

Polyhalogenated Monoterpenes from a Tasmanian Collection of the Red Seaweed *Plocamium cartilagineum*

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Two new polyhalogenated monoterpenes (3*E*,7*E*)-8-bromo-(2*E*)-chloromethylene-(5*R**,6*R**)-dichloro-6-methyloctadien-1-al (**1**) and (1*Z*,3*E*,7*E*)-9-bromo-(1*Z*,5*R**,6*R**,9)-tetrachloro-6-methyloctatriene (**2**), together with two known compounds (**3** and **4**), were isolated and identified from the red alga *Plocamium cartilagineum* collected along the eastern coast of Tasmania. The structures were established by spectroscopic techniques.

The red algal *Plocamium* genus occurs in cool to temperate seas in many parts of the world, and numerous halogenated monoterpenes, including acyclic, cyclic, and tetrahydropyran derivatives, have been reported from it.^{1–4} Acyclic halogenated monoterpenes were first reported from the digestive gland of the sea hare *Aplysia californica*;^{5–7} they were possibly obtained from the animal's algal diet.

In this paper, we report the structure elucidation of two new (**1** and **2**) and two known (**3** and **4**) acyclic polyhalogenated monoterpenes from *Plocamium cartilagineum* (L.) Dixon (Gigartinales, Plocamiaceae)^{8,9} from two Tasmanian East Coast collections, one collected by scuba diving and the other collected as drift from a beach.

P. cartilagineum was initially collected by scuba diving at Mayfield Bay and analyzed. However, subsequent attempts to obtain significantly more material by that method were unsuccessful. Five months later, immediately after a storm in the area, more of the seaweed was deposited on Schouten Beach, and this was collected. Schouten Beach is approximately 14 km NNE from Mayfield Bay, and both are part of the western side of Great Oyster Bay. Both collections gave the same two new (**1**, **2**) and two known (**3**, **4**) acyclic polyhalogenated monoterpenes.

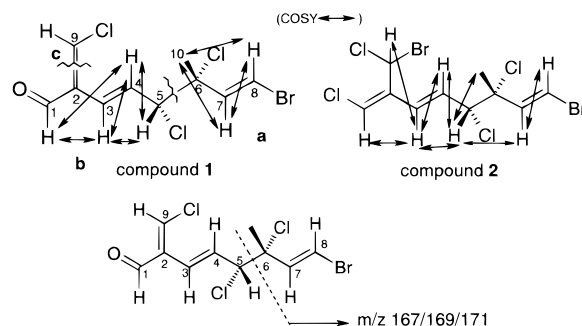
Freeze-dried *P. cartilagineum* was extracted with CH₂Cl₂ and fractionated by dry-column flash Si gel chromatography¹⁰ to give three major fractions eluted by petroleum ether with increasing percentages of EtOAc. The least polar fraction contained nonhalogenated hydrocarbons (not further investigated) and, from the beach collection, elemental sulfur. The second fraction contained one known compound (**3**) identified by comparison of its mass spectral fragmentation obtained by GCMS with literature values.¹¹ The last fraction, which was a mixture of polyhalogenated monoterpenes, was rechromatographed by MPLC to afford two main fractions. The first of these fractions contained a known compound (**4**) identified by comparison of its mass spectral fragmentation obtained by GCMS with published data.¹² Purification of the second fraction by HPLC and preparative TLC yielded two new compounds, (3*E*,7*E*)-8-bromo-(2*E*)-chloromethylene-(5*R**,6*R**)-dichloro-6-methyloctadien-1-al (**1**) and (1*Z*,3*E*,7*E*)-9-bromo-(1*Z*,5*R**,6*R**,9)-tetrachloro-6-methyloctatriene (**2**).

Compound **1** was obtained as a pale yellow oil. The CIMS gave C₁₀H₁₄BrCl₃NO [M + NH₄]⁺, which corresponds to a

molecular formula of C₁₀H₁₀BrCl₃O, indicating four degrees of unsaturation. An aldehyde group was observed at 9.55 ppm and 1726 cm⁻¹ in the ¹H NMR and IR spectra, respectively. ¹³C NMR data of **1** (Table 1) showed signals for all 10 of the carbons, which, with the DEPT spectrum, were assigned as being one methyl, seven methines (five olefinic, one aldehyde, and one with an attached chlorine), and two nonprotonated carbons.

All C–H one-bond connections in **1** were confirmed by gHMBC. The chemical shift of C-8 at 110.7 ppm indicated that a bromine atom must be attached.^{3,11,13} A ¹H–¹H gCOSY correlation of H-7 and H-8, H-7 and H-10 long-range, and H-8 and H-10 long-range allowed assignment of fragment **a** as shown. This structural moiety was supported by MS data, with a characteristic fragmentation^{3,11,13–16} to give the base peak at *m/z* 167, 169, 171.

