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Identification of Arsenobetaine as a Major Arsenic Compound in the Muscle of a Pelagic Shark, *Carcharodon carcharias*[*1]

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A water-soluble arsenic compound was isolated from the dorsal muscle of a great white shark *Carcharodon carcharias* by using chromatographic techniques. The isolated compound was confirmed as arsenobetaine by use of thin-layer chromatography, infrared spectrometry and FAB mass spectrometry. The compound accounted for nearly 94% of the water-soluble arsenic in the muscle.

1. Introduction

Higher levels of arsenic have been found in marine animals than in terrestrial ones[1]. The arsenic occurs mainly as non-toxic organoarsenic compounds[2~4]. Only small amounts of inorganic arsenic are reported to be present in marine animals[5,6]. Results from many studies on the chemical form of arsenic lead us to assume that arsenobetaine is the most widely distributed chemical species of arsenic in fish, shellfish, and other marine animals. Our previous paper[7] showed that arsenobetaine is virtually the sole arsenic species found in white muscle of 2 species of pelagic shark, blue pointer *Isurus oxyrinchus* and whitetip shark *Carcarhinus longimanus*.

This paper deals with the isolation and identification of arsenobetaine from white muscle of another pelagic shark, great white shark *Carcharodon carcharias*.

2. Materials and Methods

2·1 Material

A specimen of great white shark, *Carcharodon carcharias*, was obtained off Sumatra in the Indian Ocean in November 1983, and the dorsal white muscle was stored at −40℃ until use. The arsenic concentration of the muscle was 5.2 μg/g(wet-weight basis).

2·2 Extraction and purification of water-soluble arsenic compound

About 3960 g of myocommata-free muscle was extracted with chloroform-methanol (2:1 v/v) mixture (18.1*l*×2). After the addition of water to the extract, the aqueous phase was used for the following purification.

The aqueous phase (water-soluble fraction) was first applied to a Dowex 50W-×8 (H^+ form) column (4.2×45cm), and eluted with wa-

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ter (1*l*) followed by 1.5M aq. ammonia (1.2*l*). Unless otherwise stated, elution was carried out at a flow rate of 1m*l*/min, 15m*l*-fractions were collected, and the arsenic in eluate was detected by flameless graphite furnace atomic absorption spectrometry (Nippon Jarrel Ash, AA-845) throughout the experiment.

Further purification was achieved by passage of the arsenic-containing fraction (1.5M aq. ammonia effluent) through the columns of AG 1-×8 (OH^- form, 4.2×50 cm), active carbon (1.9×10cm), and Sephadex G25F (1.9×50cm) successively. The passing, which contained arsenic, was then chromatographed on a column (1.9×90cm) of Dowex 50W-×8 (pyridinium form) equilibrated with 0.1 M pyridine-formic acid buffer solution (pH 3.1). The same buffer solution was employed as a eluant at a flow rate of 0.4m*l*/min and 5 m*l*-fractions were pooled. Gel filtration on Sephadex G25F was again carried out for the final purification.

2·3 Analyses of water-soluble arsenic compound

Thin layer chromatography was performed on a precoated cellulose plate (0.1mm, Funakoshi Yakuhin Co., Ltd.). Five solvent systems, *i.e.* ethyl acetate-acetic acid-water (3:2:1), chloroform-methanol-aq. ammonia (28%) (2:2:1), 1-butanol-acetone-formic acid (85%)-water (10:10:2:5), 1-butanol-acetone-aq. ammonia (28%) -water (10:10:2:5), and 1-butanol-acetic acid-water (4:2:1) were used for development. The Dragendorff reagent was employed for visualization of the spot. IR spectra were recorded on a Shimadzu IR-27G spectrophotometer. Mass spectra were measured on a jeol JMS-DX 300 equipped with fast atom bombardment. Arsenic was determined by arsine evolution-electrothermal atomic absorption method after the sample was digested with a mixture of nitric, sulfuric and perchloric acids.

3. Results and Discussion

The white residue (33 mg) was finally isolated by the vacuum evaporation of the eluate from a Sephadex column. When analysed by TLC with 5 solvent systems, the isolated compound gave a single spot positive to the Dragendorff reagent in each solvent, and arsenic was detected only in the spot by atomic absorption spectrometry. The Rf value of the compound in each solvent system agreed very closely with that of synthetic arsenobetaine. The IR spectrum of the isolated compound was identical with that of synthetic arsenobetaine. As Fig.1 shows, the compound gave the molecular ion peak at m/z 179, which was also a base peak, and a fragment peak at m/z 135 ($M^+ - CO_2$) by FAB mass spectrometry. This spectrum was identical with that of synthetic arsenobetaine.

