Letters to the Editor

Speciation of Arsenic Compounds in Marine Life by High Performance Liquid Chromatography Combined with Inductively Coupled Argon Plasma Atomic Emission Spectrometry

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The high concentration of arsenic in marine life has stimulated interest in the chemical forms of arsenic. It is important to understand the cycling of the element in the environment and the toxicological implications of eating marine-derived food. Recent research works have revealed the presence of various methylated arsenic compounds which included arsenobetaine[1,2], arsenocholine[3], and arsenic-containing ribofuranosides.[4-6]

A simple and sensitive method for analyzing these materials is to use high performance liquid chromatography with inductively coupled argon plasma atomic emission spectrometric detection (HPLC-ICP). The basic ideas appeared in earlier reports.[1,2,7] However, the standards used in these reports were limited to five commercially available arsenic compounds: Arsenate, arsenite, methane arsonate, cacodylate and arsenobetaine. Hence there remained a difficulty in assigning peaks when the method was applied to marine biological samples. In the present paper we describe the separation of twelve arsenic compounds which had been identified or whose presence was suspected in the marine environment.

Experimental

Materials

Sodium arsenate (I), sodium arsenite (II), methane arsonic acid (III) and cacodylic acid (IV) were purchased from Wako Pure Chemical Ind., Japan. Arsenobetaine (VIII), Arsenocholine (VII), tetramethylarsonium iodide (VI) and trimethylarsine oxide (V) were synthesized according to the literature[1,3,8] using trimethylarsine (Alfa Products, USA). Four arsenic-containing ribofuranosides were isolated from brown algae, *Hizikia fusiforme* (XI)[9] and *Laminaria japonica* (IX, X, XII).[10]

HPLC

A JASCO HPLC apparatus (Trirotar-V) was used with three different columns: Gel permeation column (Asahipak GS-220, 7.6×500 mm), cation exchange column (Nucleosil SA, 4.6×250 mm) and anion exchange column (Nucleosil SB, 4.6×250 mm).

ICP

The HPLC effluent was directly connected to ICP (Seiko-Denshi JY-38) and arsenic emission was monitored at the wavelength of 193.8 nm. For the HPLC-ICP analysis, a fresh sample (1 g) was homogenized with methanol/water (50:50, 10 ml) and filtered. After repeated extraction, the extract was evaporated to dryness and re-dissolved in 5 ml of water. Then an aliquot of the solution was injected to the HPLC-ICP system.

Isolation and identification of tetramethylarsonium ion

Soft tissue of cockle (*Meretrix lusoria*, Japanese name "Hamaguri", 600 g) was extracted with methanol (2 l×2). The extract was evaporated, re-dissolved in water and subjected to cation exchange chromatography (Dowex 50W-X8), Sephadex G-10, CM Sephadex C-25, Sephadex G-10 and finally to gel permeation HPLC (Asahipak GS-220). ^{1}H-NMR was recorded on a JEOL JNM GX-400 FT-NMR spectrometer operating at 400 MHz.

Results and Discussion

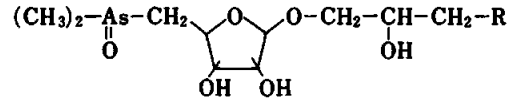
The retention times of twelve standard arsenic componuds, obtained by four different chromatographies, are shown in Table 1. It was difficult to separate all these compounds on single or double columns. The retention times of the compounds on the gel permeation column (LC-4) were not affected by the presence of electrolyte. On the contrary, the retention times in ion exchange chromatography sometimes showed slight changes; in these cases, samples were re-examined by co-injection with authentic standards.

Chromatograms of selected samples are shown in

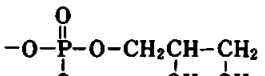
Table 1 Retention times of twelve arsenic compounds in four different liquid chromatographies

Compound[a]	Retention time[b]/min			
	LC-1	LC-2	LC-3	LC-4
(I)	2.9	3.3	11.0	11.1
(II)	4.1	4.2	4.8	18
(III)	2.9	3.3	5.3	11.5
(IV)	3.9	4.6	7.2	11.9
(V)	—[c]	6.0	5.0	13.1
(VI)	—	10.8	5.0	14.5
(VII)	—	9.6	3.8	14.8
(VIII)	9.8	4.6	4.3	12.8
(IX)	5.1	4.2	3.8	12.3
(X)	2.8	3.3	6.0	11.0
(XI)	2.9	3.6	3.8	11.4
(XII)	2.9	3.3	4.8	10.5

a. Compounds are as follows: (I) AsO_4^{3-}, (II) AsO_3^{3-}, (III) $CH_3AsO_3^{2-}$, (IV) $(CH_3)_2AsO_2^-$, (V) $(CH_3)_3AsO$, (VI) $(CH_3)_4As^+$, (VII) $(CH_3)_3As^+CH_2CH_2OH$, (VIII) $(CH_3)_3As^+CH_2COO^-$. Arseno-sugars,



