45.7
1.5	1.5	—	—	90	56.0
1.0	—	3.0	—	90	22.3
1.0	1.0	—	1.0	90	45.2

[a] Sample size 0.5 g.

Recovery is poor and a probable explanation for these low results is that the organoarsenicals present in the marine reference material are not decomposed by the various acid combinations to a form which is readily reduced by sodium tetrahydroborate $NaBH_4$ to allow detection by HG AA. (A similar situation was observed by Raptis *et al.*[17] who attempted to decompose fish solubles by refluxing with nitric acid.) More rigorous conditions are therefore necessary if HG AA techniques are to be used for subsequent analysis. This suggestion is supported by the increased recovery found when sulfuric acid is added to the mixture. The possibility of pressure build-up in the bomb leading to explosions led to abandoning the pursuit of more forcing conditions.

Analysis of the digestate with GF AA shows good recovery of arsenic in the two NRCC marine standards studied — DOLT-1 and DORM-1 (Table 3). This reinforces the suggestion that incomplete decomposition is the cause of the low recoveries found by using HG AA.

The recoveries shown in Table 3 were obtained by using 0.2 g of the dry material and 6 cm^3 of 69% nitric acid, and a microwave radiation time of 2 min. For routine analysis of the marine samples, 1 g of the homogenized wet material in 6 cm^3 of 69% nitric acid was irradiated for 2 min.

The UV–HG AA technique was used to analyze solutions such as chromatographic fractions containing arsenicals.[13]

Table 3 Recovery of arsenic in standard reference materials by microwave digestion followed by HG AA

Sample	Arsenic certified ($\mu g\ g^{-1}$)	Arsenic found ($\mu g\ g^{-1}$)
DOLT-1	17.7 ± 2.1	17.2 ± 0.7
DORM-1	10.1 ± 1.4	10.2 ± 0.5

Arsenic speciation in clams

The extraction and separation procedures outlined in Schemes 1–3 rely heavily on the work of others.[6,10,16,21–25] Particular note should be made of the use of a strong cation exchange resin for preliminary separation of the arsenicals into 'HCl', 'NH_4^+', and 'H_2O' fractions.

Soft tissues of the Manila clam *(Venurupis japonica)*, Native littleneck clam (*Protothaca staminea*), Soft-shelled clam *(Mya arenaria)*, Horse clam (*Schizathoerus nutalli*) and Butter clam (*Saxidomus giganteus*) were extracted. A summary of the weights and arsenic contents of the clam soft tissues and extracts is given in Table 4.

Extraction of the soft tissue removes 68% of the arsenic in the Manila clam, 81% in the Native littleneck

Table 4 Extraction of soft tissues of the Manila, Native littleneck, Soft-shelled, Horse and Butter clam

	Manila clam	Native littleneck clam	Soft-shelled clam	Horse clam	Butter clam
No. of clams pooled	66	21	4	2	5
Weight of soft tissue extracted (g)	168	165	134	198	522
Arsenic content of soft tissue (μg)	170	248	169	396	1200
Residue after methanol extraction (g)	27	25	17	42	73
Arsenic content of residue (μg)	49	47	29	168	460
Arsenic content of methanol extract (μg)	116	200	138	227	730
Total arsenic extracted (%)	68	81	79	57	60

clam, 79% in the Soft-shelled clam, 57% in the Horse clam, and 60% in the Butter clam. When the gum obtained after evaporation of methanol is dissolved in water and extracted with diethyl ether, 3% of the total arsenic in the Manila clam is ether-soluble whilst 4% of the total arsenic in the Native littleneck clam is extracted into ether. The percentage of the total arsenic extracted into ether for the Soft-shelled clam, Horse clam and Butter clam is 1%. These percentages of ether-soluble arsenic are comparable with those found by others in clam tissue,[26] Western rock lobster[24] and shell fish;[27] and higher amounts up to 17% are found in crabs.[16,18] No further work has been carried out on the ether extracts in this or other investigations.

The elution patterns of the water-soluble arsenic compounds in the five species of clams on Sephadex LH-20 (2.5 cm × 60 cm) are similar, eluting at retention volumes between 250 and 325 cm^3. Standard arsenobetaine, arsenocholine iodide, and tetramethylarsonium iodide elute from the column with similar retention volumes between 225 and 325 cm^3, so the arsenicals in the clams do not seem to be strongly associated with other molecules in the extracts.

