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A NEW POLYHALOGENATED MONOTERPENE FROM THE RED ALGA *PLOCAMIUM CARTILAGINEUM*

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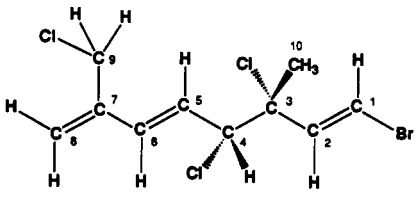
ABSTRACT.—From a sample of the red alga *Plocamium cartilagineum*, four polyhalogenated monoterpenes [**1**–**4**] were isolated and characterized, with (3*R**,4*R**)-1-bromo-7-chloromethyl-3,4-dichloro-3-methyl-1*E*,5*E*,7-octatriene [**1**] being a new compound. Also reported are antifungal, antibacterial, and molluscicidal activities, as well as the brine shrimp toxicity of these metabolites.

Algae of the genus *Plocamium* are known to yield numerous polyhalogenated monoterpenes that vary for a given species depending on collection location and season (1–4). The ecological function of many of these molecules is as yet unclear, although such compounds appear to have considerable activity in the natural environment of the plants (5) and have been proven to be active in pharmaceutical and agrochemical testing (6,7).

The current examination of a sample of *Plocamium cartilagineum* (L.) Dixon (Plocamiaceae) obtained from northern Spain provides yet another example of the seasonal/locational variation of the secondary metabolites within a previously investigated species in this genus. Along with three previously reported metabolites **2**–**4**, a new compound **1** is reported. The metabolites **2**–**4** show a wide range of biological activity, as previous reports (5–7) and the present experiments show. The biological activity of **1** is consistent with these findings. Unambiguous ^{1}H- and ^{13}C-nmr data are reported for compounds **2** and **4** for the first time.

Compound **1** was obtained as a yellow oil. Its Ft-ms contained mass peak groupings corresponding to $[M-Br]^+$ (*m/z* 237, 239, 241), $[M-Br-HCl]^+$ (*m/z* 201, 203, 205), $[M-C_6H_7Cl_2]^+$ (*m/z* 167, 169, 171), and $[M-C_4H_5ClBr]^+$ (*m/z* 149, 151, 153), clearly indicating a molecular formula of $C_{10}H_{12}Cl_3Br$. The



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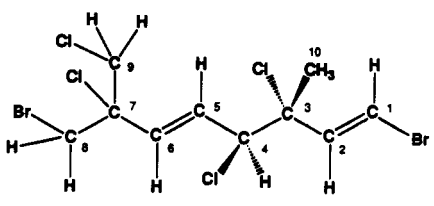

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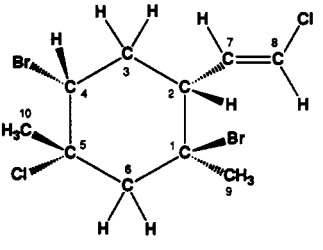

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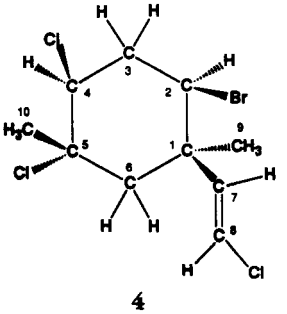

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presence of six sp^2 carbon resonances in the ^{13}C-nmr spectrum of **1** for three carbon-carbon double bonds dictated that the molecule be acyclic. ^{1}H-nmr homonuclear double-resonance experiments allowed four discrete spin systems to be discerned: $-C{=}CH_2$, $-CH_2X-$, $-CH{=}CH-CHX-$, and $-CH{=}CHX$, which accounted for all but two of the ten carbon atoms contained in **1**. The remaining two carbon atoms were a tertiary methyl group (1.77, 28.0 ppm) and a quaternary carbon (72.2 ppm). Further, the ^{1}H- and ^{13}C-nmr data revealed the presence of a vinyl bromide moiety (δ 6.57, 110.5 ppm), a vinyl chloromethyl function (δ 4.22, 4.16, 43.9 ppm) and a tertiary methyl group (1.77, 28.0 ppm) bonded to the quaternary allylic, chlorine-bearing carbon (72.2 ppm). This accounted for all but one of the halogen functions within **1**. The remaining chlorine atom can only be positioned at C-4 (69.1 ppm). This information, together with the fact that **1** has an uv maximum at 228 nm, typical for a conjugated-diene system, allowed only one possible structure for compound **1**. The proposed structure contains two chiral centers (C-3 and C-4) and two double bonds (Δ^1 and Δ^5) that required stereochemical assignment. The double bonds were both assigned an *E* configuration on the basis of the interproton coupling constants, which were >12 Hz. By application of empirical rules suggested by Mynderse and Faulkner (8) and Crews (9), the stereochemistry at the centers C-3 and C-4 was assigned, on the basis of the ^{13}C-nmr chemical shift of C-10 (9) and the positive optical rotation of **1** (8), as 3*R** and 4*R**, respectively. Compound **1** was thus assigned as (3*R**,4*R**)-1-bromo-7-chloromethyl-3,4-dichloro-3-methyl-1*E*,5*E*,7-octatriene.