These findings show that arsenobetaine is the major water-soluble arsenic compound also in the muscle of a great white shark *Carcharodon carcharias* as well as in *Isurus oxyrinchus* and *Carcarhinus longimanus*. About 98% of the total arsenic in the muscle was found in the water-soluble fraction; moreover, 96% of the water-soluble arsenic was recovered in aq. ammonia fraction from the Dowex 50W-×8 resin. Almost all the arsenic in aq. ammonia fraction passed through the columns of AG 1-×8, active carbon and Sephadex G25F. No arsenic compound other than arsenobetaine was found in any fraction during the purification procedure. On the basis of these findings, arsenobetaine was estimated to account for nearly 94% of the water-soluble arsenic in the muscle.

As to elasmobranch, the presence of arsenobetaine is so far reported in muscle of *Prionace glauca*[8], *Carcarhinus obscurus*[9], *Carcarhinus longimanus*[7], and *Isurus oxyrinchus*[7]. Considering the facts that the sharks are at the highest stage of marine trophic level and arsenobetaine is exclusively the major arsenic compound in them, we can assume that arse-

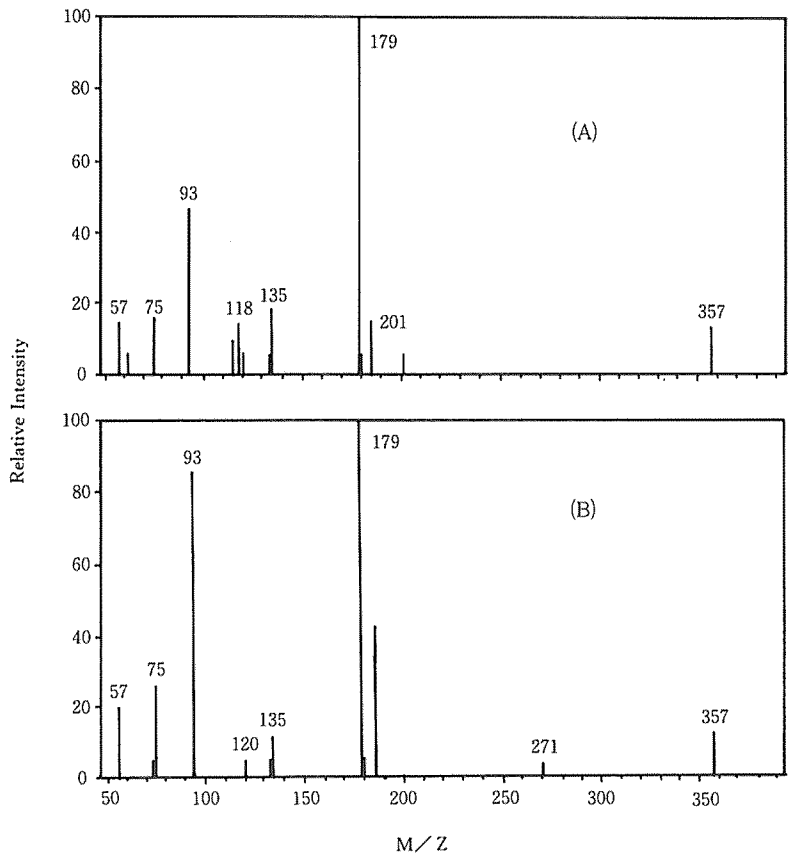

Fig. 1. FAB mass spectra of (A) the water-soluble arsenic compound from great white shark and (B) synthetic arsenobetaine.

nobetaine is probably the end product of arsenic metabolism in marine ecosystems. All the sharks whose arsenic species was studied so far, however, were pelagic.

There is no information on the arsenic species of demersal sharks. In order to confirm the above assumption, it is therefore necessary to elucidate if arsenobetaine would be a major arsenic compound also in demersal sharks.

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ホホジロザメ筋肉における主要な水溶性ヒ素化合物としてアルセノベタインの単離

花岡研一・松田裕之・貝瀬利一・田川昭治

ホホジロザメ背部肉からクロマトグラフィーによって水溶性ヒ素化合物を単離した。薄層クロマトグラフィー，赤外およびFAB-マススペクトルによって、この化合物をアルセノベタインと同定した。この化合物は、筋肉中の水溶性ヒ素のほぼ94％を占めていた。