APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 5, 427–430 (1991)

SHORT PAPER

# Conversion of arsenobetaine by intestinal bacteria of a mollusc *Liolophura japonica* chitons

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**The intestinal micro-organisms of *Liolophura japonica* chitons converted arsenobetaine [$(CH_3)_3As^+CH_2COO^-$] to trimethylarsine oxide [$(CH_3)_3AsO$] and dimethylarsinic acid [$(CH_3)_2AsOOH$] in the arsenobetaine-containing 1/5 ZoBell 2216E medium under aerobic conditions, no conversion being observed in an inorganic salt medium. This conversion pattern of arsenobetaine→trimethylarsine oxide→dimethylarsinic acid was comparable with that shown by the microorganisms associated with marine macroalgae. On the other hand, no conversion was observed in either medium under anaerobic conditions.**

**Keywords: Arsenobetaine, trimethylarsine oxide, dimethylarsinic acid, degradation, bacteria, micro-organisms, chitons**

## INTRODUCTION

Arsenobetaine was found for the first time in a marine animal, the western rock lobster, by Edmonds *et al.* in 1977[1] and has been considered as the final metabolite of arsenic in marine food chains. In recent years, we have dealt with the microbial degradation of this compound to clarify arsenic circulation in marine ecosystems. Those degradation experiments have been performed with the micro-organisms occurring in sediments[2–7] or those associated with marine macroalgae.[8] Higher degradation activity was shown in sedimentary micro-organisms, where arsenobetaine was completely degraded to inorganic arsenic. These results led us to the following hypothesis: that there is a marine arsenic cycle that begins with the methylation of inorganic arsenic on the way to arsenobetaine and terminates with the complete degradation of arsenobetaine to inorganic arsenic.[4,7] In order to prove, however, the ubiquity of microbial degradation of arsenobetaine, we must confirm the ubiquitous occurrence of arsenobetaine-decomposing micro-organisms in various sources of micro-organisms in marine environments.

We were interested in the intestines of marine animals as the possible source of arsenobetaine-decomposing micro-organisms other than sediments or algae. Thus, the intestinal micro-organisms of *Liolophura japonica* chitons were investigated in this paper. This animal was chosen in the first place, because of its interesting nature; it accumulates arsenic mainly as arsenobetaine—nevertheless it is an algal feeder.

## MATERIALS AND METHODS

### Chitons

*Liolophura japonica* chitons were collected from the coastal waters of Yoshimi, Shimonoseki, Japan, in January and May 1990. Intestines from several of them were gathered and mixed as thoroughly as possible.

### Cultivation

Two culture media have been used so far in this series of microbial degradation experiments of arsenic compounds.[2–8] These were also used in this study. They are 1/5 ZoBell 2216E (as g $dm^{-3}$ filtered seawater: peptone 1.0; yeast extract 0.2, pH 7.5) and an aqueous solution of inorganic salts

0268-2605/91/050427-04$05.00


*Received 24 January 1991*
*Revised 4 June 1991*

at pH 7.5 [as g $dm^{-3}$; sodium chloride (NaCl) 30.0; calcium chloride ($CaCl_2.2H_2O$) 0.2; potassium chloride (KCl) 0.3; iron (II) chloride ($FeCl_2.nH_2O$) 0.01; phosphates ($KH_2PO_4$) 0.5 and ($K_2HPO_4$) 1.0; magnesium sulphate ($MgSO_4.7H_2O$) 0.5; and ammonium chloride ($NH_4Cl$) 1.0]. For the aerobic experiments, arsenobetaine [$(CH_3)_3As^+CH_2COO^-$, 50 mg] and the chiton intestines (*ca* 0.01 g) were added to each medium (25 $cm^3$) in a 50-$cm^3$ Erlenmeyer flask. The flasks were kept at 25 °C in the dark under an atmosphere of air without shaking. For anaerobic experiments, about 5 $cm^3$ of liquid paraffin was placed on the surface of each mixture. Mixtures autoclaved at 120 °C for 20 min served as controls for both aerobic and anaerobic experiments. Filtered aliquots from the mixtures in the flasks were withdrawn over intervals of several days of incubation. The arsenic compounds in the diluted aliquots were fractionated by high-performance liquid chromatography.

### High-performance liquid chromatography

Arsenobetaine and its metabolites were separated on a high-performance liquid chromatograph (TOSOH Co., CCP 8000 series, TSK Gel ODS-120T column, 4.6 mm × 250 mm) under the same conditions as those which have been used in the degradation experiments so far.[4] The mobile phase consisted of a 11.2 mmol $dm^{-3}$ solution of sodium heptanesulphonate in water/acetonitrile/acetic acid (95:5:6, by vol.).[9] Fractions were collected and an aliquot of each fraction was injected into a graphite furnace atomic absorption spectrometer as described previously.[4]

### Purification and identification of the metabolite

About 5 $cm^3$ of the incubated medium containing the metabolites was taken from the flask and applied to a cation-exchange column Dowex 50W-×8 (100–200 mesh, 1 cm × 50 cm) equilibrated with 0.1 mol $dm^{-3}$ pyridine–formic acid buffer (pH 3.1) and eluted with the same buffer and 0.1 mol $dm^{-3}$ pyridine, successively. Arsenic-containing fractions were pooled and freeze-dried.

