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The chemical form and acute toxicity of arsenic compounds in marine organisms

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A method for the separation and identification of inorganic and methylated arsenic compounds in marine organisms was constructed by using a hydride generation/cold trap/gas chromatography mass spectrometry (HG/CT/GC MS) measurement system.

The chemical form of arsenic compounds in marine organisms was examined by the HG/CT/GC MS system after alkaline digestion. It was observed that trimethylarsenic compounds were distributed mainly in the water-soluble fraction of muscle of carnivorous gastropods, crustaceans and fish. Also, dimethylated arsenic compounds were distributed in the water-soluble fraction of Phaeophyceae. It is thought that most of the trimethylated arsenic is likely to be arsenobetaine since this compound released trimethylarsine by alkaline digestion and subsequent reduction with sodium borohydride.

The major arsenic compound isolated from the water-soluble fraction in the muscle and liver of sharks was identified as arsenobetaine from IR, FAB MS data, NMR spectra and TLC behaviour.

The acute toxicity of arsenobetaine was studied in male mice. The LD_{50} value was higher than 10 g kg^{-1}. This compound was found in urine in the non-metabolized form. No particular toxic symptoms were observed following administration. These results suggest that arsenobetaine has low toxicity and is not metabolized in mice. The LD_{50} values of other minor arsenicals in marine organisms, trimethylarsine oxide, arsenocholine and tetramethylarsonium salt, were also examined in mice.

Keywords: Arsenic, marine organisms, arsenobetaine, methylarsenic analysis, LD_{50}, acute toxicity, arsenocholine, tetramethylarsonium, trimethylarsine oxide

INTRODUCTION

Large amounts of arsenic have been observed, more often in marine organisms than in terrestrial ones. Lunde reported that the major part of such arsenic appeared to be in water-soluble organoarsenic compounds.[1,2] Edmonds *et al.* isolated arsenobetaine from the tail muscle of the western rock lobster, *Punulinus longipes cygnus*, and structurally characterized it in 1977.[3] Subsequently, it was recognized that arsenobetaine was widely distributed in several marine animals.[4–8] Also a few kinds of methylated arsenic compounds, viz. arsenocholine,[9–11] tetramethylarsonium ion[12] and arsenosugars,[13–15] were found in some marine organisms.

People have eaten many marine products since ancient times in Japan, which is surrounded by the ocean, so it is certain that they were exposed to many arsenicals. However, poisoning from arsenicals derived from marine products has not been reported. For the toxicological study of arsenic ingested in the human body through sea foods, it is necessary to investigate the chemical form of arsenic in the sea foods, since the toxicological effects of these arsenic compounds is dependent on their chemical form.

Although there are many reports of these water-soluble organoarsenicals in marine organisms, there are fewer reports on analytical methods for these organoarsenic compounds, because of the identification and quantification of water-soluble organoarsenic compounds in marine organisms is generally carried out with complicated techniques after laborious purification of extracts. For the toxicological investigation, there are few reports on biological studies with organoarsenic compounds from marine organisms.

In this paper, methods for the determination, distribution and acute toxicity of organoarsenic compounds in marine organisms are described.

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EXPERIMENTAL

Chemicals

Arsenobetaine, arsenocholine and trimethylarsine oxide were prepared according to previous papers.[16–18] Tetramethylarsonium iodide was synthesized from trimethylarsine with methyl iodide. The other arsenicals were obtained from Tri Chemical Corp., Kanagawa, Japan.

