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MARINE NATURAL PRODUCTS—XVI

POLYHALOGENATED ACYCLIC MONOTERPENES FROM THE RED ALGA *PLOCAMIUM* OF ANTARCTICA

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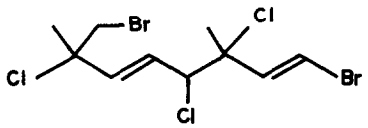
Abstract—Examination of the red alga *Plocamium* sp. collected near the Antarctic penninsula yielded three new acyclic halogenated monoterpenes (1–3). A structure revision is proposed for a compound previously reported to have structure 1. X-ray diffraction provided the structure of 1 including absolute stereochemistry. The structures of 2 and 3 followed from proton and ^{13}C NMR spectral interpretation.

Red algae have been found to contain many halogenated terpenes,[1,2] with species of the genus *Plocamium* being an exceedingly rich source of acyclic and cyclic halogenated monoterpenes.[1,2] In their investigation of the macroscopic algae of the Antarctica Peninsula, DeLaca *et al.*[3] collected and sent a large sample of a *Plocamium* sp. to us for chemical examination. In this paper we wish to describe a family of polyhalogenated acyclic compounds isolated from this alga.

Plocamium sp. was collected subtidally by divers on Anvers Island near Antarctica. The alga had been air-dried and ground when it was received so that identification of the species was impossible. The ground alga was extracted with hexane in a Soxhlet extractor. Removal of the hexane gave a crude extract. The PMR spectrum of the extract showed it to be a mixture of several compounds, but tlc examination of the extract indicated only one major spot. When the tlc plate was sprayed with spores of the fungus *Cladosporium cucumerinum* and allowed to incubate,[4] strong antifungal activity was seen in this area. Repeated open column chromatography of the crude extract on silica gel using hexane as the elutant gave two major fractions, A and B. Each fraction contained two compounds. Repeated reverse phase high pressure liquid chromatography (hplc) of fraction A gave a pure crystalline $C_{10}H_{13}Br_2Cl_3$(1) compound and a $C_{10}H_{13}BrCl_4$(2) compound. Repeated reverse phase hplc of fraction B gave two pure compounds, $C_{10}H_{12}BrCl_5$(3) and $C_{10}H_{12}Br_2Cl_4$(4).

Examination of the 90 MHz PMR spectrum ($CDCl_3$) of 1 revealed that it was closely related or identical to the polyhalogenated monoterpene 5 isolated from the sea hare *Aplysia californica* by Ireland *et al.*[5]



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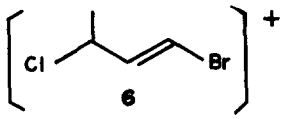
The large difference in m.p. (1, 48.5–49.0° and 5, 20°) and slight differences in the reported spectral data led us to believe the two compounds were actually different. Comparison of the ^{13}C NMR spectra of these two compounds suggested that they differed in their halogen substitution.

The mass spectrum of 1 did not display a molecular ion halogen cluster, but did display a cluster from the loss of a halogen atom (Br or Cl) corresponding to the formula $C_{10}H_{13}Br_2Cl_2$. Ireland[5] reported the base peaks of the related compound 5 at *m/e* 167, 169, 171 represented as ion 6.[5]



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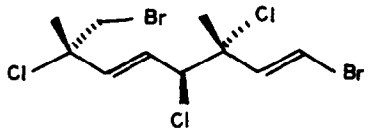
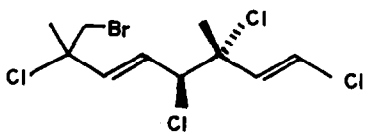


1



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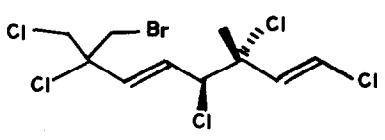

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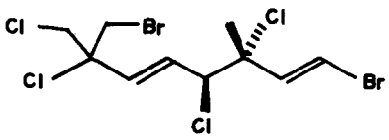

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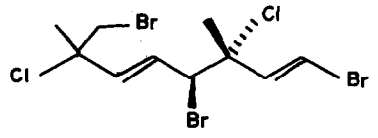
The mass spectrum of 1 showed large peaks at these mass units, but they were not the base peaks. The base peak of 1 appeared at *m/e* 78.