R: -OH (IX), $-SO_3^-$ (X), $-OSO_3^-$ (XI)



(XII).

b. Chromatographic conditions: LC-1; Nucleosil SA (4.6×250 mm), sodium phosphate buffer (pH. 6.8, 50 mM), 1 ml/min. LC-2; Nucleosil SA (4.6×250 mm), sodium phosphate buffer (pH 6.8, 37.5 mM)+tetramethylammonium chloride 0.67 mM, 1 ml/min. LC-3; Nucleosil SB (4.6×250 mm), sodium phosphate buffer (pH 6.8, 50 mM), 1 ml/min. LC-4; Asahipak GS-220 (7.6×500 mm), sodium phosphate buffer (pH 6.8, 50 mM), 1 ml/min.
c. Not eluted.

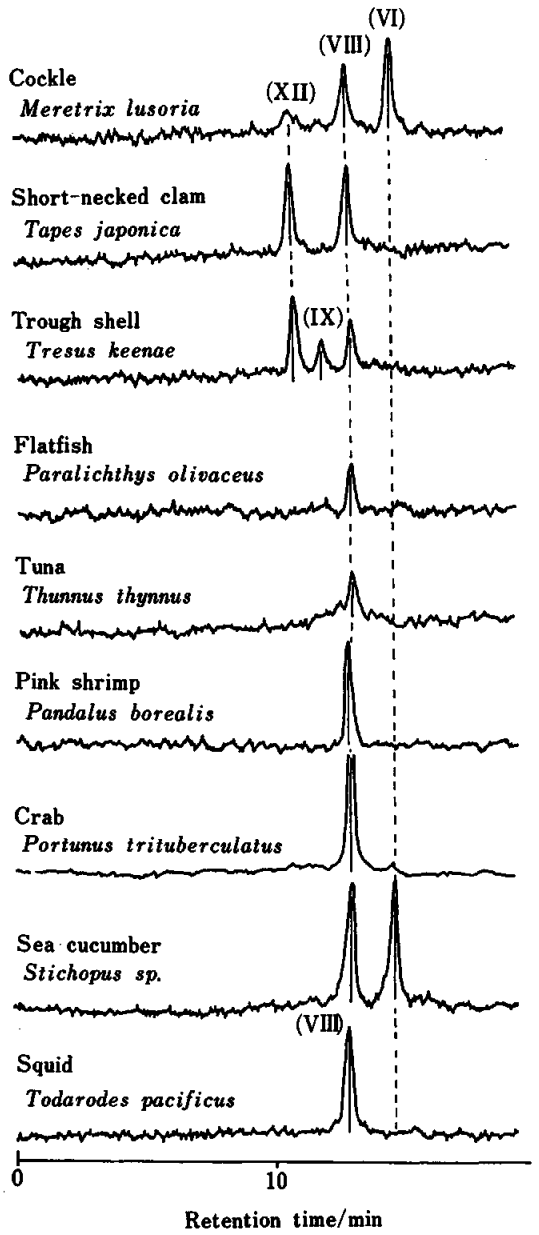


Fig. 1 Chromatograms of the extracts of marine animals on Asahipak GS-220 (LC-4). See Table 1 for column conditions.

Fig. 1. Arsenobetaine (VIII) was present in all marine animals examined. In flatfish, tuna, shrimp and squid, it was the only arsenical detected. It is noteworthy that no arsenocholine (VII) was detected in the shrimp despite a previous report of its presence.[3] Our results are in accord with those of Penrose[11] and Lunde[12] who both reported a single arsenic compound in the shrimp, *Pandalus borealis*. Arsenobetaine was one of the major arsenic components in sea cucumber, cockle, short-necked clam and trough shell.

One of the arsenic-containing ribofuranosides (XII) was detected in cockle, snail, trough shell, oyster, short-necked clam and abalone tissues, perhaps reflecting their habit of feeding on phytoplanktons and algae. A tentative identification of another arsenic-containing ribofuranoside (IX) in abalone and trough shell was also made.

The cockle contained a strongly basic arsenic compound, which was identified as tetramethylarsonium ion (VI) by its HPLC retention times. This identification was confirmed by the ^{1}H-NMR on the purified compound (broad singlet methyl signal at 1.90 ppm relative to DSS). Tetramethylarsonium ion was also found in the crab and sea cucumber. This compound, which represents the end product of arsenic methylation, has not previously been reported from an animal source.

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