APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **13**, 95–99 (1999)

Occurrence of a Few Organo-arsenicals in Jellyfish

Ken'ichi Hanaoka,[1]* Walter Goessler,[2] Toshikazu Kaise,[3] Hirokazu Ohno,[1] Yukiko Nakatani,[1] Shunshirow Ueno,[1] Doris Kuehnelt,[2] Claudia Schlagenhaufen[2] and Kurt J. Irgolic[2]

[1]Department of Food Science and Technology, National Fisheries University, Nagata-honmachi 2-7-1, Shimonoseki 759-6595, Japan

[2]Institute for Analytical Chemistry, Karl-Franzens-University Graz, Universitätsplatz 1, A-8010 Graz, Austria

[3]Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji City, Tokyo 192-0355, Japan

Water-soluble fractions containing arsenic compounds were extracted with chloroform–methanol (2:1) from two kinds of jellyfish, *Aurelia aurita* and *Carybdea rastonii*. After defatting, each water-soluble fraction was subjected to analysis by HPLC–GFAA (column: ODS 120-T) and HPLC–ICP MS (column: Supelcosil LC-SCX) for arsenicals. Arsenobetaine was detected with both analytical systems as the major arsenic compound. Besides arsenobetaine, the tetramethylarsonium ion and arsenocholine were also detected by HPLC–ICP MS. The major arsenical in each jellyfish purified by ion-exchange chromatography using Dowex 50W $\times$ 8 (H^+ form) and Dowex 50W $\times$ 8 (pyridinium form) was confirmed to be arsenobetaine by thin-layer chromatography.

Keywords: arsenobetaine; tetramethylarsonium ion; arsenocholine; jellyfish; HPLC–ICP MS

Received 26 January 1998; accepted 11 June 1998

INTRODUCTION

Arsenobetaine was the first naturally occurring organoarsenical to be isolated from a marine animal (western rock lobster).[1] Since then, this compound has been shown to occur in marine organisms including blue shark[2] (Chondrichthyes), school whiting[3] (Osteichthyes), sea cucumber[4] (Mollusca) and various kinds of zooplankton.[5] At present, arsenobetaine is accepted to be present ubiquitously in marine animals, independently of their feeding habits and trophic level.[6–11] In animals of the highest trophic levels, arsenobetaine accounts for almost all of the water-soluble arsenic compounds.

We are interested in jellyfish because they may play an important role in the circulation of substances in marine ecosystems: they secrete mucus to capture suspended matter around them and allow the mucus blobs formed in seawater to sink to the bottom. This phenomenon promotes the circulation of substances including arsenic and means that arsenicals occurring in the suspended matter and the mucus itself are always supplied to detritovores on the bottom.

As the first step in investigating the contribution of jellyfish to the circulation of arsenic in marine ecosystems, the chemical form of the water-soluble arsenic compounds extracted from jellyfish were investigated. Two species of jellyfishes, *Aurelia aurita* and *Carybdea rastonii*, were chosen as the samples because of their frequent appearance along seashores in Japan. Furthermore, they are a little different in their feeding habits: while *A. aurita* feeds mainly on zooplankton, *C. rastoni* feeds on fry and small fish in addition to zooplankton. In other words, *C. rastoni* belongs to a trophic level analogous to carnivora. This means that *C. rastoni* will take arsenobetaine up at a higher rate through its food. In this study, the arsenic compounds occurring in the two species of jellyfish were investigated to elucidate their chemical structure and to clarify the effect of feeding habits on the relative abundance of the different structures.

* Correspondence to: K. Hanaoka, Department of Food Science and Technology, National Fisheries University, Nagata-honmachi 2-7-1, Shimonoseki 759-65, Japan.

 CCC 0268–2605/99/020095–05 $17.50

MATERIALS AND METHODS

Jellyfish

Nine *Aurelia aurita* (a total of 1371 g wet weight) and eight *Carybdea rastonii* (a total of 77 g wet weight) were collected from the coastal waters of Yoshimi in front of the National Fisheries University.