Also isolated from this algal species were the previously reported metabolites **2** (9), **3** (11,12) and **4** (10,13). The unambiguous assignments of the ^{13}C- and ^{1}H-nmr data for these three compounds were made from the results of short-range ^{13}C-^{1}H 2D-correlation experiments (Bruker XHCORR) with the relevant delays optimized for J_{XH} = 136 Hz. The assignment of the corresponding data for compound **1** was also made from the results of a short-range ^{13}C-^{1}H 2D correlation experiment, this time in the inverse mode (Bruker INVPHBIRD with GARP decoupling, and delays again optimized for J_{XH} 136 Hz).

Antifungal and antibacterial activities of **1–4** were investigated by tlc-bioautographic tests, utilizing *Penicillium oxalicum, Bacillus subtilis, Micrococcus luteus*, and *Escherichia coli* as test organisms. As indicated in Table 1, no ap-

TABLE 1. Results of the Biological Assays Performed with Compounds **1–4**.

Compound[a]	*Penicillium oxalicum*[b]	*Biomphalaria glabrata*[c] 100% Mortality	*Artemia salina*[d] %Mortality
1	0.1	n.a.[e] (6)	n.a. (80)[f]
2	0.1	n.a. (6)	n.a. (80)
3	10	2.5	100 (100), 10 (20)
4	0.6	5	120 (100), 12 (26)

[a]None of the test compounds exhibited any anti-bacterial activity at up to 24 μg towards *Bacillus subtilis, Micrococcus luteus*, or *Escherichia coli*.

[b]Minimum concentration for inhibition on the tlc plate in μg.

[c]Concentration in ppm.

[d]Dose in μg/ml.

[e]n.a. = not active up to the dose expressed in parentheses.

[f]Dosage and (mortality).

preciable antibacterial activity could be observed for any of these compounds. Previous tests with polyhalogenated terpenes on marine microorganisms also revealed no significant antibacterial activity.[1] All compounds, however, exhibited pronounced antifungal properties against *P. oxalicum*, at levels as low as 0.1 μg for compounds **1** and **2**. The cyclic monoterpenes **3** and **4** exhibited potent toxicity towards both *Biomphalaria glabrata* and *Artemia salina*, while the acyclic compounds **1** and **2** were not toxic towards these organisms at the concentrations tested.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were recorded with a Perkin-Elmer 141 polarimeter using hexane as solvent. Ir spectra were measured on a Perkin-Elmer 781 infrared spectrometer as liquid films. Uv spectra were recorded in hexane on a Perkin-Elmer Lambda 3 uv/vis spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were measured at 300 and 75.5 MHz, respectively, on a Bruker AMX-300 spectrometer in $CDCl_3$ with TMS (δ 0) as the internal standard. 2D correlated spectroscopy was undertaken for compounds **2**, **3**, and **4** on a Bruker AMX-300-nmr instrument and for compound **1** on a Bruker AMX-500-nmr spectrometer. Ft-ms were recorded on a Spectrospin CMS 47X spectrometer, equipped with a 4.7 Tesla magnet and external ion source, operating at 70 eV. Hplc was carried out with a Waters 6000A solvent delivery system connected to a Rheodyne hplc injector and a Knauer differential refractometer. Hplc columns were from Knauer (250 mm × 8 mm, LiChrosorb Si60, 5 μm and 250 mm × 4 mm, Spherisorb ODS II, 5 μm). Silica (tlc-Silica 60GF 15 μm, Merck) was used for vacuum liquid chromatography, while aluminium backed sheets coated with silica $60F_{254}$, 0.2 mm thick (Merck), were used for tlc.