The CMR spectrum ($CDCl_3$) of 1 was very informative as to its halogen substitution. The chemical shift of C_1 (Table 2) indicated a vinyl bromide functionality.[6] The chemical shift of the methine carbon, C_4, indicated that a Cl was attached to it.[7] The chemical shift of C_8 indicated a bromomethylene carbon.[8] From mass spectral considerations, a Cl must be attached to the quaternary carbon, C_3. The remaining halogen bearing carbon was the quaternary carbon, C_7. Neither the carbon or mass spectral data was sufficient to determine its halogen substitution.

In order to determine the halogen substitution at C_7 and to determine the conformation and absolute configuration of 1, a single crystal X-ray diffraction study was undertaken. A stereo-pair drawing of 1 is shown in the figure. All bond lengths and bond angles fall into normal ranges and the molecule is fully extended so that both double bonds lie in the same plane. The X-ray structure established that a Cl was attached to C_7.

The structure of our compound 1 was identical to that reported for 5. Dr. Faulkner kindly supplied a sample of their compound for comparison. PMR and CMR spectra were obtained for both compounds (Tables 1 and 2) on the same instruments. The spectra were practically identical except for the small downfield shift of the H_c resonance (0.08 ppm) in 5 relative to 1 (in the PMR) and an upfield shift of 8 ppm of the C-4 resonance (in the CMR). These spectral differences are consistent with a Br at C-4 rather than a Cl as proposed by Ireland *et al.*[5] We propose that the structure of their compound be changed to 7.



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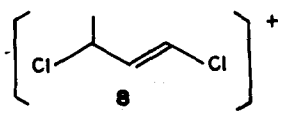
The 90 MHz PMR spectrum of 2 was nearly identical to that of 1. The difference was a slight (~0.2 ppm) upfield shift of the resonance of the terminal olefinic protons (Table 1). This shift is indicative of the replacement of a terminal Br by a Cl. The CMR spectra of 1 and 2 were also almost identical (Table 2). The resonance of the terminal olefinic carbon bearing halogen was shifted downfield indicating the proposed substitution. The mass spectrum of 2 again did not display a parent ion cluster. The highest mass cluster observed was attributed to the loss of Cl and a high resolution mass measurement on this fragment showed a molecular formula of $C_{10}H_{13}BrCl_3$. The base peak displayed an ion cluster at *m/e* 123, 125, 127 characteristic of two Cl atoms and was assigned to ion 8. The structure 2 was consistent with the data.



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The PMR spectrum of 3 indicated its structural resemblance to 2. Its spectrum lacked the Me singlet at 1.84 δ (C_9 Me) seen in the spectrum of compound 2 and instead showed the appearance of a new methylene singlet at 3.95 δ. Since the chemical shifts of the two methylenes differed, they must have different halogen substitution. The CMR spectrum was consistent with this hypothesis with the appearance of two triplets (off-resonance) at 49.7 ppm (CH_2Cl) and 37.3 ppm (CH_2Br). The mass spectrum showed no molecular ion but did show an ion cluster for which a high resolution mass measurement established a formula of $C_{10}H_{12}BrCl_4$. The base peak cluster of 3 appeared at *m/e* 123, 125, 127 characteristic of ion 8.

The PMR spectrum of 4 was almost identical to that of 3 except for a small downfield shift of the terminal AB quartet (Table 1). The chemical shifts assigned to the terminal olefin functionality in the PMR and CMR spectra of 4 were very similar to those values found for 1. This indicated a terminal vinyl bromide. The mass spectrum of 4 indicated a loss of Cl to give a cluster indicative of the formula $C_{10}H_{12}Br_2Cl_3$. The base peak cluster appeared at *m/e* 167, 169, 171, again indicative of ion 6.

