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BIOACTIVE COMPOUNDS OF THE MARINE HEMICHORDATE *PTYCHODERA FLAVA* OF THE MADRAS COAST

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ABSTRACT

A wide variety of halometabolites is ubiquitous in the marine biome. There are about 500 naturally occurring halogenated compounds which are produced by a wide variety of marine biota. Although iodine has been associated with the hemichordates, chemical characterisation has revealed the occurrence of bromo-organic compounds. In order to update the information on the chemical nature of the hemichordates, a review on its chemical constituents has been given.

It has been reported that the toxicity of the crude extract of the hemichordates is depended on the strength of the extract. High concentrations of the extract inhibited the physiological and other life processes. An attempt has been made in this paper to characterise the chemical species that may be responsible for such a behaviour. Using the NMR and IR spectrometers, the chemical structure of the bio-dynamic natural chemical substances was studied. The study revealed the presence of aromatic, $-CH_2$ proton and $-OH$ proton groups. Absence of $-NH_2$ proton and the presence of $-CH_2$ $-CH_3$ groups are considered significant. The possible ecophysiological significance of these chemical compounds have been suggested.

INTRODUCTION

THE STUDIES of Azariah *et al.* (1975) indicated that the secretion of *Ptychodera flava* may contain bioactive compounds. The effect of crude extract of *P. flava*, collected during the breeding and non-breeding seasons was studied with reference to the growth of diatoms *Amphora coffeaformis* and *Cyclotella meheghiniana* and development of eggs of the polychaete worm *Hydroides elegans*. These studies showed that low concentration of the extract stimulated the divisions of the cells of the diatoms and cleavage of eggs of *H. elegans*, while higher concentrations inhibited the diatom multiplication (Azariah and Pillai, 1985). The extract of *P. flava* when intramuscularly administered may either accelarated or depress the level of copper in the haemolymph of *Scylla serrata*. The above studies indicated the presence of biodynamic substances in the extract of *P. flava*.

Ashworth and Cormier (1967) attempted to characterise the chemical compounds responsible for the production of the characteristic iodoform-like odour in the beds of hemichordates and found 2-6 dibromophenol in large concentration, amounting to 10-15 mg/organism. Later Higa (1976) and Scheuer (1976) isolated blue pigments from the body of *P. flava lysanica* and characterised the chemical nature of the pigments as 6, 6-dibro-

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moindigotin, 5, 7, 5, 7-tetrabromo 6, 6-dimethoxyindigotin and 5, 7, 6-tribromo 6-methoxyindigotin. In addition, they also detected a colourless compound 5, 7-dibromo 6-methoxyindole. However, Higa and Scheuer (1975) failed to identify the presence of 2, 6-dibromophenol which was reported to be present in *P. flava* (Ashworth and Cormier, 1967). But they identified a simplest bromophenol, 2, 4, 6-tribromophenol. It is of interest, therefore, to characterise the bioactive compounds present in *P. flava* inhabiting in the Krusadi Island waters. The paper reports the presence of ethyl phenol and indocresol compounds.

Materials and Methods

Adult specimens of *P. flava* were collected from the Galaxea Lagoon of the Krusadi Island. Entire specimens were gently removed from their burrow and washed repeatedly in seawater. The animals were left undisturbed for a period of 2 to 3 hours so as to clear the gut contents. Animals without any sand particle in the gut were further processed for various experiments. Dry samples of *P. flava* were well powdered and made into transparent pellets by adding Potassium bromide (KBr). The procedure of Williard *et al.* (1974) was followed for the preparation of the sample for Infra-red spectroscopy and Nuclear Magnetic Resonance. The IR spectrum of each pellet was recorded using Perkin Elmer 1983 spectrometer. The IR spectrum obtained is the plot of percentage transmittance (Y-axis) verses frequency (cm^{-1}, X-axis).

The dry samples of *P. flava* dissolved in cadmium chloride ($CdCl_3$) were kept in a quartz tube. The quartz tube was placed between the poles of a powerful magnet and irradiated with radio frequency energy. At a particular combination of frequency and magnetic field strength, energy is absorbed and the nucleus is flipped from one spin state to another. The absorption of energy is recorded as peak signal in the NMR spectrum.

All the NMR spectra were recorded using the Varian model EM 390, 90 MAL NMR spectrometer, using cadmium chloride as solvent and tetramethyl silane as the internal standard.

Results

The NMR spectral values and the IR stretching frequency (cm^{-1}) region for the chemical compounds present in the *P. flava* are given in Table 1.

