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Arsenic compounds in marine sponge (*Haliclona permolis, Halichondria japonica, Halichondria okadai* and *Haliclona* sp. white) from Seto Inland Sea, Japan†

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Marine sponges were collected at Hiro or Hatami, Hiroshima Prefecture, Aki Nada in the Seto Inland Sea, Japan. Arsenic compounds were extracted with methanol/water (1:1, v/v) from freeze-dried samples of the marine sponges *Haliclona permolis, Halichondria japonica, Halichondria okadai* and *Haliclona* sp. white. The extracts were analyzed by high-performance liquid chromatography, with an inductively coupled plasma mass spectrometer serving as an arsenic-specific detector. Arsenobetaine 5 and the two arsenosugars as 6 and 7 were identified in all samples targeted, and these three compounds accounted for 74 to 96% of the water-soluble arsenic in all four species of sponge. Arsenosugar was the major arsenic species in all four species of sponge. The most abundant arsenic compound in all four species of sponge was arsenosugar 7, and arsenosugar 6 was only a minor component. The arsenobetaine and arsenosugar concentrations were considerably higher in the three sponges from demospongiae (*H. permolis, H. japonica* and *H. okadai*) compared with the single species from calcispongiae (*Haliclona* sp. white).

Keywords: sponge; Seto Inland Sea, Japan; arsenobetaine; arsenosugars; HPLC–ICP/MS

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INTRODUCTION

Marine organisms are more arsenic rich than sea water. Marine animals contain high concentrations of arsenobetaine[1] and generally smaller amounts of arsenosugar,[2,3] and tetramethylarsonium ion.[5] Arsenocholine[4] is almost a trace constitutent. However, previous studies have focused on arsenic in higher animals. Not only for a better understanding of the arsenic cycle in the marine ecosystem, but also from the viewpoint of comparative biochemistry, it is important to elucidate the chemical structure of arsenic compounds contained in taxonomically lower animals.

Arsenobetaine [$(CH_3)_3As^+CH_2COO^-$, Fig. 1] was found to be the major arsenic species in three species of sponges (*Halichondria okadai, Halichondria japonica, Spirastrella insignis*) by using high performance liquid chromatography–inductively coupled mass spectrometry (HPLC–ICP) analysis.[1] Recently, arsenosugar was found to be the major arsenic species in sponges from Philippines.[6] However, information on arsenic species in sponges is still limited.

The present paper deals with the chemical forms of arsenic in four species of marine sponges (the demospongiae *Haliclona permolis, H. japonica, H. okadai* and the calcispongiae *Halichlona* sp. white) from the Seto Inland Sea, Japan, by using HPLC–ICP/MS.

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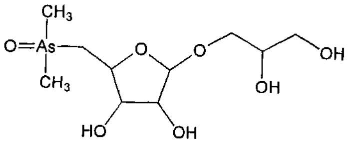
(1) Arsenite AsO_3^{3-} **(2)** Arsenate AsO_4^{3-}

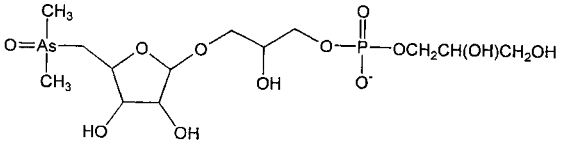
(3) Monomethyl arsonate $CH_3AsO_3^{2-}$

(4) Dimethyl arsinate $(CH_3)_2AsO_2^-$

(5) Arsenobetaine $(CH_3)_3As^+CH_2COO^-$



(6) Arsenosugar



(7) Arsenosugar

Figure 1 Arsenic standards used in the present study.