Arsenic content

After samples had been digested with nitric, sulfuric and perchloric acids, their arsenic content indicated in Table 1 was determined by arsine evolution–electrothermal atomic absorption spectrometry as described previously.[12]

Extraction and purification of arsenic compounds

Each jellyfish was extracted twice with 10 times its volume of chloroform–methanol (2:1); water was then added to the extract to reach a water/chloroform–methanol ratio of 1:4, and the mixture was shaken for 2 min and stored overnight.[13] The major arsenic compound extracted in the water-soluble arsenic fraction (upper phase) from each jellyfish was purified by ion-exchange chromatography: the water-soluble arsenic fraction was placed on a cation-exchange resin Dowex 50W × 8 (50–100 mesh, H^+ form) column (2.0 cm × 11.0 cm, 35 cm^3) and eluted with 180 cm^3 of water, 180 cm^3 of 2.0 mol dm^{-3} pyridine and 180 cm^3 of 1.0 mol dm^{-3} HCl, successively. The pyridine fraction, in which a large proportion of the arsenic was eluted, was then chromatographed with Dowex 50W × 2 cation-exchange resin (200–400 mesh, pyridinium form) using a 1cm × 50 cm column equilibrated with 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1). The sample was then successively eluted with 200 cm^3 of the same buffer, 200 cm^3 of 0.1 mol dm^{-3} pyridine and 200 cm^3 of 1.0 mol dm^{-3} HCl.

Table 1 Total, water-soluble, lipid-soluble and residual arsenic content in *A. aurita* and *C. rastoni*

	Arsenic content (μg g^{-1} wet weight)			
Species	Total	Water-soluble	Lipid-soluble	Residual
A. aurita	0.039	0.032	0.006	0.001
C. rastoni	0.135	0.117	0.010	0.005

High-performance liquid chromatography–graphite furnace-atomic absorption spectrometry (HPLC–GFAA)

A Tosoh CCP 8000-series chromatograph was used for the chromatographic separation of arsenicals using an ODS 120T column (4.6 mm × 250 mm; Tosoh Co. Ltd) with a mobile phase of 11.2 mmol dm^{-3} sodium heptanesulfonate in water–acetonitrile–acetic acid (95:5:6, by vol.; flow rate, 0.8 cm^3 min^{-1}; sample size, 5 mm^3).[14] Fractions were collected for 25 s and injected into the graphite-furnace atomic absorption spectrometer and analyzed as described previously. A mixture of the authentic arsenic compounds (all with 100 mg As dm^{-3}) was also fractionated.

High-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP MS)

A Hewlett-Packard 1050 solvent delivery unit and a 100-μl injection loop of a Rheodyne six-port injection valve was used. The arsenic compounds were separated at a flow rate of 1.5 cm^3 min^{-1} on a Supelcosil LC-SCX anion-exchange column (250 mm × 4.6 mm i.d.) with a pyridine–formic acid buffer (pH 3.1). The exit of the column was connected to a hydraulic high-pressure nebulizer HHPN (Knauer, Berlin, Germany) via 30 cm of 1/16 in. PEEK capillary tubing (0.25 mm i.d). A VG Plasma Quad 2 Turbo Plus (VG Elemental, Winsford, UK) inductively coupled plasma mass spectrometer (ICP MS) served as an arsenic-specific detector.

Confirmation of the metabolite

The major arsenic compound purified by ion-exchange chromatography was subjected to thin-layer chromatography performed on a cellulose thin layer (Avicel SF, thickness 0.1 mm; Funakoshi Yakuhin Co., Ltd.). In order to confirm the position of the purified arsenic compound, the cellulose thin layer was removed at 5-mm intervals. Each of the removed samples was added to a portion of 20% ethanol, mixed with a vortex mixer for 20 s and analyzed by graphite-furnace atomic absorption spectrometry. Dragendorff reagent was used to authenticate synthetic arsenobetaine (Trichemical Co. Ltd).