Sample preparation

The marine biological samples for arsenic analysis were collected on the coast of the Miura Peninsula in Kanagawa and the coast of Shimonoseki in Yamaguchi, Japan, from March to October in 1987. Tissues (5–10 g) were extracted with a mixture of chloroform and methanol (2:1) for inorganic and methylated arsenic analysis. Water was added to the extracts and then water-soluble and lipid-soluble fractions were transferred to the test-tubes and evaporated to near-dryness under nitrogen gas, and were heated with 2 mol sodium hydroxide at 85 °C for 3 h. The aqueous solution was neutralized with dilute hydrochloric acid. This procedure was described fully in a previous paper.[17]

Analytical procedure

Total arsenic

Each tissue (1 g) was digested with 10 cm^3 of nitric acid (61% w/w) on a hot plate at below 100 °C until the evolution of brown fumes ceased. After cooling, a mixture of 5 cm^3 of nitric acid, 3 cm^3 of sulphuric acid (97% w/w) and 5 cm^3 of perchloric acid (60% w/w) was added and the mixture was heated until dense fumes of sulphur trioxide appeared. After cooling, solutions were diluted with water (20 cm^3) and neutralized with dilute ammonium hydroxide. The degraded solution was transferred into a 100 cm^3 volumetric flask. 2 cm^3 of 36% (w/w) hydrochloric acid, 4 cm^3 of 20% (w/w) potassium iodide and 4 cm^3 of 20% (w/w) ascorbic acid were added to the solution, and the solution was made up to 100 cm^3 with water. Total arsenic was determined by reduction of arsenic to arsine with the fully automated continuous arsine generation system using sodium borohydride and an atomic absorption spectrophotometer equipped with a heated quartz tube.

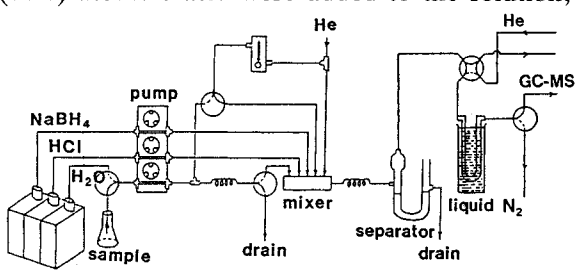

Figure 1 The hydride generation and cold-trap system for the generation and collection of arsines.

Hydride generation/cold trap/gas chromatography-mass spectrometry system (HG/CT/GC MS)

Arsenic trioxide, methylarsonic acid, dimethylarsinic acid by trimethylarsine oxide were converted to the corresponding arsines by reduction with sodium borohydride. Arsenic compounds in marine organisms, after treatment with an alkaline solution and neutralization, were reduced in a fully automated hydride generation system. The hydride generator was connected to a stainless-steel U-shaped tube packed with quartz wool and wrapped with nichrome wire. The U-tube was precooled with liquid nitrogen and the generated arsines were collected in the U-tube. The coolant was then removed and the U-tube was heated at 200 °C to transfer the arsines into the GC MS equipped with a silicone OV-17 glass column for selective ion monitoring (SIM) at m/z 76 for AsH_3, 78 for AsH_3, 90 for CH_3AsH_2, 90 for $(CH_3)_2AsH$, 103 for $(CH_3)_3As$ and 120 for $(CH_3)_3As$. The apparatus for the fully automated continuous reduction of arsenic compounds is illustrated in Fig. 1.

Identification of water-soluble organoarsenic compounds in the tissue of sharks

The muscle and liver of two species of shark, *Squalus brevirostris* and *Mustelus manazo*, were extracted with a mixture of chloroform and methanol (2:1). The total arsenic content of each tissue is as follows: *S. brevirostris*: muscle 44.3 μg As g^{-1}, liver 20.5 μg As g^{-1}; *M. manazo*: muscle 17.3 μg As g^{-1}, liver 16.2 μg As g^{-1}.

The water-soluble arsenic compounds in the methanolic fraction of each tissue was purified by chromatography on Dowex 50W × 8, AG 1 × 8, active carbon, Sephadex G-25F and Dowex 50W × 8 columns. Finally, the water-soluble arsenic compound was isolated on a preparative thin-layer chromatography column of cellulose (TLC). This arsenic compound was identified by IR, NMR, FAB MS spectra and TLC behaviour.

