Short Communication

Identification of Arsenobetaine, a Water Soluble Organo-arsenic Compound in Muscle and Liver of a Shark, *Prionace glaucus*

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Arsenic compounds have been recognized as potential poisons. It is well known that arsenic is widely distributed in the biosphere and, especially, marine animals and plants accumulate arsenic much more than terrestrial organisms. Therefore, for those people where fish and seaweed constitute a major part of the diet, *e.g.*, Japanese, the amount of arsenic taken is a serious problem.^{1,2)} In our previous paper,³⁾ we described that more than 90% of arsenic in shark was in the organic form and unknown in character. In this paper we are reporting that a water soluble organo-arsenic compound isolated from the muscle and liver of a shark, *Prionace glaucus*, has been identified as arsenobetaine as shown in Fig. 1(a). Recently, Edmonds *et al.*⁴⁾ reported the isolation and identification of this compound from the tail muscle of the western rock lobster, *Panulirus longipes cygnus* George. The presence of arsenobetaine both in crustacea and elasmobranchii is of particular interest since it suggests that the compound would be distributed in a wide variety of marine organisms.

A female shark, 233 cm in total length, was caught by the Tanseimaru, the ship of our university, in March 1979 near Miyake-jima

Island in the north west Pacific Ocean. The average total arsenic contents in the muscle and liver were 37.4 and 27.7 $\mu\text{g/g}$ wet wt., respectively, which were determined by arsine vaporization atomic absorption spectrometry after digestion of the sample with mixed acids, HNO_3 , H_2SO_4 and HClO_4 .

After Bligh and Dyer's fractionation⁵⁾ of the homogenized samples, the aqueous fractions were treated by high performance liquid chromatography (HPLC) with either the column of Nagel-Nucleosil 10 SA cation or 10 SB anion exchange resin. The HPLC eluent was directly connected to the nebulizer inlet of an inductively coupled plasma (ICP) spectrometer to monitor the emission intensity of arsenic at 193.7 nm. The combined HPLC-ICP system⁶⁾ is particularly suitable both for separation and identification of arseno-compounds. As for reference materials, arsenobetaine was synthesized according to the

FIG. 1. High Performance Liquid Chromatogram Monitored by Inductively Coupled Plasma Emission Spectrometry.

(a) aqueous fraction of shark muscle.

(b) references; 1, arsenobetaine; 2, arsenite; 3, MMA; 4, DMA; 5, arsenate.

Column, Nagel-Nucleosil 10 SB anion exchange resin; eluent, 0.025 M phosphate buffer, pH 7.4.

method of Edmonds *et al.*⁴⁾ While other arsenic compounds, such as monomethyl arsonate (MMA), dimethyl arsinat (DMA), and inorganic As (III) and As (V) compounds were of commercial origin.

As for the muscle sample, 80% of arsenic was detected in the aqueous fraction, 10% in the residue and almost none in the lipid fraction. On the other hand, for liver, 40% was found in the aqueous fraction and 45% in the lipid fraction. Fig. 1(a) shows the result of the aqueous fraction of muscle by HPLC-ICP with an anion exchange resin column with the elution pattern of the reference materials being compared in Fig. 1(b). The same result was obtained in this treatment for the aqueous fraction of liver. The first elution peak in Fig. 1(a) was the main arsenic compound and the retention time agreed well with that of the synthesized arsenobetaine. When the cation exchange resin column in HPLC-ICP was adopted for the aqueous fraction of liver, the main peak appeared last as shown in Fig. 2(a) and the retention time also agreed with the authentic sample. In addition to chromatographic characterization, the main component in the aqueous fraction of both muscle and liver gave the molecular ion peak, m/e 179, by FD mass spectrometry. Thus, the chemical structure of arsenobetaine is confirmed as shown in Fig. 1(a) which is an analog of betaine where nitrogen is replaced by arsenic.

The chemical status of organo-arsenic compounds in the biosphere including natural water has so far been extensively studied and recognized mainly as methylated products, such as MMA, DMA and trimethylarsine (TMA).⁷⁾ The present work shows that arsenobetaine is clearly the major arsenic compound in the higher marine animal instead of the so far recognized lower methylated compounds. Therefore, this finding together with the presence of arsenolipids in marine algae reported by Cooney *et al.*⁸⁾ is very suggestive for the further research on the biosynthesis and metabolism as well as the physiological roles of organo-arsenic compounds.

FIG. 2. High Performance Liquid Chromatogram Monitored by Inductively Coupled Plasma Emission Spectrometry.

(a) aqueous fraction of shark liver.

(b) references: 1, arsenate; 2, MMA; 3, DMA; 4, arsenite; 5, arsenobetaine.

Column, Nagel-Nucleosil 10 SA cation exchange resin; eluent, 0.025 M phosphate buffer, pH 7.4.

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