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VARIATIONS IN THE HALOGENATED MONOTERPENE METABOLITES OF *PLOCAMIUM CARTILAGINEUM* AND *P. VIOLACEUM**

JON S. MYNDERSE and D. JOHN FAULKNER
Scripps Institution of Oceanography, La Jolla, CA 92093, U.S.A.

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Key Word Index—*Plocamium cartilagineum*; *P. violaceum*; Plocamiaceae; red algae; halogenated monoterpenes; variation in metabolite content.

Abstract—Observed variations in the halogenated monoterpene content of *Aplysia californica* were due to digestion of *Plocamium cartilagineum* and *Plocamium violaceum*. Whereas *Plocamium violaceum* was chemically homogeneous, some variation in the composition of halogenated monoterpene composition of individual plants of *P. cartilagineum* was observed.

During the course of our studies on the chemical constituents of the digestive gland of the sea hare *Aplysia californica*, we observed considerable quantitative and qualitative variations in the halogenated monoterpenes and sesquiterpenes obtained from different collections of animals [1, 2]. Although we could show that some chemical transformations occur within the digestive system of *A. californica* [3], the variations in chemical content are mainly due to variations in the algal diet of *A. californica* [1]. In particular, the variations in the monoterpene content [4] of individual *A. californica* showed that there should be several algal sources of halogenated monoterpenes in the La Jolla area.

In a subsequent study, we found only two major sources of halogenated monoterpenes in the La Jolla area, namely the red algae *Plocamium cartilagineum* Dixon and *P. violaceum* Farlow. We have reported two major metabolites **1** and **2** from *P. violaceum* [5, 6] and twelve metabolites **5–16** from *P. cartilagineum* [7]. Other halogenated monoterpenes [2, 8] previously isolated from *A. californica* were not detected in this study. We were intrigued to find that various bulk samples of *P. cartilagineum* collected in La Jolla contained different ratios of halogenated metabolites. In a parallel study, Crews has observed that collections of *Plocamium* from different locations in the vicinity of Santa Cruz contained different metabolites [9, 10].

In considering the possible causes of these variations, we had first to eliminate the possibility that the compounds observed were the result of chemical decomposition during extraction. It is known that some monoterpenes isolated from terrestrial sources are actually artifacts of isolation. The situation for halogenated monoterpenes is potentially worse, since dehydrohalogenation reactions are autocatalytic. We therefore compared the compounds resulting from our extraction procedure with those from the low-temperature extraction procedure, which is considered by Banthorpe *et al.* [11] to be least likely to cause decomposition.

We set out to investigate possible causes of the observed variations by analyzing the halogenated monoterpene content of individual plants collected from various depths and locations between September 1974 and March 1975. We preserved and identified voucher samples of each plant and noted those plants bearing cystocarps or tetraspores. Since the quantity of plant material required for a GLC analysis of the halogenated monoterpenes was so small, we could perform several analyses on the same plant and could compare different parts of the same plant. The results obtained in this study provide a reasonable explanation of the variations in chemical content of the bulk samples of *Plocamium* sp. and of the variation in monoterpene content of individual *Aplysia*.

The GLC traces resulting from the liquid nitrogen–hexane, $CHCl_3$–MeOH, and Et_2O extraction procedures of one individual specimen of *Plocamium violaceum* are reproduced in Fig. 1. The three GLC traces from the *P. violaceum* specimen showed the same monoterpene constituents in very nearly the same proportions, irrespective of the extraction procedure. We concluded that all the components observed were genuine secondary metabolites of *P. violaceum*. Four of the peaks in each GLC trace were identified as the cyclic monoterpenes **1–4** by coinjection of authentic samples and by GC–MS. For the purpose of qualitative comparative studies of intraspecies variation of halogenated monoterpenes in *Plocamium* species, we chose ether extraction of dried specimens, the simplest procedure.

In the case of *Plocamium cartilagineum*, the GLC traces resulting from the three different extraction procedures revealed some variation of components (Fig. 2). However, the halogenated monoterpenes **5–14** were all detected in a liquid nitrogen–hexane extract (Fig. 2a) and must be considered true secondary metabolites. The relative concentrations of the halogenated monoterpenes **5–14** were comparable for both liquid nitrogen–hexane and ether extraction procedures. None of the additional peaks observed in the ether trace were due to halogenated monoterpenes. Some chemical reactions which could have occurred during the extraction procedure were the dehydrochlorination of **14** to obtain **15** or **16**, epimerization at C-4 affecting the **6**:**7**, **9**:**10**, and **12**:**13** ratios or

*Taken from the Ph.D. Thesis of J. S. Mynderse, UCSD (1975).