Acute toxicity of arsenic compounds

Five-week-old male ddY mice were used for acute toxicity examinations. The mice were quarantined for one week in a temperature-controlled

room at 23 ± 2 °C and relative humidity 55 ± 5%. Pelleted dry diet (CE2: Clea Japan Inc., Japan) and tap-water were fed *ad lib*. Arsenicals were administered orally with distilled water to the animals. The toxicity symptoms were observed at all times after 5 h following administration and subsequent observations were made at intervals of 1 h for 24 h and then daily for seven days. The LD_{50} values were calculated by the probit method.

RESULTS AND DISCUSSION

Measurement of arsines by the HG/CT/GC MS system[17]

The arsines were separated by gas chromatography and identified by GC MS in the SIM mode. Arsenite, methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide were found to be stable in hot aqueous sodium hydroxide and were quantitatively converted to arsine (99.8%), methylarsine (98%), dimethylarsine (99.1%) and trimethylarsine (99.1%) respectively, by reduction with sodium borohydride. The SIM chromatograms for the arsines are shown in Fig. 2. The calibration curves of peak area versus amount of arsenic for arsine, methylarsine, dimethylarsine and trimethylarsine were linear from 0.3 ng to 300 ng of arsenic. The detection limit after sodium borohydride reduction is 0.1 ng As g^{-1} of biological sample. Arsenobetaine was quantitatively converted to trimethylarsine (99.8%); but arsenocholine formed little trimethylarsine

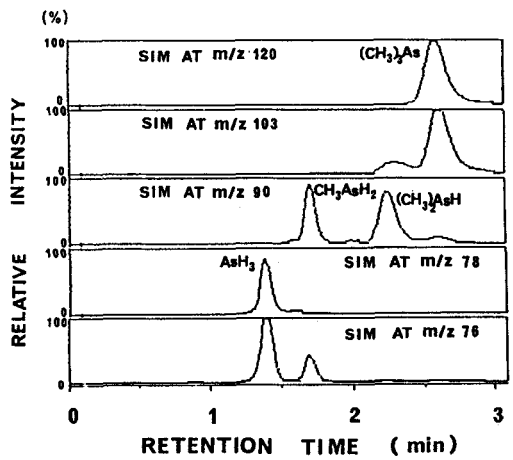

Figure 2 The SIM chromatograms of arsine, methylarsine, dimethylarsine and trimethylarsine (10 ng arsenic each) after introduction of a solution containing arsenite, methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide into the hydride generation system.

(0.8%), on treatment of the sample with hot base and subsequent reduction with sodium borohydride.

Total arsenic contents in marine organisms[19]

Sixty specimens of marine organisms were examined for accumulated arsenic. Phaeophyceae contained high levels (*Laminaria japonica* 44.3 μg g^{-1}; *Hizikia fusiforme* 41.3 μg g^{-1}; *Undaria pinnatifida* 38.3 μg g^{-1}) and a species of Rhodophyceae also contained high levels (*Porphyra tenera* 69.9 μg g^{-1}). These were mainly representative of the edible seaweeds in Japan. The arsenic content of the muscle of carnivorous gastropods and crustacea was outstandingly high (*Kellettia lischkei* 125.9 μg g^{-1}; *Reishia bronni* 123.8 μg g^{-1}, *Babylonia japonica* 61.6 μg g^{-1}; *Panulirus japonicus* 48.9 μg g^{-1}, *Plagusia dentipes* 46.9 μg g^{-1}, *Penaeus japonicus* 65.8 μg g^{-1}). The arsenic content in plankton-feeding bivalves and herbivorous gastropods, which feed on Phaeophyceae, was fairly low when compared with that of carnivorous gastropods.