Table 2 R_f values of the purified arsenic compounds from *A. aurita* and *C. rastoni*

	R_f		
Solvent system	*A. aurita*	*C. rastoni*	Arsenobetaine
Ethyl acetate–acetic acid–water (3:2:1)	0.85	0.87	0.86
Chloroform–methanol–28% aq. ammonia (2:2:1)	0.81	0.80	0.82
1-Butanol–acetone–formic acid–water (10:10:2:5)	0.69	0.70	0.70
1-Butanol–acetone–28% aq. ammonia–water (10:10:2:5)	0.45	0.43	0.45
1-Butanol–acetic acid–water (4:2:1)	0.72	0.73	0.74

RESULTS

Arsenic content

Total, water-soluble, lipid-soluble and residual arsenic contents of each jellyfish are shown in Table 1. The total arsenic in *A. aurita* (0.039 μg g^{-1} wet weight) and *C. rastonii* (0.135 μg g^{-1} wet weight), especially in the former, was lower than in other marine animals. The major part of the total arsenic, 82% (*A. aurita*) or 87% (*C. rastoni*), was water-soluble. A few percent of the residual arsenic could not be extracted with chloroform–methanol in both jellyfish.

HPLC–GF AA and HPLC–ICP MS

The water-soluble arsenic fraction from each jellyfish was analyzed by HPLC–GFAA with the authentic arsenic compounds [retention times (RT), s: As(III) 225–300; As(V) 150–225; methanearsonic acid 225–300; dimethylarsinic acid 325–400: arsenobetaine 525–625; trimethylarsine oxide 725–850; tetramethylarsonium ion 1125–1275]. A single arsenic peak whose RT agreed with that of arsenobetaine was detected in each fraction. In addition, 95.9% (*A. aurita*) or 91.8% (*C. rastoni*) of the total arsenic in the water-soluble fraction injected was recovered in each eluate from the ODS column, indicating that arsenobetaine is the major arsenical in the water-soluble fraction. One major arsenic peak was also detected in both jellyfish by HPLC–ICP MS (Fig. 1). The RT of this peak agreed with that of arsenobetaine and an arsenosugar, 5′-dimethylarsinoyl-5′-deoxyribosylglycerol, although the latter compound was not authenticated in the present study. In addition, a few small peaks were found for *C. rastoni*, and the RT of one agreed with that of the tetramethylarsonium ion. Several small peaks were found for *A. aurita* and the RTs of two of these agreed with those of the tetramethylarsonium ion and arsenocholine.

Purification of the major arsenic compound

The major arsenic compound extracted from each of *A. aurita* and *C. rastonii* was purified by ion-exchange chromatography using Dowex 50W × 8 (H^+ form) and Dowex 50W × 2 (pyridinium form). The arsenic compound was eluted from Dowex 50W × 8 with a pyridine solution and from Dowex 50W × 2 with pyridine–formic acid buffer (pH 3.1). The purified arsenic compound was chromatographed on cellulose thin layers together with the synthetic arsenobetaine. As shown in Table 2, the R_f value of the compound agreed with that of synthetic arsenobetaine in the five solvent systems tested.

From the results of HPLC–GFAA, HPLC–ICP MS and thin-layer chromatography, the purified compound was concluded to be arsenobetaine.