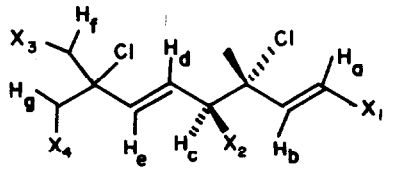
Assignments of the ^{13}C NMR spectra for all of the compounds could be accomplished by consideration of the chemical shifts and off-resonance multiplicities.[9-11] True coupling constants for 3 were obtained by a gated decoupled spectrum.[12] A knowledge of $^1J_{CH}$ enables an unambiguous distinction of carbons similar in chemical shift, but differing in substituent electronegativity.[13] The highest field olefinic resonance was assigned to C_1 because it had the largest $^1J_{CH}$ which would be expected for a carbon attached to a halogen.[14] By analogy, the highest field olefinic signals in 1, 2 and 4 could be assigned to C_1.

In an off-resonance decoupled spectrum, the residual coupling constant, J^r, is proportional to the chemical shift difference of the proton absorption and the decoupler frequency offset. If two proton resonances differ in chemical shift, the carbons to which they are attached can be distinguished by examination of J^r.[15] H_2 appeared at lower field than H_5 or H_6 and examination of J^r permitted assignment of C_2. Of the two remaining olefinic resonances, the lowest field signal was assigned to C_5 because it was adjacent to C_7, a quaternary carbon. This downfield shift has been well documented for olefinic carbons.[16] Assignment of the two quaternary resonances in each compound were made by consideration of structural changes made. Changes at C_1 should affect C_3, and changes made at C_8 should affect C_7.

The absolute configuration of 1 was established by X-ray analysis. The relative configuration at C_3 and C_4 of 1 allows both the chlorines and the R groups to adopt *trans* positions relative to the 3, 4 bond. This also seems to be the preferred conformation in solution in view of the C_{10} proton and carbon chemical shifts. Mynderse has reported the chemical shift of this C_{10} Me group is sensitive to the C_3–C_4 relative stereochemistry.[6] Crews has found that the carbon chemical shift can be a much more sensitive tool,[17] and has reported a Me shift difference of 3 ppm between the (*R, S*) and (*R, R*) configurations. The proton and carbon chemical shifts indicate that 2, 3 and 4 have the same relative stereochemistry (*R, S*) as 1 at the 3 and 4 positions.

During the preparation of this manuscript, it came to our attention that Crews[17,18] had found 4 in *Plocamium oregonum*. Direct comparison of the compounds was not possible since his compound had been used up in the

Table 1. Proton chemical shifts, 90 MHz ($CDCl_3$)



	X_1	X_2	X_3	X_4	H_a	H_b	H_c	H_d	H_e	H_f	H_g	CH_3
1	Br	Cl	Br	H	6.58(d)	6.41(d)	4.46(dd)	5.99	5.93	3.70(s)	1.84(s)	1.75(s)
					J_{ab}=13.5Hz		J_{cd}=5.0Hz J_{ce}=3.0Hz	J_{de}=16.0Hz				
2	Cl	Cl	Br	H	6.42(d)	6.16(d)	4.47(dd)	5.99	5.93	3.70(s)	1.84(s)	1.76(s)
					J_{ab}=13.0Hz		J_{cd}=7.0Hz J_{ce}=2.0Hz	J_{de}=16.0Hz				
3	Cl	Cl	Br	Cl	6.44(d)	6.15(d)	4.48(dd)	6.12	6.05	3.95(s)	3.83(s)	1.75(s)
					J_{ab}=13.0Hz		J_{cd}=6.0Hz J_{ce}=1.5Hz	J_{de}=16.5Hz				
4	Br	Cl	Br	Cl	6.59(d)	6.39(d)	4.48(dd)	6.13	6.04	3.95(s)	3.83(s)	1.75(s)
					J_{ab}=13.5Hz		J_{cd}=6.0Hz J_{ce}=1.0Hz	J_{de}=16.5Hz				
7	Br	Br	Br	H	6.58(d)	6.40(d)	4.54(dd)	6.02	6.89(m)	3.70(s)	1.82(s)	1.79(s)
					J_{ab}=13.5Hz		J_{cd}=2.0Hz J_{ce}=8.0Hz					