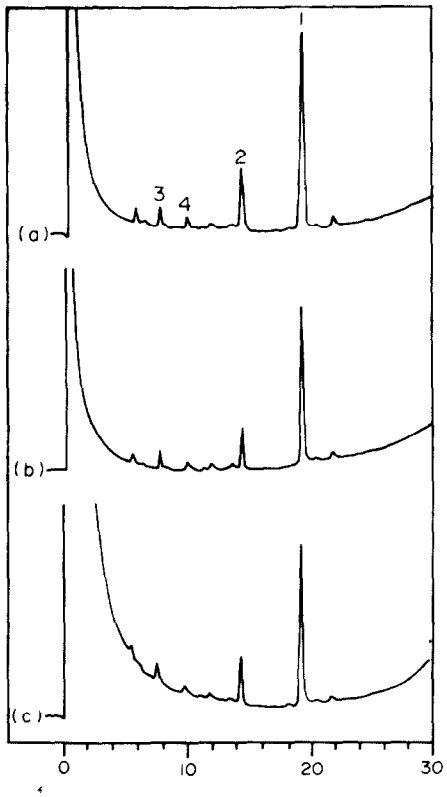


Fig. 1. GLC traces of three extracts of a single specimen of *Plocamium violaceum*. (a) Liquid N_2–hexane extract; (b) $CHCl_3$–MeOH extract; (c) Et_2O extract of dry alga. (See Experimental for details of GLC analysis, retention times in minutes).

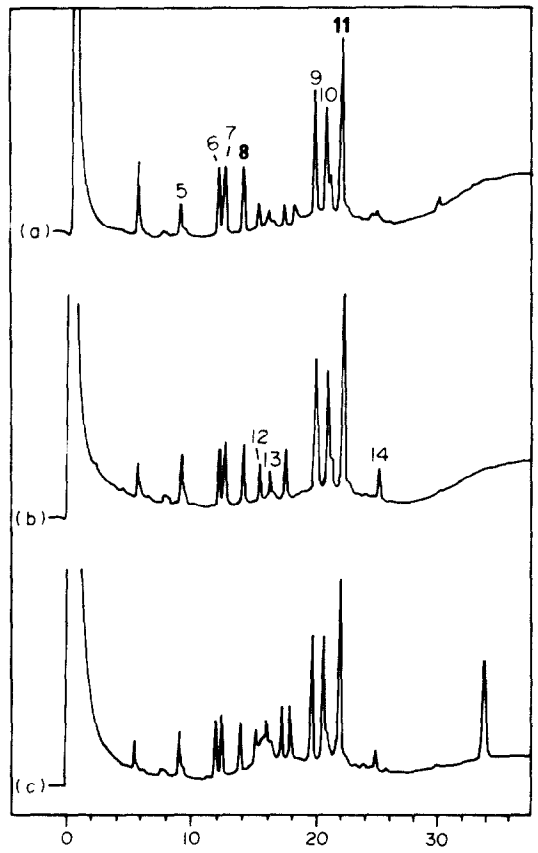
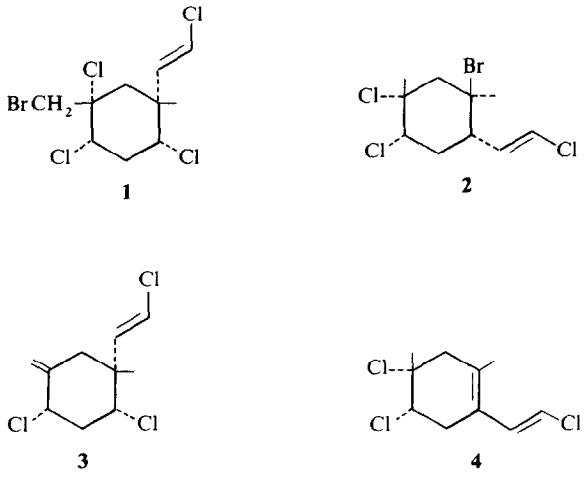


Fig. 2. GLC traces of three extracts of a single specimen of *Plocamium cartilagineum*. (a) Liquid N_2–hexane extract; (b) $CHCl_3$–MeOH extract; (c) Et_2O extract of dry alga.