FAB mass spectrometry (FAB mass, JEOL JMS DX-300 mass spectrometer equipped with a fast atom bombardment ion source and xenon atoms at 6 keV) was used for the confirmation of the structure of purified metabolites.

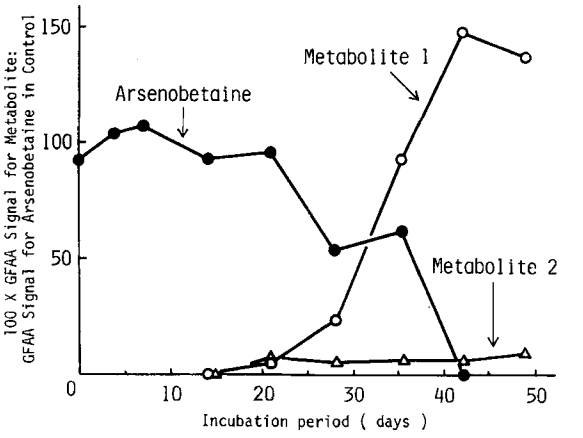


**Figure 1** The conversion of arsenobetaine in a ZoBell medium added with intestine of *Liolophura japonica* during aerobic incubation at 25 °C.

## RESULTS

### Conversion of arsenobetaine by intestinal bacteria

Figure 1 shows the time course pattern of arsenobetaine and its metabolites. Arsenobetaine was converted only in ZoBell/aerobic incubation. Two types of metabolites began to appear on day 20 of incubation. One had an HPLC-retention time which agreed with that of trimethylarsine oxide (13–15 min, provisionally called metabolite 1) and the other had the same retention time as that of dimethylarsinic acid (6.0–7.5 min, metabolite 2). Metabolite 1 rapidly increased after 42 days of incubation with complete disappearance of arsenobetaine. Metabolite 2 did not increase to any large extent throughout the incubation period.

A prelimary test had been performed with viscera of the chitons, which were incubated at 37 °C for 10 days to investigate their metabolic pathway to convert arsenobetaine to other arsenic compounds. Although trimethylarsine oxide was detected besides arsenobetaine in the incubated viscera by high performance liquid chromatography, it was not detected in the control added with toluene (1%). This fact may indicate that there is no metabolic pathway to convert arsenobetaine to

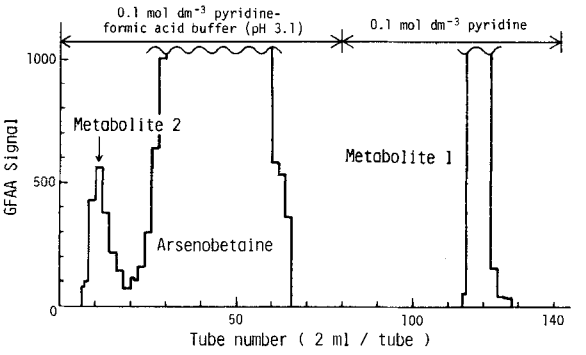


**Figure 2** Cation-exchange chromatographic separation (Dowex 50W-X8, pyridinium form) of metabolite 1 and metabolite 2 derived from arsenobetaine by the intestinal microorganisms of *Liolophura japonica*.

trimethylarsine oxide or dimethylarsinic acid. These findings led us to the conclusion that the conversion of arsenobetaine shown in this study may be resulted from the microbial action.

### Isolation of the metabolite

About 5 $cm^3$ of the aerobically incubated (35 days) ZoBell medium was applied to a cation-exchange resin column (Dowex 50W-X8) after filtration. As shown in Fig. 2, metabolite 2 was eluted with 0.1 mol $dm^{-3}$ pyridine–formic acid buffer and metabolite 1 with 0.1 mol $dm^{-3}$ pyridine. Metabolite 1 was subjected to FAB mass spectrometry to confirm its structure. Because of small quantity of metabolite 2, physicochemical analysis was not performed with it.

### Identification of the metabolite

FAB mass spectra of metabolite 1 and synthetic trimethylarsine oxide proved these two compounds to be identical, with *m/z* 137 $[(CH_3)_3AsOH^+]$, the most intense peak (Fig. 3). On the basis of this result and high-performance liquid chromatographic behaviour, metabolite 1 was confirmed as trimethylarsine oxide.