Water- and lipid-soluble arsenic in marine organisms[17, 19]

Most of the arsenic in the marine organisms was in the water-soluble fraction and the methylated form. Lipid-soluble arsenic was found ubiquitously, but its content was fairly low compared with water-soluble arsenic. The water-soluble trimethylated arsenic was widely spread in marine animals, and this was the main component of water-soluble arsenic. It was thought this trimethylated arsenic was likely to be arsenobetaine, since arsenobetaine released trimethylarsine by alkaline digestion and subsequent reduction with sodium borohydride, but arsenocholine does not give trimethylarsine by the same procedure.[17] The content of water-soluble dimethylated arsenic was noticeably high in seaweeds. It was thought that this dimethylated arsenic was a degradation product of the alkaline digestion of arsenosugars having a $(CH_3)_2AsO$ moiety. The averages of these arsenic contents in each group are summarized in Table 1.

Identification of arsenobetaine in the muscle and liver of sharks[20, 21]

About 75–98% of the total arsenic in the muscle and liver tissues was found in the water-soluble fraction. Each of the isolated materials from the sharks gave a single spot which was positive to the

Table 1 Arsenic content in marine organisms (μg As g^{-1})

Species	Tissue	Water-soluble				Lipid-soluble			Total As
		IOA	MA	DMA	TMA	MA	DMA	TMA	
Demospongia	Whole	0.05	0.29	4.84	1.88	0.03	0.08	0.02	8.11
Coelenterata	Whole	0.12	0.01	ND	0.12	ND	ND	ND	0.46
Echinodermata	Ovary	ND	ND	0.21	1.34	ND	0.14	0.03	2.11
	Muscle	ND	ND	1.22	13.28	ND	ND	ND	23.43
	Viscera	0.16	ND	0.32	1.11	ND	0.37	0.18	2.96
Mollusca	Muscle	ND	0.02	0.10	2.34	ND	ND	ND	4.49
	Viscera	ND	ND	0.07	2.31	ND	0.01	0.54	2.97
Conch (H)	Muscle	ND	0.02	0.21	2.51	0.02	0.16	0.01	1.89
	Viscera	ND	0.03	1.10	7.29	0.03	0.36	0.09	5.74
Bivalvia (P)	Whole	ND	ND	0.29	2.38	ND	0.16	0.15	4.29
Conch (C)	Muscle	ND	0.12	1.62	54.39	0.01	0.13	0.56	67.12
	Viscera	ND	0.39	3.04	58.82	0.05	1.01	0.42	68.38
Fish (P)	Muscle	ND	0.02	0.01	2.25	ND	ND	0.01	2.71
	Viscera	ND	0.06	0.11	1.82	0.03	0.08	0.03	2.22
Fish (H)	Muscle	ND	ND	ND	0.03	ND	ND	ND	0.29
	Viscera	ND	ND	0.22	0.12	ND	0.04	ND	1.95
Fish (C)	Muscle	ND	ND	0.02	1.63	ND	0.02	0.08	2.14
	Viscera	ND	ND	0.19	2.15	ND	0.12	0.22	3.45
Crustacea	Muscle	ND	ND	0.11	18.40	ND	0.05	0.31	23.36
Seaweeds									
Phaeophyceae		0.59	ND	15.56	1.35	ND	0.98	0.03	28.59
Rhodophyceae		ND	ND	8.03	0.16	ND	0.16	ND	18.67
Chlorophyceae		ND	ND	0.42	0.10	ND	ND	ND	0.73

Abbreviations: IOA, inorganic arsenic; MA, methylated arsenic; DMA, dimethylated arsenic; TMA, trimethylated arsenic; ND, Not detectable; H, herbivorous; P, plankton feeder; C, carnivorous.
Total arsenic yields are greater than the sum of the water soluble and lipid soluble arsenics. It is thought that a portion of arsenic in the tissue of marine organisms is not extracted with water or chloroform–methanol, i.e. it might be bonded with their tissues.