Table 1. *Infra-red (IR) and Nuclear Magnetic Resonance (NMR) spectral values obtained for* P. flava *and probable chemical compounds*

Nature of Chemical compound		Spectral value
NMR analysis	Aromatic	6.5-6.9
	$-CH_3$	2.1
Spectral value (δ)	—OH	3.0
	$-NH_2$	—
	$-CH_2-CH_3$	2.1 and 3.5
IR Stretching	Aromatic	1653-1640
	OH	3394-3220
	NH	—
Frequency (cm) region	C-Cl	—
	C-Br	—
	C-I	521
Probable chemical compound	Ethyl phenols	Iodocresol

Samples of *P. flava* subjected to analysis of NMR spectroscopy indicated the presence of aromatic protons (6.5-6.9 δ CH_3 proton (2.1δ) and OH proton (3.0δ). The sample was characterised by the absence of NH_2 proton. A feature of special mention is occurrence of — CH_2CH_3 protons (2.1 and 3.5δ). The IR studies provided confirmatory evidence for the occurrence of aromatic and

-OH group due to the absorbance in the range of 1653-1640 cm^{-1} and 3394 cm^{-1} respectively. In addition the IR spectrum revealed the presence of C-1 group. The occurrence of iodine moiety (C-I) was confirmed by the absorbance at 521 cm^{-1}. On the basis of the above results, it is reasonable to infer that the *P. flava* may contain ethyl phenol and iodocresol.

Discussion

It is known that halogenated organic compounds are ubiquitous in the biosphere. As a result, a wide variety of halometabolites have been isolated from marine organisms. There are about 550 naturally occurring halogenated compounds which are produced by 250 biological organisms (Siudo and De Bernardis, 1973 ; Strunz, 1976 ; Faulkner, 1977). The number of chlorinated compounds exceed 150 including 50 brominated substances. On the other hand, fluorinated and iodinated compounds are few in number. Bromine bound with organic substances occurring as bromo-organic compounds varies between 3 and 50 ppm (Lunde, 1973). Bromine containing metabolites have been identified in hemichordates *Balanoglossus biminiensis*.

The characterisation of ethyl phenol and iodocresol in *P. flava* differs from earlier studies (Ashworth and Cormier, 1977 ; Higa and Scheuser, 1976, 1977 ; Higa *et al.*, 1980) who have reported the occurrence of bromo and chloro compounds. The cause for the absence of bromophenol and other biochemicals in *P. flava* of Krusadi Island is not known. It is likely, there may be species differences in phylum Hemichordata.

According to Higa and Scheuer (1976), the compound responsible for the reportedly iodoform—like odour of *P. flava* are 3-chloroindole and trace amount of 3-bromoindole and 6-bromo, 3-chloroindole. They have observed differently coloured animals during their collection. Those which were blue in colour, had only a faint odour. The search for the substances responsible for the iodoform-like odour of *P. flava* led to the isolation of four halogenated indoles. Of the four chemical compounds. 3-chloroindole and 3-bromoindole were responsible for the odour, while 6-bromo 3-chloroindole and 3, 5, 7 tribromoindole were virtually odourless compounds (Higa and Scheuer, 1977 ; Higa *et al.*, 1980). The chemical structure of the synthetic 6-bromochloro 3, 5, 7 tribromoindole were compared with the *Ptychodera* metabolites which indicated a similarity in all physical and chemical properties.

A feature of interest that is worth consideration is the variation in the occurrence of halogenated phenols and indole constituents within the members of the hemichordata. Hitherto six species of hemichordates have been subjected to chemical analysis. They were *Ptychodera flava lysanica*, *P. flava*, *Glossobalanus* sp., *Balanoglossus curnosces*, *B. misakiensis* and *B. biminensis*. Higa *et al.* (1980), in a comparative study on hemichordates of Japanese waters found species differences in the chemical compounds. They reported that the bromophenols are closely associated with *Balanoglossus* whereas 3-haloindoles are closely associated with the genera *Ptychodera* and *Glossobalanus*. On the basis of their study they suggested that the distribution of different halometabolites among different species may be due to variation in competing halogens and oxidation process which operate in common precursors. The chemical picture obtained from *P. flava* of Indian waters is quite different from that of its counterparts of the world. The total number of 18 compounds belonging to phenols, hydroquinones and indoles have been identified in hemichordates (Higa *et al.*, 1980). The results reported in the present study is different in that none of the 18 compounds were present in *P. flava* of Indian waters. NMR studies

revealed that the bromophenols were absent in *P. flava*. In the present study, the occurrence of iodocresol and ethyl phenol compounds have been recorded. But iodo groups are linked with cresols, a related component of phenol. The distribution of different halometabolites linked with phenols, hydroquinone and indoles are differently present in related species of hemichordates. From the foregoing consideration, intraspecies variation in the occurrence of halogenated compounds in hemichordata is evident. The apparent variation in the halogenated chemical moieties among various species of hemichordates may be attributed to the difference in the occurrence of haloperoxidase.

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