DISCUSSION

The fact that *C. rastonii* feeds on fry or small fish means that it belongs to a higher trophic level than *A. aurita*, which feeds mainly on zooplankton: the former may take arsenobetaine at a higher rate through its food than the latter. Almost all arsenic, however, accumulated as arsenobetaine in both species, regardless of the difference in feeding habits. To explain this phenomenon, one must take into account not only the pathway in their tissues that converts some arsenicals to arsenobetaine, but also the direct uptake of arsenobetaine from seawater. Gailer *et al.*[15] placed blue mussel (*Mytilus edulis*) in seawater spiked with arsenobetaine, and found that almost all the experimentally accumulated arsenic in the tissue was in the form of arsenobetaine. On the other hand, we recently showed the occurrence of arsenobetaine in micro-suspended substances collected from seawater that had been passed through a 5-μm plankton net.[16]

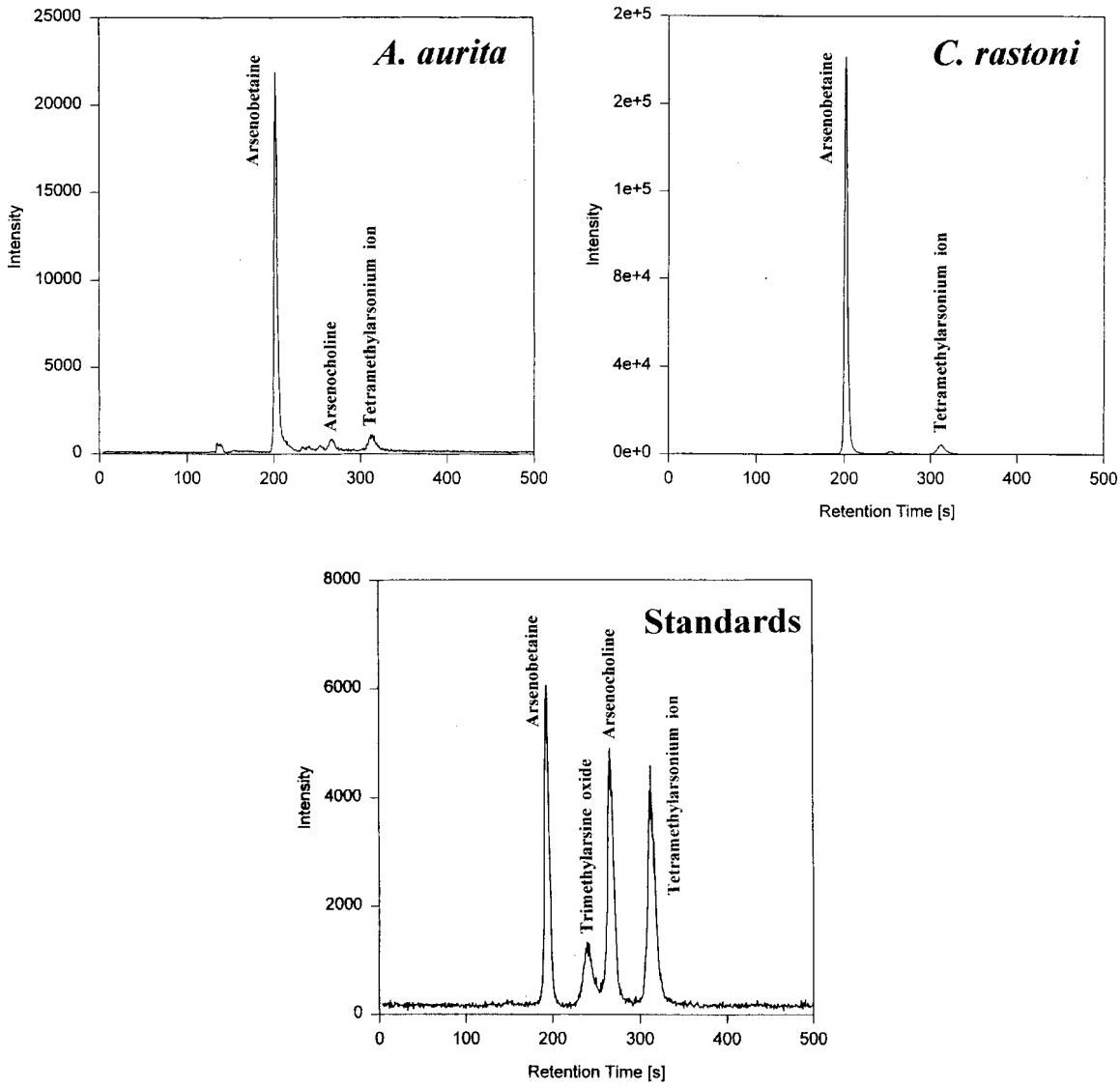

Figure 1 HPLC–ICP MS chromatogram of the water-soluble fraction containing arsenic compounds extracted from *A. aurita* and *C. rastoni*.