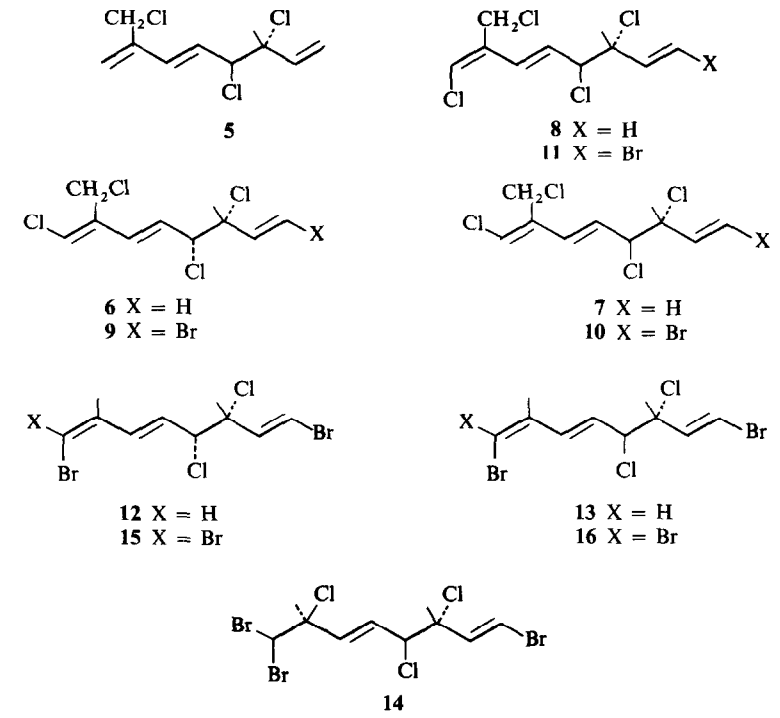


isomerization of the $\Delta^{7,8}$ olefinic bond resulting in changing **7**:**8** or **10**:**11** ratios. Comparison of Figs 2a and 2c revealed that none of these reactions had occurred, justifying use of the ether extraction procedure. Since ether effectively extracts the halogenated monoterpenes, we were prejudiced against using $CHCl_3$–MeOH, which extracts considerably more colored, non-volatile and polar materials that can contaminate the GLC column and cause peak broadening.

We found that there could be considerable variation of halogenated monoterpene content of *P. cartilagineum*. In order to determine the extent of variation in halogenated monoterpene composition within the two species of *Plocamium*, we periodically sampled both species at Bird Rock, La Jolla, between September 1974 and March 1975.

The ether extracts of all specimens of *Plocamium violaceum* contained the same components. For 28 specimens examined in detail, violacene (**1**) was always the major component, and, with a single exception, violacene-2 (**2**) was the second most abundant component. The minor components **3** and **4** were among the six most abundant components in all specimens.

Careful analysis of the GLC traces due to ether extracts of individual specimens of *P. cartilagineum* collected at Bird Rock revealed three 'patterns' of monoterpene composition. Three specimens (Figs 3a–c) contained three isomers of $C_{10}H_{11}Cl_5$ (**6**, 7 and **8**) and three isomers of $C_{10}H_{10}BrCl_5$ (**9**, **10** and **11**), as well as other halogenated monoterpenes. Two specimens (Figs 3d and 3f) contained 7Z isomers (**6**, **7**, **9**, **10**) but lacked the corresponding 7E isomers (**8**, **11**), while two specimens (Fig. 3e) contained 7E isomers but no 7Z isomers. Of 21 specimens examined in detail, eight contained all compounds, seven lacked the 7E isomers, five lacked the 7Z isomers and one contained two unknown compounds as the major components. The GLC traces of five specimens of *P. cartilagineum*



