Short Paper

Identification of Arsenobetaine in
Muscle of Roundnose Flounder
*Eopsetta grigorjewi**1

Marine organisms have been shown to bioaccumulate the arsenic in the organic forms according to their trophic levels. Arsenobetaine has been isolated from many marine animals in higher trophic levels since 1977,¹⁾ and considered to be the most stable organo-arsenic derivative. We reported in the preceding paper²⁾ the isolation and identification of arsenobetaine as a major water-soluble organoarsenic compound from marine organisms in the highest trophic level, blue pointer *Isurus oxyrinchus* and whitetip shark *Carcharhinus longimanus*. The present paper deals with again the isolation and identification of the compound from a marine organism in higher trophic level, roundnose flounder *Eopsetta grigorjewi*.

White muscle (480 g, 20.1 μg As/g wet weight) of the fish obtained as freshly as possible from a market was subjected to the extraction of water-soluble organoarsenic compound. The extract was chromatographed by successive use of Dowex 50W- $\times$ 8 (H^+ form), AG 1- $\times$ 8 (OH^- form), active carbon and Sephadex G-25F equilibrated with water. The major water-soluble organoarsenic compound was eluted with water from all columns except Dowex 50W- $\times$ 8 on which it was adsorbed and eluted with 1.5 M ammonia. The extraction of the water-soluble organoarsenic compound and detection of arsenic in the eluate were performed as described previously.²⁾ The infrared spectrum of the compound obtained by the above chromatography was identical with that of glycinebetaine as in the case of blue pointer and whitetip shark. In order to separate the arsenic compound from glycinebetaine, three purification methods were carried out, e.g., cellulose column chromatography (Avicel SF, Funakoshi Yakuhin Co., Ltd.), cation exchange chromatography using concave gradient elution system (AG 50W- $\times$ 8, H^+ form, 1.0–2.9N HCl) and cation exchange chromatography (Dowex 50W- $\times$ 8, pyridinium form, eluted with 0.1 M pyridine-formic acid buffer of pH 3.1) which was recommended by SHIOMI *et al.*³⁾ for the purification of arsenobetaine. The last one was the most effective for separation of them, although the other two partly removed the glycinebetaine from the compound. The sample was rechromatographed successively by use of Dowex 50W- $\times$ 8 (pyridinium form) and active carbon.

Thin layer chromatography was applied to the identification of the purified arsenic compound on cellulose plate (Avicel SF, Funakoshi Yakuhin Co., Ltd.) using five solvent systems, 1; ethyl acetate-acetic acid-water (3:2:1), 2; chloroform-methanol-ammonia (28%) (2:2:1), 3; 1-butanol-acetone-formic acid (85%)-water (10:10:2:5), 4; 1-butanol-acetone-ammonia (28%)-water (10:10:2:5) and 5; 1-butanol-acetic acid water (4:2:1). The single spot, of which Rf value

Table 1. Rf values in thin layer chromatography of the water-soluble arsenic compound from roundnose flounder and synthetic arsenobetaine

Sample	Rf value				
	1	2	3	4	5
Roundnose flounder	0.73	0.75	0.64	0.82	0.69
Synthetic arsenobetaine	0.74	0.75	0.64	0.81	0.70

Fig. 1. Infrared spectra of (B) the water-soluble arsenic compound from roundnose flounder and (A) synthetic arsenobetaine.

was identical with that of synthetic arsenobetaine, was detected with Dragendorff reagent and arsenic was detected only from the part of the spot by atomic absorption spectrophotometry in each solvent system (Table 1). Infrared spectra of the purified arsenic compound and synthetic arsenobetaine were recorded with SHIMADZU IR 27-G spectrophotometer in KBr disk (Fig. 1). The comparison of these spectra, though small peaks attributable to the presence of impurities were observed in the spectrum of the isolated compound, confirmed the water-soluble organoarsenic compound from roundnose flounder to be arsenobetaine.

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References

- 1) J.S. EDMONDS and K.A. FRANCESCONI: *Tetrahedron Letters*, **18**, 1547–1550 (1977).
- 2) K. HANAOKA and S. TAGAWA: *Bull. Japan. Soc. Sci. Fish.*, **51**, 681–685 (1985).
- 3) K. SHIOMI, A. SHINAGAWA, H. YAMANAKA, and T. KIKUCHI: *Bull. Japan. Soc. Sci. Fish.*, **49**, 79–83 (1983).

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*1 ムシガレイ普通筋における水溶性ヒ素化合物としてアルセノベタインの同定.

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