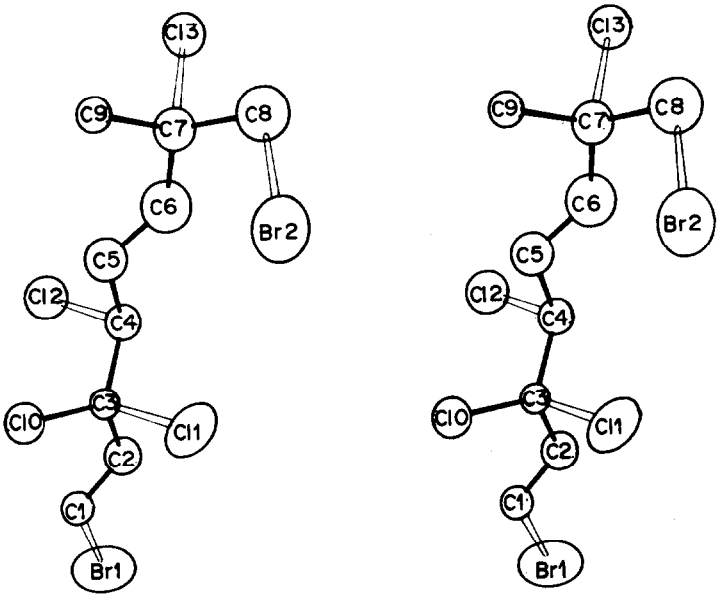
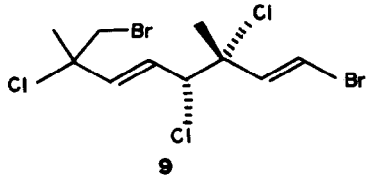


Fig. 1. Stereo-pair drawing of 1.

structure proof. In addition, Crews found 9 a stereoisomer of 1.



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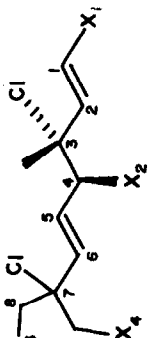
Many of the halogen containing metabolities of marine algae have been found to exhibit antibacterial and antifungal properties.[1,19] Fenical has proposed that these compounds are produced to discourage invertebrate predators and as a defense against parasitic microflora.[1] Compounds 1–4 were tested individually against the fungus *Cladosporium cucumerimum* on a TLC plate[4] and were found to have moderate activity.

EXPERIMENTAL

Optical rotations were determined on a Jasco ORD-CD spectrometer with a one centimeter cell (1 ml). M.ps were determined on a Thomas-Hoover m.p. apparatus and is uncorrected. IR spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. PMR and CMR spectra were recorded on a

Table 2. Carbon chemical shifts, 22.6 MHz ($CDCl_3$)



	X_1	X_2	X_3	X_4	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	CH_3
1	Br	Cl	Br	H	110.1	138.6	71.6	67.6	127.4	138.0	66.9	41.5	27.5	25.5
2	Cl	Cl	Br	H	122.5(d)	135.0(d)	70.4(s)	67.9(d)	127.5(d)	138.0(d)	66.9(s)	41.5(t)	27.6(q)	25.7(q)
3	Cl	Cl	Br	Cl	122.6(d)	134.8(d)	70.2(s)	67.4(d)	130.3(d)	133.5(d)	68.8(s)	37.3(t)	49.7(q)	25.4(q)
4	Br	Cl	Br	Cl	110.3	138.6	71.4	67.7	130.4	133.6	68.9	37.3	49.6	25.3
7	Br	Br	Br	H	110.0	138.9	71.2	60.1	128.1	137.4	66.9	41.6	27.8	26.3

Bruker WH-90 multinuclear spectrometer. The frequency offset in the off-resonance experiments was at -4δ with a power of 3340 Hz. Low resolution mass spectra were run on a Finnigan 1015 S/L spectrometer. High resolution mass spectra were recorded by Dr. Kai Fang, Department of Chemistry, UCLA. High pressure liquid chromatography was performed on a Waters Associates instrument with the M-6000 pump.