Dragendorff reagent on cellulose TLC obtained with the five solvent system. The R_f value of each was the same as that a synthetic arsenobetaine and arsenic was detected only from the spot by graphite furnace–atomic absorption spectrophotometry (GF AA). The IR, NMR and FAB mass spectra of the isolated arsenicals were essentially identical with those of synthetic arsenobetaine. The IR and FAB mass spectra were shown in Figs 3 and 4.

Acute toxicity and excretion

Arsenobetaine[16]

In the mouse group given 10 g kg^{-1} of arsenobetaine, the animals showed a decrease of spontaneous motility and a decrease of respiration, but these symptoms disappeared after 1 h. In the fractionation of the arsenic compound in the urine on columns of ion-exchange resins, the fraction numbers containing the arsenical agreed

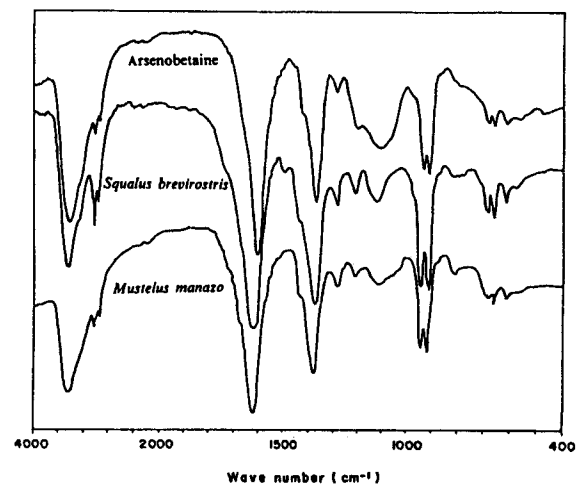

Figure 3 Infrared spectra of the water-soluble arsenic compounds isolated from liver tissues of *Squalus brevirostris* and *Mustelus manazo*.

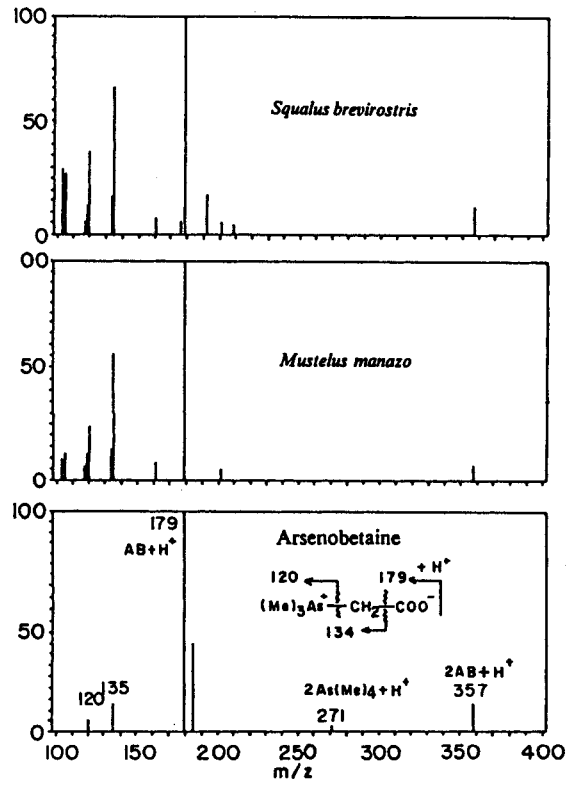

Figure 4 FAB mass spectra of the water-soluble arsenic compounds isolated from liver tissues of *Squalus brevirostris* and *Mustelus manazo*.

with those from a previous experiment using authentic arsenobetaine and no other fraction contained an arsenical. The arsenic compound in urine gave a single spot with iodine vapour on a TLC chromatogram obtained with a developing solvent system (n-butanol/acetic acid/water, 60:15:25). The R_f value of the spot was the same as that of synthetic arsenobetaine and arsenic was detected only from this spot by GF AA. The single arsenic compound excreted in the urine was examined by FAB MS and by IR spectroscopy. They were identical to those of arsenobetaine. Arsenobetaine was absorbed from the digestive tract immediately after oral administration and excreted rapidly in urine without metabolism. The LD_{50} value of arsenobetaine was above 10 g kg^{-1}.

