

HALOGENATED MONOTERPENES FROM *PLOCAMIUM COCCINEUM*
OF NORTHWEST SPAIN¹

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The red algae of the genus *Plocamium* are known to be an important source of halogenated monoterpenes (1). In the course of a program aimed at the isolation of secondary metabolites from marine organisms of our coast,¹ we have undertaken the study of *Plocamium coccineum* Lyngb., a species which, to our knowledge, has not been investigated. In this paper we describe the isolation of six known halogenated monoterpenes, **2-7**, and a new one, **1**, which we have named coccinene, having the violacene type skeleton (1).

RESULTS AND DISCUSSION

MeOH extracts of fresh *P. coccineum* were concentrated under vacuum and the residue partitioned between hexane and H₂O. The hexane extracts were chromatographed on a silica gel column, giving a fraction containing a mixture of halogenated compounds which could be separated into five major fractions by preparative hplc (normal phase). Reverse-phase hplc of those fractions gave compounds **1-7**.

The minor components (less than 5% of the mixture) **3** (2), **4** (3), **5** (4), **6** (5), and **7** (5) had already been isolated from other *Plocamium* species and were identified by their spectroscopic and physical data. Compound **2** accounts for 65% of the total mixture and was identified by direct comparison with an authentic sample isolated from *Plocamium cartilagineum* (2). Monoterpene **1** (30% of the total mixture) is a crystalline solid

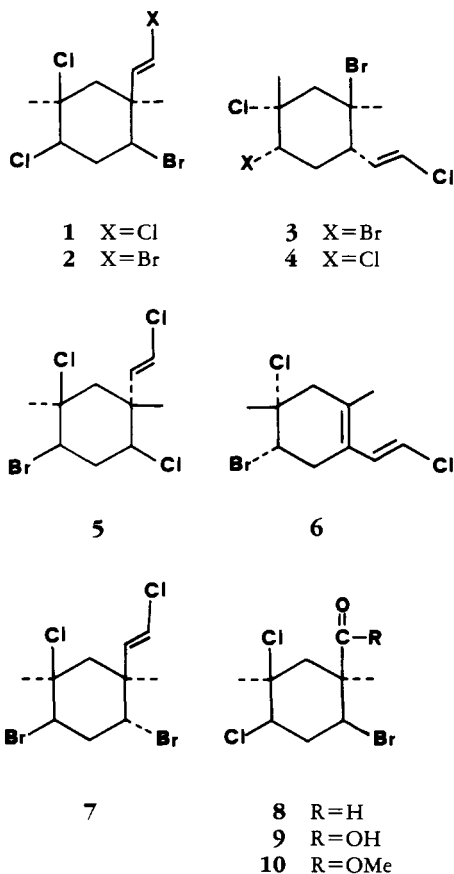
whose mass spectrum corresponds to a C₁₀H₁₄BrCl₃ molecular formula. Its pmr spectrum (250 MHz) is quite similar to that of **2**, with the exception of the olefinic protons that resonate as an AB quartet centered at $\delta_A=6.15$ and $\delta_B=6.21$ ppm ($J=13.8$ Hz), which indicates an *E* configuration. The cmr spectrum of **1** displays two signals for the olefinic carbons at $\delta=120.6$ and 135.4 ppm, while in **2**, these signals appear at 108.4 and 139.7 ppm. For this compound, all the above data suggest structure **1** has a chlorine substituent at the vinyl group. Further support for this structure was obtained from chemical evidence. Ozonolysis of **1** and **2** gave the same aldehyde **8** which did not display a molecular ion cluster in the mass spectra. Therefore, this aldehyde **8** was further characterized by oxidation with Bu₄NMnO₄ (6) to give acid **9** which, upon treatment with CH₂N₂, produced ester **10**, identified from its spectroscopic data (ms, nmr, ir).

From a chemosystematic point of view, it might be of interest to point out that only cyclic halogenated monoterpenes have been found in this alga and that its overall composition seems to remain unchanged by the time of the year (April to October), the mode (dredging or intertidally), and the exact location of the collection (from Bastiagueiro in La Coruña to Patos in Vigo), which is in contrast with earlier findings (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected, pmr and cmr spectra were taken in CDCl₃ solutions, and chemical shifts are given in ppm with TMS as the internal standard, coupling constants are given in Hz. The spectra were registered with a Varian CFT-20

¹Part II in the series "Marine Natural Products from the Galician Coast." For part I, see L. Castedo, E. Quiñoá, and R. Riguera, *An. Quím. (C)*, **79**, 454 (1983).



spectrometer operating at 80 MHz for proton and 20 MHz for ^{13}C carbon, and a Bruker WM 250 operating at 250.13 MHz. Mass spectra were run on a "Kratos MS-25." Ir spectra were measured with a Pye Unicam 1100 in CHCl_3 . Optical rotations were taken on a Perkin-Elmer 141 polarimeter. A Waters Associates 6000A chromatograph was used for the hplc separations on Whatman Partisil and Partisil ODS columns.

PLANT MATERIAL.—*P. coccineum* was collected along the coast of N.W. Spain between April and October, intertidally at Bastiagueiro, Aguiño, La Lanzada and Patos, and by dredging at Ria de Arosa. The different batches were shown by hplc to have the same composition in compounds 1-7. The alga was authenticated by Prof. X. Niels (Instituto de Oceanografía, Vigo) by comparison with an authentic specimen.

EXTRACTION AND ISOLATION.—Fresh *P. coccineum* (5 kg) was extracted several times with MeOH. The extracts were concentrated in vacuo, and the residue was partitioned between H_2O and hexane. The organic phase upon evaporation produced 18 g of an oil which was chromatographed on a silica gel column. Elution with hexane gave 5 g of a mixture of terpenes 1-7.