PLANT MATERIAL.—The algal material was obtained in June, 1989, in L'Estartit, Spain. Plants growing at 0–5 m depth were collected, deep frozen and, on return to the laboratory, freeze-dried. A voucher specimen is deposited at the Department of Pharmacy, ETH Zurich (voucher number CT189B).

EXTRACTION AND ISOLATION.—The dry algal tissue (67 g) was exhaustively extracted to yield 2 g of a CH_2Cl_2-soluble material. Vacuum liquid chromatography (vlc) (14) of the crude extract on Si gel, using hexane with increasing proportions of EtOAc as eluent, afforded 13 fractions of 75 ml each. Tlc and ^{1}H-nmr examination of these fractions indicated that fractions 1–6 were of further interest.

Compound **1**.—Hplc separation of combined fractions 1 and 2 from the vlc, using RP-18 material with MeOH-MeCN-H_2O (64:9:27) as eluent, afforded compound **1** as a clear oil (2 mg, 0.003%): $[\alpha]^{22}D$ +4.4° ($CHCl_3$, c = 0.16); ir ν max (film) 2920, 1510, 1370, 1020, 950 cm^{-1}; uv λ max (hexane) nm (log ε) 228 (3.8); ^{1}H nmr ($CDCl_3$, 300 MHz) δ 1.77 (s, 3H, H-10), 4.16 (d, J = 12.0 Hz, 1H, H-9), 4.22 (d, J = 12.0 Hz, 1H, H-9′), 4.48 (d, J = 8.8 Hz, 1H, H-4), 5.30 (s, 1H, H-8), 5.42 (s, 1H, H-8′), 5.91 (dd, J = 8.8, 15.8 Hz, 1H, H-5), 6.29 (d, J = 15.8 Hz, 1H, H-6), 6.43 (d, J = 13.5 Hz, 1H, H-2), 6.57 (d, J = 13.5 Hz, 1H, H-1); ^{13}C nmr ($CDCl_3$, 75.5 MHz) 28.0 (q, C-10), 43.9 (t, C-9), 69.1 (d, C-4), 72.2 (s, C-3), 110.5 (d, C-1), 121.7 (t, C-8), 126.6 (d, C-5), 133.6 (d, C-6), 137.3 (d, C-2), 140.7 (s, C-7) ppm; Ft-ms *m/z* (rel. int.) 241 (1), 239 (4), $[M-Br]^+$ 237 (5), 205 (2), 203 (11), $[M-Br-HCl]^+$ 201 (17), 171 (22), 169 (100), $[M-C_6H_7Cl_2]^+$ 167 (78), 153 (3), 151 (21), $[M-C_4H_5BrCl]^+$ 149 (28), 133 (32), 129 (31), 117 (19), 116 (15), 115 (38), 113 (99), 105 (12), 104 (11), 103 (16), 102 (30), 101 (10), 91 (46).

Hplc separation of combined fractions 3–6 from the vlc on Si gel, with hexane and $CHCl_3$ (47:3) as eluent afforded pure compounds **2–4**.

Compound **2**.—Compound **2** (oregonene A) (9) (22 mg, 0.033%): ^{1}H nmr ($CDCl_3$, 300 MHz) δ 1.75 (s, 3H, H-10), 3.79 (d, J = 11.0 Hz, 1H, H-8), 3.87 (d, J = 11.0 Hz, 1H, H-8′), 3.92 (d, J = 11.7 Hz, 1H, H-9), 3.98 (d, J = 11.7 Hz, 1H, H-9′), 4.49 (d, J = 7.7 Hz, 1H, H-4), 6.07 (d, J = 15.3 Hz, 1H, H-6), 6.19 (dd, J = 7.7, 15.3 Hz, 1H, H-5), 6.41 (d, J = 13.5 Hz, 1H, H-2), 6.57 (d, J = 13.5 Hz, 1H, H-1); ^{13}C nmr ($CDCl_3$, 75.5 MHz) 25.1 (s, C-10), 37.3 (t, C-8), 49.6 (t, C-9), 67.1 (d, C-4), 68.9 (s, C-7), 71.4 (s, C-3), 110.4 (d, C-1), 130.3 (d, C-5), 133.5 (d, C-6), 138.5 (d, C-2) ppm.