MATERIALS AND METHODS

The marine sponges (*H. permolis, H. japonica, H. okadai* and *Haliclona* sp. white) were collected near Hiro and Hatami in the Aki Nada, Seto Inland Sea, Japan, during August 1998. The whole sponge body (whole wet weights of *H. permolis, H. japonica, H. okadai* and *Haliclona* sp. white 10 g, 13 g, 15 g and 12 g respectively) were kept frozen at −20 °C until analysis. The sponge samples were freeze-dried and ground to a powder. Each of the freeze-dried sponge samples (50 to 100 mg dry weight) was weighed into a centrifuge tube. To each tube was added 5 ml of methanol/water (1:1, v/v), and the tube was sonicated for 10 min. After centrifugation (2000 rpm for 10 min), the extract was removed by a Pasteur pipette. The extraction process was repeated five times for each sample; the extracts were combined evaporated to dryness and dissolved in 2 ml of water. The solution was filtered through a 0.5 µm disposable filter unit (Millipore Columngard-LCR13), and an aliquot of the solution (25 µl) was injected into the HPLC–ICP/MS system (Yokogawa HP 4500 type, Japan).

HPLC–ICP/MS analysis was conducted as reported previously[7] using an Inertsil ODS column (4.6 × 250 mm^2; GL Science, Japan). Arsenic compounds were eluted with 10 mM tetraethylammonium hydroxide–4.5 mM malonic acid–0.05% methanol (pH 6.8).

Quantification was performed by comparing the peak area of each compound with that of a known concentration of arsenic compounds standard. Interference from Cl ($^{40}Ar^{35}Cl^+$ shows the same *m/z* 75 as $^{75}As^+$) was detected by monitoring ion counts at *m/z* 77 (corresponding to $^{40}Ar^{37}Cl^+$) simultaneously. Water-soluble arsenic concentration represents the sum of all arsenic species obtained by HPLC.

The water-soluble arsenic compounds used as standards [Fig. 1, arsenobetaine (**5**), and arsenosugars (**6**), and (**7**)] were prepared as reported previously.[7]

RESULTS AND DISCUSSION

The water-soluble arsenic concentrations from *H. permolis, H. japonica, H. okadai* and *Haliclona* sp. white are shown in Table 1. Water-soluble arsenic concentrations were relatively low (less than 13 µg per gram dry weight) in all four species of sponge.

Table 1 Arsenic species in marine sponges from Seto Inland Sea, Japan[a]

	Arsenic concentration (µg/g dry weight)				
Species	Water-soluble arsenic[b]	Arsenobetaine	Arsenosugar (**6**)	Arsenosugar (**7**)	Other compounds
H. permolis	13.0 (100)	3.34 (26)	1.12 (9)	8.06 (61)	0.52 (4)
H. japonica	3.42 (100)	1.10 (32)	0.15 (4)	1.39 (41)	0.78 (23)
H. okadai	5.50 (100)	0.53 (10)	0.28 (5)	4.22 (76)	0.47 (9)
Haliclona sp. white	0.81 (100)	0.15 (19)	0.05 (6)	0.40 (49)	0.21 (26)

[a] Values in parentheses are percentages.
[b] Water-soluble arsenic represents the sum of all arsenic species obtained by HPLC.