HPLC SEPARATION.—Of the above mixture, 4.9 g was submitted to hplc on a Partisil M9 column by injecting a maximum of 100 mg each time. Elution with hexane produced, in inverse order of elution, the following five fractions: (a) 4.643 g of a mixture of 1 and 2, (b) 0.115 g of a mixture of 3 and 4, (c) 0.074 g of 5, (d) 0.016 g of 6, and (e) 0.012 g of 7. Hplc of fraction (b) on a Partisil M9 10/50 ODS-3 (reverse phase) column eluting with acetonitrile- H_2O (65:35) gave 92 mg of 3 and 22 mg of 4 (fast moving compound). Analogously 300 mg of fraction (a) were separated by elution with acetonitrile- H_2O (60:40) affording 92 mg of 1 (fast moving compound) and 208 mg of 2. Compounds 5, 6, and 7 were also purified by reverse phase hplc eluting with acetonitrile- H_2O (65:35).

Compound 1: mp 65° (*n*-hexane), $[\alpha]^{25}_{\text{D}} = -24$ (*c* 0.82, CHCl_3). Pmr δ 1.26 (s, 3H), 1.66 (s, 3H), 2.20 and 2.58 (AB system, 2H, $J = 14.3$ Hz), 2.38 (dd, 1H, $J = 12.7, 13.9$ Hz), 2.65 (dt, 1H, $J = 4.2, 13.9$ Hz), 3.84 (dd, 1H, $J = 4.0, 12.9$ Hz), 4.15 (dd, 1H, $J = 4.3, 12.5$ Hz), 6.15 and 6.21 (AB system, 2H, $J = 13.8$ Hz). Cmr δ 26.1, 30.0, 41.1, 43.3, 55.2, 56.6, 66.9, 70.6, 120.6 and 135.3. Ms m/z 318, 320, 322, 324 (M^+) 283, 285, 287 ($\text{M}^+ - \text{Cl}$) 247, 249, 251 ($\text{M}^+ - \text{Cl}_2\text{H}$) 239, 241, 243 ($\text{M}^+ - \text{Br}$) 203, 205, 207 ($\text{M}^+ - \text{BrClH}$) 167, 169 ($\text{M}^+ - \text{Cl}_2\text{BrH}_2$).

OZONOLYSIS OF 1 AND 2.—Compound 2 (50 mg) was ozonized in 2 ml of dry CH_2Cl_2 for 10 min at -78° . After that time, a few drops of Me_2S were added, and the mixture was allowed to reach room temperature. The solution was washed with H_2O , dried over anhydrous MgSO_4 , and concentrated, giving a solid which was purified by hplc on a Partisil M9 10/50 column eluting with hexane-EtOAc 2.5% giving 34 mg of aldehyde 8 mp 85° , $[\alpha]^{25}_{\text{D}} = -96$ (*c* 0.42, CHCl_3). Pmr δ 1.24 (s, 3H), 1.50 (s, 3H), 1.90-3.00 (m, 4H), 3.97 (dd, 1H, $J = 5.0, 12.5$ Hz), 4.16 (dd, 1H, $J = 4.9, 11.9$ Hz), 9.93 (s, 1H). Ms m/z 221, 223, 225 ($\text{M}^+ - \text{COClH}_2$) 178, 180, 182 ($\text{M}^+ - \text{COBrH}_2$) 141, 143 ($\text{M}^+ - \text{COClBrH}_3$).

Analogously, ozonolysis of 1 gave aldehyde 8 identical to that obtained from 2.

PREPARATION OF 9 AND 10.—To a CH_2Cl_2 solution of Bu_4NMnO_4 prepared from KMnO_4 and Bu_4NCl by the phase transfer method (6) 10 mg of 8 in 1 ml of CH_2Cl_2 was added with stirring. After stirring for 2 h, the reaction mixture was allowed to stand for another 2 h and then filtered, dried over MgSO_4 , and concentrated, giving 10 mg of acid 9, mp 168° (Et_2O); ν max 1700 cm^{-1} . Pmr δ 1.46 (s, 3H), 1.66 (s, 3H), 1.70-3.20 (m, 4H), 3.77 (dd, 1H, $J = 4.8, 12.2$ Hz), 4.16 (dd, 1H, $J = 5.1, 11.8$ Hz). Ms m/z 302, 304, 306 (M^+) 285, 287, 289 ($\text{M}^+ - \text{OH}$) 267, 269, 271 ($\text{M}^+ - \text{Cl}$) 257, 259, 261 ($\text{M}^+ - \text{CO}_2\text{H}$) 231, 233 ($\text{M}^+ - \text{Cl}_2\text{H}$) 223, 225 ($\text{M}^+ - \text{Br}$).

Acid **9** (10 mg) dissolved in Et₂O was treated overnight with an ethereal solution of CH₂N₂ giving, after the usual work-up, 10 mg of methyl ester **10** as an oil.

Pmr δ 1.40 (s, 3H), 1.56 (s, 3H), 1.80-3.10 (m, 4H), 3.73 (s, 3H), 3.76 (dd, 1H, $J=4.4$, 12.3 Hz), 4.16 (dd, 1H, $J=5.2$, 11.7 Hz). Ms m/z 316, 318, 320 (M⁺) 285, 287, 289 (M⁺-OMe) 281, 283, 285 (M⁺-Cl) 257, 259, 261 (M⁺-CO₂Me) 237, 239, 241 (M⁺-Br) 221, 223, 225 (M⁺-ClHCO₂Me) 201, 203 (M⁺-BrClH).

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NOTE ADDED IN PROOF.—A revised structure of compound **5** has been suggested in: S. Naylor, F.J. Hanke, L. V. Manes, and P. Crews, "Progress in the Chemistry of Natural Products,"

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