These results suggested that at least part of the arsenobetaine accumulated in the jellyfish was directly taken up from seawater. At the present stage, however, no definite conclusion can be reached concerning the origin of arsenobetaine accumulating in the jellyfish.

In both species, the tetramethylarsonium ion was also suggested by HPLC–ICP MS. This compound was first identified in the gills of a clam (*Meretrix lusorio*) by Shiomi *et al.*[17] and has been detected subsequently in animals by other groups as reviewed by Shiomi[11] and Francesconi and Edmonds[9]. Shiomi[11] suggested that the tetramethylarsonium ion occurs at a high frequency in lower marine animals. Although only two species were examined in this study, it is suggested that this compound is also distributed at a high frequency in jellyfish. Arsenic compounds occurring in another species of jellyfish are now under study.

Gailer *et al.*[15] also reported that *M. edulis* took up tetramethylarsonium ion in addition to arsenobetaine from seawater, and accumulated it in its tissues. On the other hand, the presence of a trace amount of the tetramethylarsonium ion in seawater

is expected from the fact that it is aerobically converted by sedimentary micro-organisms from methanearsonic acid and dimethylarsinic acid,[18] both of which are known to occur in seawater.[9] These experimental results raised the possibility that seawater is the origin of the tetramethylarsonium ion in the two jellyfish.

In *A. aurita*, arsenocholine was also suggested as a minor component. This compound is generally considered to be the precursor of arsenobetaine. It is reported to be converted to arsenobetaine in marine microorganisms,[19] shellfish,[15] fish[20] and terrestrial mammals.[21,22] The occurrence of arsenocholine was also suggested in microsuspended substances, as stated above.[16] This indicates the possible uptake of arsenocholine as well as arsenobetaine from seawater into the tissues of jellyfish, although why arsenocholine remains unchanged in *A. aurita* is not clear.

Jellyfish are generally not eaten by animals of higher trophic levels, and thus the arsenobetaine that accumulates in them may not be passed directly to these higher-level animals. Instead, it is expected that the arsenobetaine would be degraded in a multi-step process to inorganic arsenic by micro-organisms occurring in sediments,[12,23–25] suspended substances,[26] macroalgae,[27] seawater[28] etc. In other words, the arsenic may be circulating in a smaller ecosystem composed of seawater, plankton, small fish and jellyfish rather than in a general ecosystem that includes animals of all trophic levels.

Despite the fact that jellyfish are member of a limited ecosystem, they may play an important role in marine ecosystems as described above. When jellyfish tentacles come into contact with suspended matter, the jellyfish secrete mucus to capture the matter. The amorphous blobs that are formed then sink and may be consumed by detritovores when they reach the bottom. We are now investigating the arsenic compounds in the mucus of jellyfish.