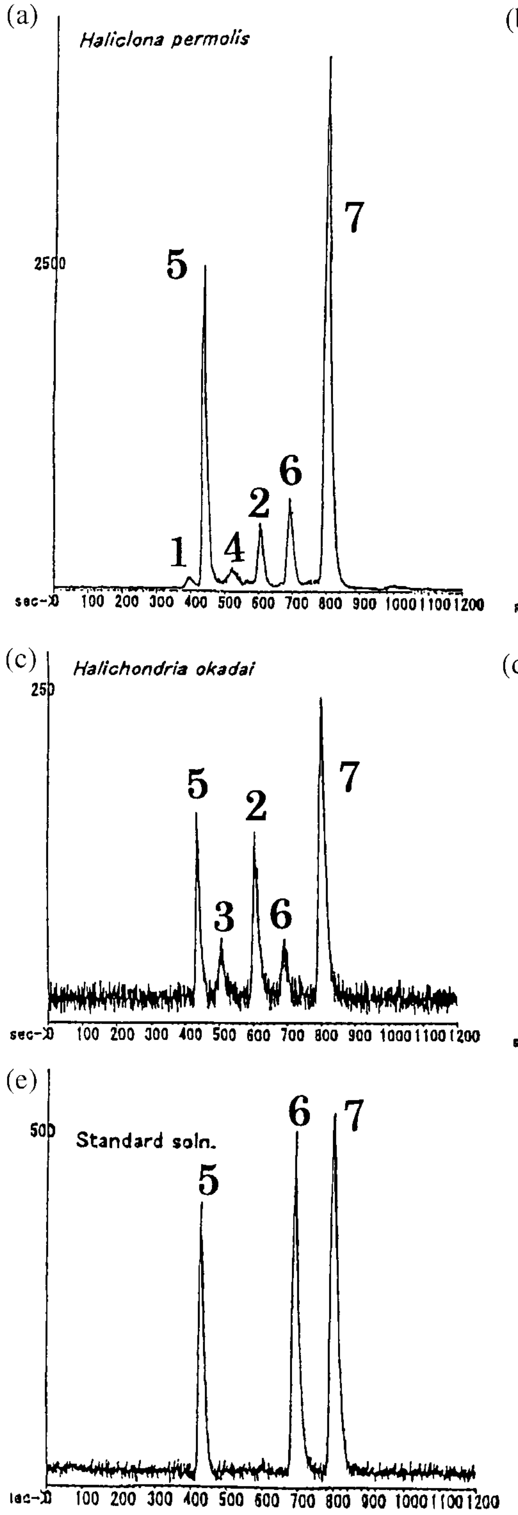


Figure 2 HPLC–ICP/MS chromatograms ($m/z = 75$) of the sponge extracts. Column, Inertsil ODS; buffer, 10 mM tetraethylammonium hydroxide–4.5 mM malonic acid–0.05% methanol (pH 6.8); flow rate, 0.75 ml min^{-1}; 25 µl of each extract was injected. (a) *H. permolis*, (b) *H. japonica*, (c) *H. okadai*, (d) *Haliclona* sp. white, (e) standard solution. The peaks are labeled as follows: (1) arsenite; (2) arsenate; (3) MMAA; (4) DMAA; (5) arsenobetaine; (6) arsenosgar (**6**); (7) arsenosugar (**7**).

It was found that the order of water-soluble arsenic concentrations in sponges was *H. permolis* > *H. okadai* > *H. japonica* > *Haliclona* sp. white. The results suggested that the higher levels may just be associated with symbionts in the sponges. Chromatograms of the HPLC–ICP/MS analysis of four sponge species are shown in Figure 2. The sponges clearly show the presence of five dominant arsenic compounds in the extract. The major water-soluble arsenic species in all four species of sponge were identified as arsenobetaine (**5**) and arsenosugar derivatives (**6** and **7**) by comparison with the standards. There are several other weak peaks in the chromatogram, and they were identified as arsenate (**2**), arsenite (**1**), monomethylarsonic acid (MMAA, **3**) and dimethylarsinic acid (DMAA, **4**).

Arsenobetaine was found to be the major arsenic species in sponges (*H. okadai, H. japonica* and *S. insignis*) from Japanese coastal areas using HPLC–ICP analysis.[1] However, the authors did not detect significant amounts of arsenosugar in sponges. Our findings, on the contrary, show that sponges from the Seto Inland Sea, Japan, contain arsenosugar as major water-soluble arsenic compounds. Recently, Yamaoka *et al.*[6] detected arsenosugar in a sponges from the Philippines, using HPLC–ICP/MS.

The extracts of various sponges (*H. permolis, H. japonica, H. okadai* and *Haliclona* sp. white) were analyzed in the same manner as above, and the results are summarized in Table 1. Water-soluble arsenic concentration represents the sum of all arsenic species obtained by HPLC. As shown in Table 1, *H. permolis, H. japonica, H. okadai* and *Haliclona* sp. white contained two arsenosugar (**6** and **7**) as major water-soluble arsenic compounds.

