ther data on its molecular and chemical properties will be necessary for the understanding of its possible role in the overcoming of the host plant resistance.

1 Acknowledgments. We acknowledge the assistance of Mr M. McNeil and P. Albersheim in doing the sugar analyses shown in this report. Financial support for this project was provided by a BARD grant 1-31-79, the French government, and the Montana Ag. Expt. Station.

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0014-4754/84/111244-04$1.50 + 0.20/0

Evidence for the presence of arsenobetaine as a major arsenic compound in the shrimp *Sergestes lucens*[1]

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Summary. The major arsenic compound in the shrimp *Sergestes lucens* was isolated and identified as arsenobetaine $(CH_3)_3A_s^+CH_2COO^-$. Arsenobetaine accounted for 80% of the total arsenic in the shrimp.
Key words. Shrimp; arsenobetaine; arsenic; marine ecosystem; marine food.

It is well known that marine organisms contain more appreciable amounts of arsenic than terrestrial organisms[2]. From the viewpoint of food hygiene, this fact evokes a serious problem for those people who consume a lot of marine organisms as food. Since the toxic effects of arsenic depend on its chemical form, recent studies concerning the arsenic in marine organisms have concentrated on the elucidation of this; the compounds which have so far been clearly identified are arsenobetaine in some animals[3–8] and peculiar arsenosugars in the brown kelp[9] and the giant clam[10]. Among crustaceans 2 species of lobsters, western rock lobster *Panulirus longipes cygnus*[3] and American lobster *Homarus americanus*[6], have been shown to possess arsenobetaine. In the shrimp *Sergestes lucens,* which is a species closely related to the lobsters, the presence of an arsenobetaine-containing oligopeptide was recently suggested, but it was not fully purified[11]. This suggestion led us to isolate and identify the major arsenic compound in this shrimp. As described below, the results revealed that the major arsenic compound in the shrimp was not an arsenobetaine-containing oligopeptide but nothing other than arsenobetaine.

For the estimation of total arsenic, samples were digested with a mixture of nitric acid, perchloric acid and sulfuric acid, dissolved in an appropriate volume of water, and applied to a Jarrell Ash inductively coupled plasma emission spectrometer (AtomComp Series 800). In the course of purification, arsenic was determined on the same spectrometer without wet-digestion.

Fresh specimens of *S. lucens* (1.2 kg, As 5.5 ppm) obtained at Tokyo Central Wholesale Market were lyophilized and ground to powder. The powdered sample was extracted 3 times with methanol and the methanolic extracts were concentrated to dryness. The residue obtained was suspended in a small volume of water and defatted 3 times with an equal volume of ether. The aqueous phase (water-soluble fraction) which contained approximately 90% of the total arsenic in the starting specimens was used for the following purification procedure. The water-soluble fraction was first subjected to adsorption chromatography on Amberlite XAD-2. The unadsorbed fraction was applied to a column of Dowex 50 × 2 (H^+ form) equilibrated with water. The major arsenic compound was adsorbed by the column and could be eluted with 0.2 N ammonium hydroxide after washing the column with water. The eluate containing arsenic was combined, concentrated to remove ammonia, and passed successively through columns of Dowex 2 × 8 (OH^- form) and Amberlite CG-50 (H^+ form) both of which were previously equilibrated with water. The arsenic fraction was then put onto a column of Dowex 50 × 2 (pyridinium form) which was equilibrated with 0.1 M pyridine-formic acid buffer (pH 3.1). When eluted with the same buffer, the arsenic compound exhibited a weak interaction with the column and was eluted in volumes between 3 and 4 times the column volume. Chromatographic separations on Amberlite CG-50 (H^+ form) and Dowex 50 × 2 (pyridinium form) were performed again. Finally, gel filtration on Bio-Gel P-2 with water yielded 5.7 mg of the purified arsenic compound. The arsenic content of the purified compound was 40% which is comparable to the calculated value for arsenobetaine (41%). When analyzed by TLC on precoated silica gel 60 plates

(Merck) with 3 solvent systems consisting of ethanol-acetic acid-water (65:1:34), 1-propanol-water (7:3), and 1-propanol-34% ammonium hydroxide (7:3), or on precoated cellulose plates (Funakoshi) with a solvent system of 1-butanol-acetic acid-water (60:15:25), the purified arsenic compound gave a single spot in each system. The spot, which was positive to iodine vapor and the Dragendorff reagent but negative to ninhydrin, appeared at an Rf value identical with that of arsenobetaine. The homogeneity of the purified compound was further evidenced by electrophoresis on cellulose acetate strips (Sartorius); with a buffer system of pyridine-acetic acid-water (1:10:89; pH 3.6), its mobility was the same as that of arsenobetaine.