REFERENCES

1. J. S. Edmonds and K. A. Francesconi, *Nature, (London)* **265**, 436 (1977).
2. S. Kurosawa, K. Yasuda, M. Taguchi, S. Yamazaki, S. Toda, M. Morita, T. Uehiro and K. Fuwa, *Agric. Biol. Chem.* **44**, 1993 (1980).
3. J. S. Edmonds and K. A. Francesconi, *Mar. Pollut. Bull.* **12**, 92 (1981).
4. K. Shiomi, A. Shinagawa, M. Azuma, H. Yamanaka and T. Kikuchi, *Comp. Biochem. Physiol.* **74C**, 393 (1983).
5. Y. Shibata, M. Sekiguchi, A. Otsuki and M. Morita, *Appl. Organometal. Chem.*, **10**, 713 (1996).
6. W. Maher and E. Batler, *Appl. Organometal. Chem.* **2**, 191 (1988).
7. J. S. Edmonds and K. A. Francesconi, *Appl. Organometal. Chem.*, **2**, 297 (1988).
8. K. Hanaoka, H. Yamamoto, K. Kawashima, S. Tagawa and T. Kaise, *Appl. Organometal. Chem.* **2**, 371 (1988).
9. K. A. Francesconi and J. S. Edmonds, Biotransformation of arsenic in the marine environment. In: *Arsenic in the Environment, Part I: Cycling and Characterization*, J. O. Nriagu (ed.), John Wiley, New York, 1994, pp. 221–261.
10. K. A. Francesconi and J. S. Edmonds, Determination of arsenic and arsenic species in marine environmental samples. In: *Arsenic in the Environment, Part I: Cycling and Characterization*, J. O. Nriagu (ed.), John Wiley, New York, 1994, pp. 189–219.
11. K. Shiomi, Arsenic in marine organisms: chemical forms and toxicological aspects. In: *Arsenic in the Environment*, Part II: *Human Health and Ecosystem Effects*, J. O. Nriagu (ed.), John Wiley, New York, 1994, pp. 261–282.
12. K. Hanaoka, T. Matsumoto, S. Tagawa and T. Kaise, *Chemosphere* **16**, 2545 (1987).
13. J. Folch, M. Lees and H. S. Stanley, *J. Biol. Chem.* **226**, 497 (1975).
14. R. A. Stockton and K. J. Irgolic, *J. Environ Anal. Chem.* **6**, 313 (1979).
15. J. Gailer, K. A. Francesconi, J. S. Edmonds and K. J. Irgolic, *Appl. Organometal. Chem.* **9**, 341 (1995).
16. K. Hanaoka, W. Gössler, K. J. Irgolic, S. Ueno and T. Kaise, *Chemosphere* **35**, 2463 (1997).
17. K. Shiomi, Y. Kakehashi, H. Yamanaka and T. Kikuchi, *Appl. Organometal. Chem.* **1**, 177 (1987).
18. K. Hanaoka, Y. Dote, K. Yosida, T. Kaise, T. Kuroiwa and S. Maeda, *Appl. Organometal. Chem.* **10**, 683 (1996).
19. K. Hanaoka, T. Satow, S. Tagawa and T. Kaise, *Appl. Organometal. Chem.* **6**, 375 (1992).
20. K. A. Francesconi and J. S. Edmonds, *Sci. Total Environ.* **19**, 59 (1989).
21. E. Marafante, M. Vahter and L. Denker, *Sci. Total Environ.* **34**, 223 (1984).
22. T. Kaise, K. Shiomi and M. Chino, *Appl. Organometal. Chem.*, **6**, 369 (1992).
23. T. Kaise, K. Hanaoka and S. Tagawa, *Chemosphere* **16**, 2551 (1987).
24. K. Hanaoka, S. Hasegawa, N. Kawabe, S. Tagawa and T. Kaise, *Appl. Organometal. Chem.*, **4**, 239 (1990).
25. K. Hanaoka, S. Tagawa and T. Kaise, *Hydrobiologia* **256/257**, 623 (1992).
26. K. Hanaoka, H. Koga, S. Tagawa and T. Kaise, *Comp. Biochem. Physiol.* **101B**, 595 (1992).
27. K. Hanaoka, K. Ueno, S. Tagawa and T. Kaise, *Comp. Biochem. Physiol.* **94B**, 379 (1989).
28. K. Hanaoka, O. Nakamura, H. Ohno, S. Tagawa and T. Kaise, *Hydrobiologia* **316**, 75 (1995).