Compound **3**.—Compound **3** (46 mg, 0.069%) had physical and spectroscopic properties identical with those previously reported (11,12).

Compound **4**.—Compound **4** (coccinene) (10,13) (90 mg, 0.134%): ^{1}H nmr ($CDCl_3$, 300 MHz) δ 1.27 (s, 3H, H-10), 1.67 (s, 3H, H-9), 2.20 (d, J = 14.4 Hz, 1H, H-6ax), 2.38 (ddd, J = 12.5, 12.9, 13.7 Hz, 1H, H-3ax), 2.58 (d, J = 14.4 Hz, 1H, H-6eq), 2.65 (ddd, J = 4.0, 4.3, 13.7 Hz, 1H, H-3eq), 3.84 (dd, J = 4.0, 12.9 Hz, 1H, H-2), 4.15 (dd, J = 4.3, 12.5 Hz, 1H, H-4), 6.14 (d, J = 13.8 Hz, 1H, H-8), 6.22

[1]P. Alino, personal communication.

(d, $J = 13.8$ Hz, 1H, H-7); ^{13}C nmr ($CDCl_3$, 75.5 MHz) 26.1 (q, C-9), 30.0 (q, C-10), 41.1 (t, C-3), 43.3 (s, C-1), 55.2 (t, C-6), 56.7 (d, C-2), 66.9 (d, C-4), 70.6 (s, C-5), 120.6 (d, C-8), 135.4 (d, C-7) ppm.

BIOASSAYS.—Molluscicidal bioassays were performed according to the procedure reported by Nakanishi and Kubo (15). Brine shrimp bioassays were performed using *A. salina* as already described (16). Tlc-bioautographic tests were performed utilizing the test organisms *P. oxalicum* (CBS 219.30), *Ba. subtilis* (ATCC 6633), *M. luteus* (ATCC 9341) and *E. coli* (ATCC 25922) as previously described (16).

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LITERATURE CITED

1. S. Naylor, F.J. Hanke, L.V. Manes, and P. Crews, *Prog. Chem. Org. Nat. Prod.*, **44**, 189 (1983).
2. J.S. Mynderse and D.J. Faulkner, *Phytochemistry*, **17**, 237 (1978).
3. P. Crews, L. Campbell, and E. Heron, *Calif. J. Phycol.*, **13**, 297 (1977).
4. J.C. Coll, B.W. Skelton, A.H. White, and A.D. Wright, *Aust. J. Chem.*, **41**, 1743 (1988).
5. V.J. Paul, J. McConnell, and W. Fenical, *J. Org. Chem.*, **45**, 3401 (1980).
6. G. Gonzàlez, V. Daria, and E. Estèvez, *Planta Med.*, **44**, 44 (1982).
7. P. Crews, B.L. Myers, S. Naylor, E.L. Clason, R.S. Jacobs, and G.B. Staal, *Phytochemistry*, **23**, 1449 (1984).
8. J.S. Mynderse and D.J. Faulkner, *Tetrahedron*, **31**, 1963 (1975).
9. P. Crews, *J. Org. Chem.*, **42**, 2634 (1977).
10. R.S. Norton, R.G. Warren, and R.J. Wells, *Tetrahedron Lett.*, 3905 (1977).
11. M.D. Higgs, D.J. Vanderah, and D.J. Faulkner, *Tetrahedron*, **33**, 2775 (1977).
12. A.G. Gonzàlez, J.M. Artega, J.D. Martin, M.L. Rodriguez, and J. Fayos, *Phytochemistry*, **17**, 947 (1978).
13. F.J. Sardina, E. Quinoà, L. Castedo, and R. Riguera, *Chem. Lett.*, 697 (1985).
14. J.C. Coll and B.F. Bowden, *J. Nat. Prod.*, **49**, 934 (1986).
15. K. Nakanishi and I. Kubo, *Isr. J. Chem.*, **16**, 28 (1977).
16. B. Baumgartner, C.A.J. Erdelmeier, A.D. Wright, T. Rali, and O. Sticher, *Phytochemistry*, **29**, 3327 (1990).

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