Collection and extraction. Plocamium sp. was collected by divers on Anvers Island near the Antarctic Peninsula. The air dried alga (2 kg) was ground in a Wiley mill and extracted with distilled hexane in a Soxhlet apparatus. The solvent was removed under reduced pressure to give 13 g of a dark green oil.

Silica gel chromatography. The crude extract (5.8 g) was applied to a 3 × 80 cm column (Grace, grade 62) and the non-polar oil eluted with hexane. Fraction 1 contained nonhalogenated hydrocarbons (0.2 g), fractions 2–4 contained compounds 1 and 2 (1.6 g) and fractions 5–9 contained compounds 3 and 4 (2.9 g).

Reverse phase high pressure liquid chromatography. Fractions 2–4 were combined and were further fractionated by repeated reverse phase high pressure liquid chromatography on a 1/8 in. × 8 ft column made by the procedure of Gilpin *et al.*[20] The mobile phase used was methanol:water (95:5) and afforded pure 1 (0.3 g) and pure 2 (1.2 g). Under the same conditions, reverse phase chromatography of fractions 5–9 yielded pure 3 (1.8 g) and 4 (0.9 g).

Compound 1 m.p. = 48.5–49.0°; $[\alpha]_D = -46.3°$ ($c = 1.03$, $CHCl_3$); IR (μ) 3.35, 6.15, 7.25, 9.25, 11.60; PMR (90 MHz, $CDCl_3$) δ 1.75(3H, s), 1.84(3H, s), 3.70(2H, s), 4.46(1H, dd, J = 5.0, 3.0 Hz), 6.01–5.91(2H, m), 6.41(1H, d, J = 13.5 Hz), 6.58(1H, d, J = 13.5 Hz); CMR ($CDCl_3$) ppm 25.5, 27.5, 41.5, 66.9, 67.6, 71.6, 110.1, 127.5, 138.0, 138.6; Mass spectrum *m/e* 361, 363, 365, 367, (M^+–Cl), 229, 231, 233, (M^+–C_4H_5BrCl), 167, 169, 171, ($C_4H_5BrCl^+$), 115, 79 (BP).

Compound 2 $[\alpha]_D = -48.8°$ ($c = 0.86$, $CHCl_3$); IR (μ) 3.35, 6.16 7.25, 9.30, 11.90; PMR (90 MHz, $CDCl_3$) δ 1.76(3H, s), 1.84(3H, s), 3.70(2H, s), 4.47(1H, dd, J = 7.0, 2.0 Hz), 6.01–5.91(2H, m), 6.16(1H, d, J = 13.0 Hz), 6.42(1H, d, J = 13.0 Hz); CMR ($CDCl_3$) ppm 25.7(q), 27.6(q), 41.5(t), 66.9(s), 67.9(d), 70.4(s), 122.5(d), 127.5(d, J^r = 43.0 Hz), 135.0(d, J^r = 51.0 Hz), 138.0(d, J^r = 43.0 Hz); Mass spectrum *m/e* 317, 319, 321, 323, (M^+–Cl), 229, 231, 233, (M^+–$C_4H_5Cl_2$), 123, 125, 127, ($C_4H_5Cl_2^+$) (BP), 115, 117. High resolution mass measurement. Calc. for $C_{10}H_{13}$ $^{79}Br^{35}Cl_3$: 316.9253. Obs: 316.9267 ± 0.002.