Arsenocholine[22]

Decrease of respiration and spontaneous motility was observed in the group administered at 12 g kg^{-1}. The animals exhibited ataxia. Finally, they showed paralysis of the hind legs after 20 min. The single metabolite of arsenocholine in the urine was identified by retention time as arsenobetaine using HPLC ICP and also by the FAB mass spectrum. It was thought arsenocholine in the tissues was metabolized to nontoxic arsenobetaine. The LD_{50} value of arsenocholine was 6.5 g kg^{-1}.

Trimethylarsine oxide[23,24]

In the group administered trimethylarsine oxide at the lethal dose of 14.4 g kg^{-1}, a garlic-like odour was definitely smelled in the exhalation of the animals after 2–3 min, and it continued for a few hours. The expired air was trapped in a U-tube cooled with liquid nitrogen for 3 s and flashed into a GC MS with heating at 200 °C after the removal of the coolant. The mass spectrum of the odorous substance was essentially identical with that of trimethylarsine. The animals exhibited irritability, and subsequently ataxia and respiratory depression, followed by acceleration of spontaneous motility, and they occasionally showed startle motility. When the arsenic compound in the urine was purified with ion-exchange resins and Sephadex LH20, white crystals were obtained. The FAB mass spectrum of the crystals gave the same spectrum of trimethylarsine oxide. The LD_{50} value of trimethylarsine oxide was 10.6 g kg^{-1}.

Tetramethylarsonium iodide[25]

Immediately after administration at lethal doses of 1.1 g kg^{-1} as tetramethylarsonium iodide, the mice exhibited an acceleration of spontaneous motility which was frequently accompanied by grooming. The spontaneous motility was inhibited in a few minutes and instead vasodilation and respiratory depression appeared, followed by mild ataxia with tremor. Finally the animals showed severe tremor with tonic convulsion and salivation and died of respiratory arrest after several fits of gasping. When the urine was analysed by HPLC ICP, the retention time of the arsenical in urine coincided well with that of tetramethylarsonium iodide. The only arsenical excreted in the urine was tetramethylarsonium ion obtained by FAB MS. The LD_{50} value of tetramethylarsonium iodide was 0.9 g kg^{-1}.

The median lethal dose values of these arsenic compounds summarized in Table 2.

CONCLUSION

A method for the separation and identification of inorganic and methylated arsines was established using GC MS equipped with a hydride generation

Table 2 LD_{50} values of arsenic compounds

Arsenical	Species	LD_{50} (g kg^{-1})	95% confidence limits (g kg^{-1})
Arsenic trioxide	As(III)	34.5×10^{-3} (16)	$(32.4–37.7) \times 10^{-3}$
Methylarsonic acid	$(CH_3)As$	1.8 (23)	1.7–1.9
Dimethylarsinic acid	$(CH_3)_2As$	1.2 (23)	1.0–1.3
Trimethylarsine oxide	$(CH_3)_3As$	10.6	9.4–11.5
Arsenobetaine	$(CH_3)_3As$	>10	
Arsenocholine	$(CH_3)_3As$	6.5	5.8–7.2
Tetramethylarsonium iodide	$(CH_3)_4As^+$	0.9	0.8–1.0

cold-trap system. This method was applied to extracts from marine organisms. The water-soluble trimethylated arsenic was widely spread in marine animals, and was the main component of water-soluble arsenic. It was thought this trimethylated arsenic was likely to be arsenobetaine. On the other hand, the amounts of water-soluble dimethylated arsenic was high in seaweeds. It was thought dimethylated arsenic was derived from arsenosugars. The acute toxicity of arsenobetaine is very low. It must be considered significantly safe for human consumption of arsenic-containing marine animals from the viewpoint of food hygiene.

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