In all four samples, arsenobetaine was detected (Table 1). In accordance with the reports by Shiomi *et al.*[1] and Yamaoka *et al.*,[6] *H. okadai, H. japonica, S. insignis, Haliclona* sp., *Jaspis* sp. and *Subertes* sp. were found to accumulate the highest amounts of arsenobetaine. Arsenobetaine has been widely found as a major arsenic compound in marine higher animals. It was found that the order of arsenobetaine concentrations in sponges was *H. permolis* > *H. japonica* > *H. okadai* > *Haliclona* sp. white.

On the other hand, arsenosugar derivatives (the two arsenosugars as **6** and **7**) were detected in *H. permolis, H. japonica, H. okadai* and *Haliclona* sp. white. The two arsenosugars **6** and **7** were at low concentrations in the calcispongiae *Haliclona* sp. white. The above difference in arsenosugars might therefore be attributable to sponge species differences.

Edmonds and Francesconi[8] isolated and identified arsenic-containing ribofuranosides (so-called arsenosugars: **6** and **7** in Fig. 1) from the brown alga *Ecklonia radiata*. Also, arsenosugars were identified in brown alga,[9] the green alga,[9] red alga,[9] zooplankton and phytoplankton.[10]

The ratio between arsenosugars and arsenobetaine is higher in *H. permolis, H. okadai* and *Haliclona* sp. white than in *H. japonica*. Also, the concentration of arsenobetaine and the two arsenosugar **6** and **7** in the calcispongiae *Haliclona* sp. white was much lower than in the other sponges. It was found that the order of arsenosugar concentrations in sponges from the Seto Inland Sea, Japan, was **7** > **6**. The different proportions of arsenosugars may reflect the different symbionts in the various sponges examined. The meaning of this specific character is not clear at this stage. From the standpoint of metabolism and cycling of arsenic in the marine food web, it is especially interesting that a fairly large amount of the unknown compound was detected in sponges that feed on phytoplankton-containing arsenosugar derivatives.

On the other hand, sponges are known to contain symbiotic cyanobacteria, which were attributed as the source of the arsenosugar in the sponges. Clearly, more information is necessary to obtain a general view of arsenic compounds in marine sponges. In conclusion, it has been shown in the present study that sponges from Japan and the Philippines contain arsenobetaine and the two arsenosugars **6** and **7** as major water-soluble arsenic compounds, and that arsenic concentrations in the demospongiae are higher than in the calcinopongiae.

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REFERENCES

1. Shiomi K, Aoyama M, Yamanaka H, Kikuchi T. *Comp. Biochem. Physiol.* 1988; **99C**: 361.
2. Edmonds JS, Francesconi KA, Healy PC, White AH. *J. Chem. Soc. Perkin Trans. 1* 1982; 2989.
3. Shibata Y, Morita M. *Appl. Organomet. Chem.* 1992; **6**: 343.
4. Norin H, Ryhage R, Christakopoulous A, Sandstrom M. *Chemosphere* 1983; 129.
5. Shiomi K, Kakehashi Y, Yamanaka H, Kikuchi T. *Appl. Organomet. Chem.* 1987; **1**: 177.
6. Yamaoka Y, Carmona ML, Oclarite JM, Jin K, Shibata Y. *Mar. Biotechnol.* 1999; in preparation.
7. Shibata Y, Morita M. *Anal. Sci.* 1989; **5**: 107.

8. Edmonds JS, Francesconi KA. *J. Chem. Soc. Perkin Trans. 1* 1983; 2375.
9. Shibata Y, Jin K, Morita M. *Appl. Organomet. Chem.* 1990; **4**: 255.
10. Shibata Y, Sekiguchi M, Otsuki A, Morita M. *Appl. Organomet. Chem.* 1996; **10**: 713.