The arsenic-containing fractions from the LH-20 column were chromatographed on Dowex 50W × 8; elution with water, 5% ammonium hydroxide, and 2% hydrochloric acid results in varying concentrations of arsenic in the fractions (Table 5). The Soft-shelled clam and the Butter clam show the highest concentration of arsenic in the ammonium hydroxide fraction, whereas the Horse clam, the Manila clam and the Native littleneck clam contain higher amounts of arsenic in the HCl fraction. The characterization of the arsenic compounds in the fractions is discussed in the following sections.

Table 5 Fractionation of water-soluble arsenic in the Manila, Native littleneck, Soft-shelled, and Butter clam on Dowex 50W × 8

Clam	Arsenic placed on column (μg)	Arsenic in fractions (μg)[a] Unabsorbed	 NH_4OH	 HCl
Manila	100	9 (7)	22 (13)	68 (48)
Native littleneck	150	6 (3)	29 (13)	99 (48)
Soft-shelled	100	20 (10)	46 (38)	26 (20)
Horse	150	13 (6)	30 (12)	99 (39)
Butter	250	7 (2)	100 (24)	88 (21)

[a] The ratio (%) to the total arsenic concentration is given in parentheses.

Identification of arsenic compounds in the ammonium hydroxide fractions

When subjected to gel-permeation chromatography on a smaller Sephadex LH-20 column (2.5 cm × 30 cm) with water as eluant, only fractions with retention volumes of 120 and 140 cm^3 contain arsenic. Standard arsenobetaine, a compound which is commonly recovered in the ammonium hydroxide fraction,[10] elutes with a similar retention volume. The fractions, when combined and evaporated, yield light-brown syrups. Chromatography of these syrups on a Dowex 2 × 8 column (elution with water followed by 0.2 mol dm^{-3} hydrochloric acid) yields two sets of arsenic-containing fractions for the Butter clam extract and one set of arsenic-containing fractions for the other clams. The fractions with retention volume 37.5–62.5 cm^3 contain 90% of the arsenic in the Butter clam extract placed on the column whilst 4% of the arsenic is contained in the fraction with volume 112.5–125 cm^3. The fractions with retention volume 37.5–50.0 cm^3 contain all the arsenic for the other clams. For brevity, the identification of the arsenic compounds in the Butter clam extract is described as an example. The two sets of arsenic-containing fractions are termed 'major' (90%) and 'minor' (4%).

Three spots are present in the thin-layer chromatogram of the major fraction. Only the spot with R_f 0.52 contains arsenic. The R_f of standard arsenobetaine under the same conditions is also 0.52 (silica-gel plate, ethanol–acetic acid–water, 65:1:34 by vol.). Extraction of the arsenic-containing spot with methanol and evaporation of the methanol yields a residue. The NMR spectrum of a D_2O solution of this residue shows peaks at δ1.86, 3.22, 3.85, 4.32 and 4.70 ppm. This spectrum corresponds to that of a mixture of arsenobetaine and betaine. Further purification of the residue can be achieved by HPLC on a Protein-Pak column with 5 mmol dm^{-3} ammonium acetate buffer as eluant.[11] The arsenic compound elutes with the same retention time, 2.5–3.5 min, as synthetic arsenobetaine. Evaporation of the solvent yields a residue. The NMR of a D_20 solution of this residue shows singlets at 1.8 (9H), 3.2 (2H), and 4.8 ppm (HOD) and is identical with that of arsenobetaine.

To obtain further evidence that the arsenic compound is arsenobetaine, it was analyzed by mass spectrometry. The FAB mass spectrum shows peaks at *m/z* 105 $[(CH_3)_2As]^+$, 120 $[(CH_3)_3As]^+$, 134 $(M - CO_2)^+$, 135 $[(CH_3)_4As]^+$, 149 $(M - CO_2 + CH_3)^+$, 179 $(M + H)^+$ and 357 (2M + 1), among other peaks. This corresponds to the well-documented FAB spectrum of arsenobetaine.[5,6,28] The thermospray LC mass spectrum includes peaks at *m/z* 179 $(M + H)^+$, 193 $(M + CH_3)^+$, 214 $(M + H_2O + NH_4)^+$ and 329. Thus the arsenic compound in the major fraction is well identified as arsenobetaine.

The arsenic compound in the other clams (Horse clam, Manila clam, Native littleneck clam and Soft-shelled clam) is characterized as arsenobetaine on the basis of similar evidence.

As mentioned in the introduction arsenobetaine is found in a wide variety of marine invertebrates and fish. The finding of arsenobetaine in the clams reported in this study goes to confirm the virtual ubiquity of this organoarsenic compound in marine animals.