5

8 X = H
11 X = Br

6 X = H
9 X = Br

7 X = H
10 X = Br

12 X = H
15 X = Br

13 X = H
16 X = Br

14

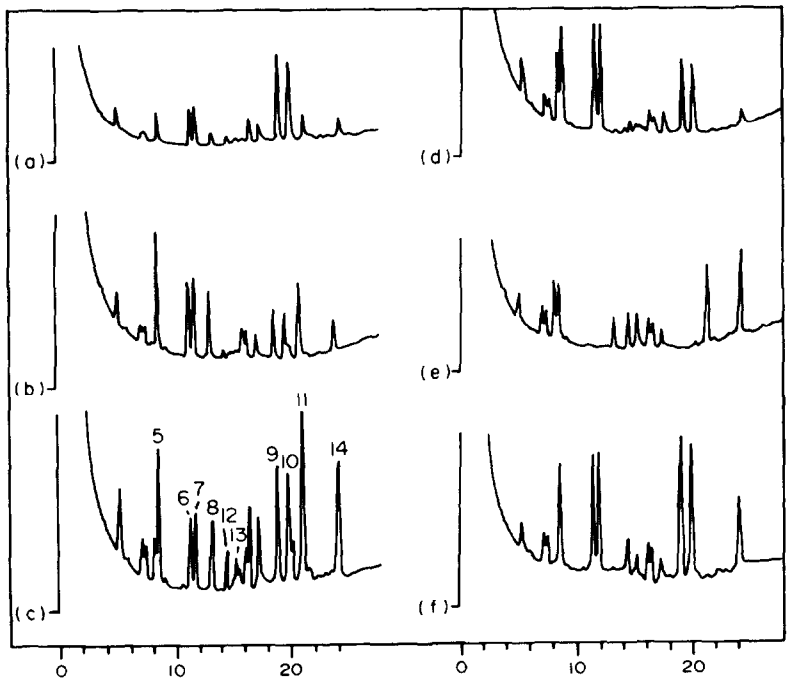


Fig. 3. GLC traces of ether extracts of six individual specimens of *Plocamium cartilagineum* collected at Bird Rock, La Jolla.

from Quast Rock (−20 m) all lacked the 7E isomers (cf. Fig. 3d), while those of 23 specimens from T Rock (−20 m) revealed 18 specimens containing all compounds and five lacking the 7E isomers.

The reasons for intraspecies variation of polyhalogenated monoterpene composition in *P. cartilagineum* are not known. We found no evidence for seasonal variation or variation due to sexual differences. The observed differences could be attributed to as little as a subtle change in a single enzymatic process.

We conclude that the variations in halogenated monoterpene content in the digestive glands of individual

Aplysia, as well as variations in monoterpene content of bulk collections of *Plocamium*, are due to variations at the individual plant level. When these results were compared with the array of metabolites which have been obtained from specimens of *P. cartilagineum* from Santa Cruz, England, Australia and Antarctica, a truly confusing picture emerges. At best, one can observe that all samples contain halogenated monoterpenes but that the metabolites show considerable variation, depending on collecting location [12].

EXPERIMENTAL

Collection of Plocamium *specimens.* Individual specimens of *Plocamium violaceum* were collected periodically from an intertidal area (*ca* 200 × 20 m) at Bird Rock, La Jolla, California from Septemeber 1974 through May 1975. Individual specimens of *Plocamium cartilagineum* were collected periodically from a small intertidal area (*ca* 1 × 2 m) at Bird Rock, La Jolla, California from September 1974 through May 1975 and from isolated rock formations (T Rock and Quast Rock) in water 20 meters deep off La Jolla, California in February and March 1975.

Extraction procedures. Individual specimens of *Plocamium cartilagineum* and *P. violaceum* were collected and checked to ensure that all thalli were continuous through the holdfast. *Ca* one-third of each specimen was quickly shaken in deionized H_2O to remove extraneous organisms and salts, blotted dry, and immediately frozen in liquid N_2. Upon return to the laboratory, the specimen was pulverized with a mortar and pestle containing liquid N_2. Pre-cooled hexane (distilled) was added. The resulting solid was pulverized and transferred with additional liquid N_2 to a pre-cooled flask, which was then placed in a freezer. After several hours in the freezer, the hexane extract was subjected to GLC analysis. Another one-third of each specimen was rinsed in deionized H_2O, blotted dry, shredded and extracted with $CHCl_3$–MeOH (1:1) in the refrigerator for several hours. The extract was then partitioned into two layers by the addition of H_2O. The $CHCl_3$ layer was rinsed with H_2O, dried and then subjected to GLC analysis. The remainder of each specimen was rinsed in deionized H_2O, blotted dry, separated into individual thalli and dried 18 hr at room temp. The individual thalli were then crushed and transferred to vials and Et_2O was added. GLC analyses were then performed on the extracts of individual thalli.

GLC analysis of Plocamium *extracts.* GLC analyses were performed on a Hewlett-Packard 402 gas chromatograph using a 183 cm × 2 mm i.d. column containing 3% SP-2250 on Supelcoport 100/120 and a flame ionization detector. The flash heater was customarily maintained at 170° and the oven temp. at 140° for 2 min after injection of the sample, then increased at 4 min to 230°. Components were identified by comparison of retention data to that of the pure compounds and, when necessary, by GC coinjection and by GC–MS on a Hewlett-Packard 5930A GC–mass spectrometer system.

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