## DISCUSSION

It has been shown for the first time that arsenobetaine is degraded to trimethylarsine oxide and probably dimethylarsinic acid, probably by intestinal microorganisms from a marine animal species, chitons. Dimethylarsinic acid was estimated only by its retention time in HPLC analysis; therefore, it must be identified by physicochemical analyses in future. The extent of degradation was not so large as that with some microorganisms occurring in the sediments, where arse-

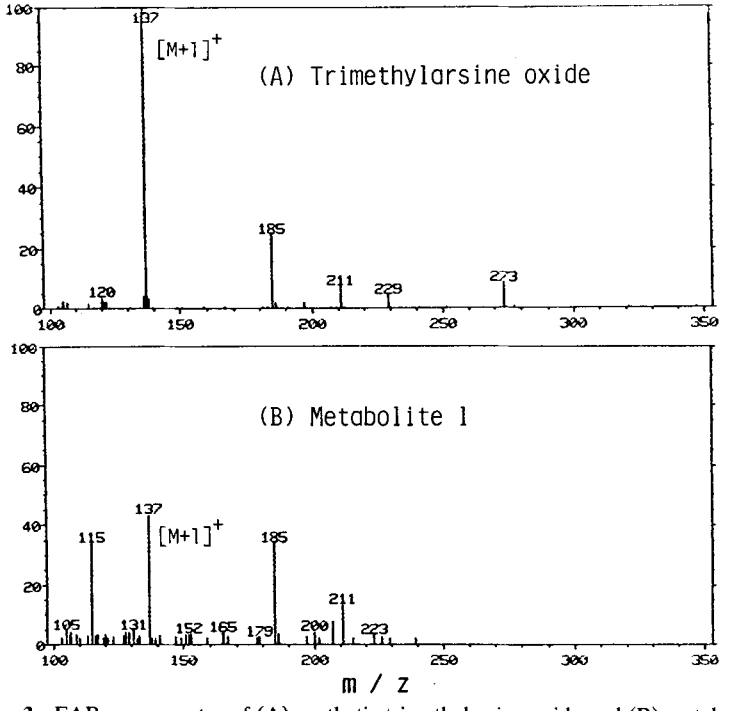


**Figure 3** FAB mass spectra of (A) synthetic trimethylarsine oxide and (B) metabolite 1.

nobetaine was completely degraded to inorganic arsenic.[6] The degradation extent was, however, comparable with that with the micro-organisms associated with marine macroalgae.[8] It is interesting that a similar conversion pattern is shown with those two sources, algae and intestine. We have not identified the micro-organisms in the intestine of the chitons or that associated with the macroalgae used. A part at least, however, of the micro-organisms in these sources may be in common to both, showing a similar conversion pattern. In order to verify this assumption, the arsenobetaine-decomposing micro-organisms occurring in those two sources need to be identified. Alternatively, conversion could result from an enzymic process derived from the chiton itself.

Trimethylarsine oxide was also reported to increase in the muscle of fish during cold storage at −20 °C.[10] Although this phenomenon is very interesting as compared with our investigation, it may have no relation to microbial activities.

No conversion was observed in an inorganic salt medium and using aerobic incubation. A difference in the extent of degradation has already been observed between these two media with arsenobetaine.[2,4-6,8] Such an extreme difference, however, as shown in this study has never been observed previously. Arsenobetaine alone may be insufficient as a carbon source for the intestinal arsenobetaine-decomposing micro-organisms to show their activity. We, however, only conclude, at the present stage, that arsenobetaine is degraded by intestinal micro-organisms under aerobic conditions.

No conversion of arsenobetaine was observed in either anaerobically incubated medium. This result was consistent with those obtained from experiments with sediments[5] or isolated arsenobetaine-decomposing bacterial strains,[7] where no or little arsenobetaine was converted in both media under anaerobic conditions. The conversion of arsenobetaine to trimethylarsine oxide implies the utilization of the carboxymethyl moiety of arsenobetaine by the micro-organisms. The conversion of arsenobetaine only in aerobic conditions may be caused by the utilization of this moiety in aerobic pathways such as the TCA cycle.

Three representative sources of marine micro-organisms have been investigated so far, including this study, i.e. sediments,[2-6] algae[8] and intestine of a marine animal. Arsenobetaine has been degraded with various pathways, in all cases under aerobic conditions. The microbial degradation of arsenobetaine may be a ubiquitous phenomenon in marine ecosystems. A major source of micro-organisms, namely suspended matter in marine ecosystems, however, still remains untouched. The degradation of arsenobetaine by micro-organisms occurring in it is now under investigation.

## CONCLUSION

Degradation of arsenobetaine was investigated in two types of growth media, 1/5 ZoBell and an inorganic salt medium, added with intestine of chitons, under both aerobic and anaerobic conditions. Arsenobetaine was degraded to trimethylarsine oxide and dimethylarsinic acid only in aerobically incubated 1/5 ZoBell 2216E medium. This extent of conversion was comparable with that shown by the micro-organisms associated with marine macroalgae and not so large as that by the micro-organisms occurring in sediments. The conversion is probably due to intestinal micro-organisms in the chiton, but an alternative process could be that it results from an enzymic process which is derived from the chiton and not from micro-organisms in the intestines.

*Acknowledgements* We express our sincere thanks to Dr T Murakami and Dr B Kimura, Laboratory of Microbiology, Department of Food Science and Technology, Shimonoseki University of Fisheries, for helpful microbiological advice.

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