The thin-layer chromatrogram of the minor fraction of the Butter clam extracts show one arsenic-containing spot, R_f 0.68. This does not correspond to the R_f value of any of the standard arsenic compounds used (arsenocholine 0.30, tetramethylarsonium iodide 0.38, and arsenobetaine 0.52). The NMR spectrum in D_2O shows a singlet at δ1.8 ppm characteristic of a methyl group attached to arsenic. This compound is not a tetramethylarsonium salt because it is known that tetramethylarsonium salts are recovered in the HCl fraction after chromatography on Dowex 50.[10] Trimethylarsine oxide is recovered in the NH_4OH fraction after chromatography on Dowex 50[5] and has an NMR spectrum with a singlet at 1.77 ppm with respect to external sodium 2,2-dimethyl-2-silapentane-5-sulfonate.[29] Thus it is more likely that the arsenic compound in the minor fraction is trimethylarsine oxide. The thermospray LC mass spectrum of this fraction supports this contention with peaks at *m/z* 137 $(M + H)^+$, 155 $(M + H + H_2O)^+$, 177 $(M + CH_3CO_2 - H_2O)^+$, 195 $(M + CH_3CO_2)^+$. Because of scarcity, further tests could not be performed.

Trimethylarsine oxide has previously been reported in fish; the concentration increased with storage. Trimethylarsine oxide also occurs as a natural component in estuary catfish[8] and accumulates in the tissues of fish as a result of oral administration of inorganic arsenic. It is suggested that the presence of this compound is dependent upon bacterial actions in the gut tract of the fish.[9] The presence of trimethylarsine oxide in Butter clams is the first reported instance of this compound in a marine bivalve and isolation and unequivocal identification is desirable. Unlike the Butter clam, no evidence for the presence of trimethylarsine oxide is found in the other four species of clams studied.

Identification of arsenic compounds in the hydrochloric acid fractions

Gel-permeation chromatography on Sephadex LH-20 (2.5 cm × 30 cm) affords one arsenic-containing peak between the elution volumes 120–160 cm^3. This is similar to the elution pattern of the compound in the ammonium hydroxide fraction, the main component of which is characterized as arsenobetaine, suggesting that the arsenic compound in HCl fraction is almost the same size as arsenobetaine. Extracts of all the five species of clams exhibit the same behaviour on LH-20.

Thin-layer chromatographic analyses show the purified HCl fractions from all five species of clams contain one major arsenic compound with R_f 0.38, which is identical with the R_f of standard tetramethylarsonium iodide. Preparative TLC and HPLC cleanup yields solid residues. The ^1H NMR spectra of the residues are identical with that of standard tetramethylarsonium iodide, i.e. a singlet at $\delta 4.8$ ppm due to $(CH_3)_4As^+$. The thermospray LC mass spectrum shows the characteristic peak at m/z 135 assigned to $(CH_3)_4As^+$. Thus the residues from the Butter clam, Horse clam, Manila clam, Native littleneck clam, and Soft-shelled clam are characterized as tetramethylarsonium salts.

The presence of tetramethylarsonium salts in a marine invertebrate, the clam *Meretrix lusoria,* was first reported by Shiomi *et al.* [10] The presence of this compound in all clams studied suggests that it may be more common in the marine environment than previously thought.[2,3] Perhaps it is formed by methylating trimethylarsine, *S*-adenosylmethionine being the likely source of Me^+ (Eqn [1]).[4]

$$Me_3As + Me^+ \rightarrow Me_4As^+ \qquad [1]$$

Arsenic compounds in the unadsorbed fractions

The thin-layer chromatogram of the concentrated unadsorbed fraction of the Manila clam shows three spots with R_f values of 0.34, 0.76 and 0.96. Only the spot with R_f 0.96 contains arsenic. Native littleneck shows three spots on TLC with R_f values of 0.78, 0.86 and 0.91; again, only one spot (R_f 0.91) contains arsenic. Butter clam extract has two spots on TLC with R_f values of 0.80 and 0.97; the spot with R_f 0.97 contains arsenic. Preparative TLC of the Butter clam extract yields about 1 mg of a light-brown residue, containing less than 1% arsenic. The thermospray LC mass spectrum of this residue is complex and further cleanup is necessary to aid characterization. Because of sample scarcity this was not possible in the present investigations. None of the arsenic compounds in the unadsorbed fractions in previous studies[10,30] has been identified to date, presumably for similar reasons.

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