The purified arsenic compound exhibited no characteristic UV absorption band. Its ^{1}H-NMR spectrum (100 MHz, D_2O) gave 2 signals at δ 1.87 (singlet, 9 H) and 3.30 (singlet, 2 H). The field desorption mass spectrum showed a molecular ion peak at m/z 179 and a base peak at m/z 135 (M^+-CO_2). These spectral data coincided well with those reported for arsenobetaine[3, 7, 12].

Judging from these results, the purified arsenic compound from the shrimp *S. lucens* was identified as arsenobetaine $(CH_3)_3A_s^+CH_2COO^-$. As mentioned above, about 90% of the total arsenic in the shrimp was found in the water-soluble fraction. In addition, 90% of the arsenic in the water-soluble fraction was adsorbed by Dowex 50 × 2 (H^+ form) and it was attributed to arsenobetaine because no arsenic compounds other than arsenobetaine were detected in any steps of the succeeding purification procedure. Therefore, arsenobetaine seemed to account for approximately 80% of the total arsenic of the shrimp.

Fukui et al.[11] previously suggested the presence of an arsenobetaine-containing oligopeptide in the shrimp. However, the suggestion should now be accepted with doubt because their final preparation was apparently contaminated with a large amount of impurities and its arsenic content was as low as 0.092%. Actually, just when we started this work, Norin et al.[12] also reported from the behavior of the compounds in TLC and electrophoresis that not an arsenobetaine-containing oligopeptide but arsenobetaine and arsenocholine were present in shrimps whose scientific names were not given. Our results prove the presence of arsenobetaine in the shrimp *S. lucens* and support those of Norin et al. We could not, however, detect arsenocholine in the shrimp. It seems reasonably safe to assume that, apart from arsenocholine, arsenobetaine is a common arsenic compound in shrimps.

All the marine animals in which the presence of arsenobetaine has been reported so far are carnivores. The present study, together with that of Norin et al.[12], confirmed the presence of arsenobetaine in shrimps which are typical non-carnivores and plankton-feeders. This finding is very interesting from the point of view of the marine ecosystem. Although the arsenic in marine animals in higher trophic levels is present chiefly in organic forms such as arsenobetaine, they cannot transform inorganic arsenic, which is the major form of arsenic in sea water, into organic arsenic in their own bodies[13]. It is very likely, therefore, that the inorganic arsenic in sea water is first incorporated and metabolized to organic arsenic compounds such as arsenobetaine and its precursor by plankton. The shrimp and other plankton-feeders will accumulate arsenobetaine directly from the plankton or will incorporate a precursor from them and convert it to arsenobetaine. Finally, carnivorous animals will get arsenobetaine from their food, including shrimps. Another pathway, from arsenosugars found in the brown kelp to arsenobetaine in marine animals, has also been suggested by Edmonds et al.[14]. For more detailed discussion on the arsenic cycle in the marine ecosystem it will be necessary to elucidate the chemical forms of arsenic in a wide variety of marine animals in connection with feeding habits.

1 We are grateful to Drs N. Fusetani and S. Matsunaga, the University of Tokyo, for estimating the ^{1}H-NMR and field desorption mass spectra of the isolated arsenic compound. We also wish to thank Dr M. Chiba, Research Station, Agriculture Canada, Vineland Station, Ontario, Canada, for revising this manuscript. This work was partly supported by a grant from the Ministry of Education, Science and Culture of Japan.

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0014-4754/84/111247-02$1.50 + 0.20/0

Alteichin: an unusual phytotoxin from *Alternaria eichorniae*, a fungal pathogen of water hyacinth[1]

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Summary. The phytopathogenic fungus *Alternaria eichorniae* attacks water hyacinth, an economically significant aquatic weed. The novel phytotoxin alteichin was isolated from liquid cultures of this fungus and its structure was deduced by X-ray crystallographic analysis. Altheichin is a doubly hydrated form of 4,9-dihydroxy perylene-3,10-quinone. A single step dehydration of alteichin to anhydroalteichin is catalyzed both by acid and by a crude enzyme preparation from water hyacinth.
Key words. Fungus, phytopathogenic; *Alternaria eichorniae;* phytotoxins; altheichin.