Compound 3 $[\alpha]_D = -20.2°$ (c = 1.19, $CHCl_3$); IR (μ) 3.23, 3.38, 6.15, 7.00, 10.31, 13.41; PMR (90 MHz, $CDCl_3$) δ 1.75(3H, s), 3.83(2H, s), 3.95(2H, s), 4.48(1H, dd, J = 6.0, 1.5 Hz), 6.16–6.11(2H, m), 6.15(1H, d, J = 13.0 Hz), 6.44(1H, d, J = 13.0 Hz); CMR ($CDCl_3$) ppm 25.4(q, J = 129.4 Hz), 37.3(t, J = 154.0 Hz), 49.7(t, J = 152.5 Hz), 67.4(d, J = 150.9 Hz), 68.8(s), 70.2(s), 122.6(d, J = 197 Hz), 130.3(d, J = 162 Hz, J^r = 44.5 Hz), 133.5(d, J = 162 Hz, J^r = 45.1 Hz), 134.8(d, J = 167 Hz, J^r = 50.1 Hz); Mass spectrum *m/e* 351, 353, 355, 357, (M^+–Cl), 123, 125, 127, ($C_4H_5Cl_2^+$), 77. High resolution mass measurement. Calc. for $C_{10}H_{12}$ $^{79}Br^{35}Cl_4$. 350.8886. Obs: 350.8878 ± 0.002.

Compound 4 $[\alpha]_D = -19.4°$ ($c = 1.21$, $CHCl_3$); IR (μ) 3.28, 3.36, 6.16, 7.03, 10.33, 13.65; PMR (90 MHz, $CDCl_3$) δ 1.75(3H, s), 3.83(2H, s), 3.95(2H, s), 4.48(1H, dd, J = 6.0, 1.0 Hz) 6.22–6.10(2H, m), 6.39(1H, d, J = 13.5 Hz), 6.59(1H, d, J = 13.5 Hz); CMR ($CDCl_3$) ppm 25.3, 37.3, 49.6, 67.7, 68.9, 71.4, 110.3, 130.4, 133.6, 138.6; Mass spectrum *m/e* 395, 397, 399, 401, (M^+–Cl), 351, 353, 355, 357, (M^+–Br), 167, 169, 171, ($C_4H_5BrCl^+$) (BP), 115, 77.

X-ray structure of 1. Three small (*ca.* 3 × 1 × 1 mm) clear orthorhombic crystals were found in the residue from slow evaporation of an aqueous EtOH soln. One of these was used to establish the space group $P2_12_12_1$ from a pair of zero layer precision photographs. The crystal was discolored to an orange-brown hue at this point and proved unsatisfactory for intensity collection, using our usual[21] 0–20 scan procedure and MoK_α-radiation. The other two crystals were utilised for this purpose, the second for the first 424 observations at which point the three standard reflections collected after every fifty scans had fallen off by 37%. The remaining data were collected on the third crystal which suffered a 55% decrease in the standards due to crystal decomposition. At this point, none of the crystals, now

orange-brown, were useful for diffraction measurements. Consequently, the unit cell constants $a_0 = 5.93(1)$ Å, $b_0 = 20.23(3)$ Å, $C_0 = 12.09(2)$ Å $\alpha = \beta = \gamma = 90°$ are based on the average of those values resulting from the three separate alignments.

After reduction of the data, 679 of the 849 unique reflections collected in an octant out to sin $\theta/\lambda = 0.481$ were accepted using the criteria of $F \geq 1.5\ \sigma_F$.

Attempts to solve the structure by Patterson methods were frustrated by a large number of overlapping peaks, hence, we resorted to a direct methods approach to locating the five halogen atoms.[22] The solutions and refinement then proceeded routinely to give the structure shown in the figure. Large anomolous dispersion contributions from the halogen atoms resulted in a weighted residual of 9.9% for the correct configuration[23] as against 10.5% for the enantiomer. The final unweighted residual was 10.7% and X was 1.85.[24]

Acknowledgements—The algal sample was provided by Dr. T. E. DeLaca (NSF-OPP-74-12139). This work was supported by the National Science Foundation CHE-74-13938. The Bruker WH-90-D-18 was supported by the NIH Biomedical Science Grant No. 5-SO5-RRO7010-19 and by the NSF Grant No. MPS75-06138.

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