Fragment **b** was determined by ¹H–¹H gCOSY correlation of H-1 and H-3 long-range, H-1 and H-4 long-range, H-3 and H-4, H-3 and H-5 long-range, and H-4 and H-5. The signal at chemical shift 69.5 ppm showed that C-5 must have a chlorine atom attached.^{3,11,13,15–17} The aldehyde proton (H-1) was connected to a vinylic proton (H-3) by gCOSY and gNOESY. Then the nonprotonated vinylic carbon (C-2) was connected to the aldehyde proton (H-1), and the aldehyde carbon (C-1) was also connected to the vinylic proton (H-3); these connections were established by gHMBC. Furthermore, data from gHMBC (Table 1) confirmed the connection of fragment **c** to fragment **b** and fragment **b** to fragment **a**, thereby securing the connectivity of **1**.



The ¹H NMR spectrum of **1** (Table 1) showed signals corresponding to three double bonds; two of them were

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Table 1. ^1H (200 MHz for **1** and 400 MHz for **2**) and ^{13}C (100 MHz for both **1** and **2**) NMR Data in CDCl_3

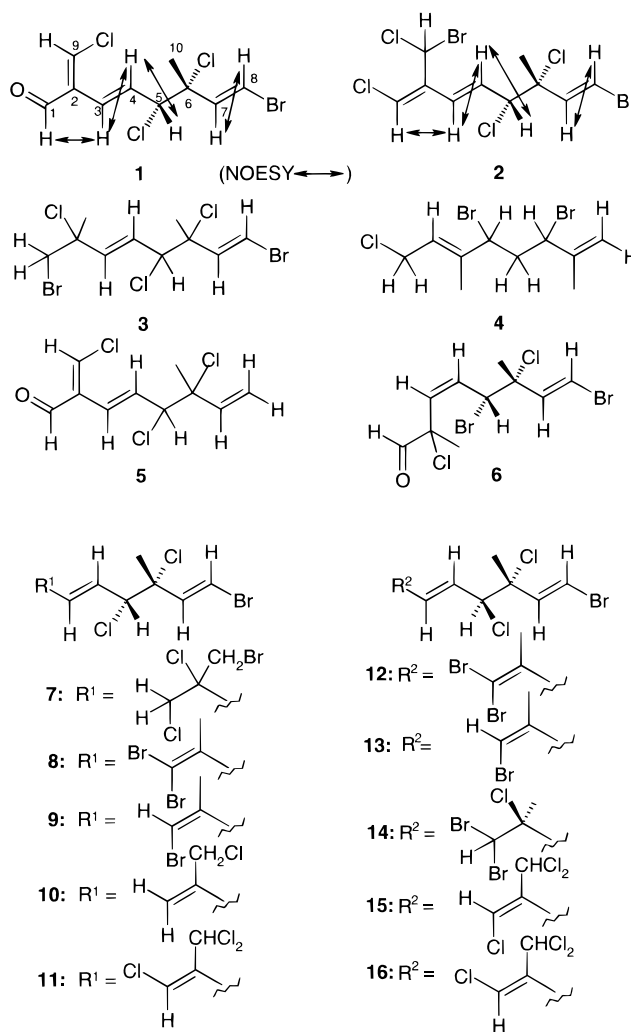
no.	^1H J (Hz)	1		2		
		^{13}C DEPT	HMBC	^1H J (Hz)	^{13}C DEPT	HMBC
1	9.55 d, 2.0	189.3, CH	3, 9	6.75 d, 0.6	124.6, CH	3
2		137.4, C	1, 9		136.0, C	3, 4
3	6.55 ddd, 0.9, 2.1, 15.9	123.0, CH	1, 5, 9	6.60 d, 16.1	124.4, CH	1, 4, 5
4	7.01 ddd, 0.6, 8.9, 15.9	133.7, CH	5	6.42 ddd, 0.7, 8.6, 16.2	137.0, CH	5, 10
5	4.54 dd, 0.8, 8.8	69.5, CH	3, 10	4.55 dd, 0.7, 8.6	69.2, CH	3, 10
6		71.6, C	5, 10		71.9, C	5, 7, 8, 10
7	6.46 d, 13.5	136.9, CH	5, 10	6.45 d, 13.3	131.5, CH	5, 8
8	6.61 d, 13.6	110.7, CH	7	6.58 d, 13.5	110.9, CH	7, 10
9	7.15 s	144.3, CH	3	6.37 s	69.6, CH	1
10	1.82 s	28.0, CH_3	5, 7	1.80 s	28.1, CH_3	7

assigned as trans on the basis of coupling constants ($J_{3,4} = 15.9$ and $J_{7,8} = 13.6$ Hz). The stereochemistry of the remaining double bond (between C-2 and C-9) was attributed to the *E* form¹⁷ by comparison of the observed chemical shift for H-9 (7.15 ppm) with the calculated chemical shift from a table of substituent constants¹⁸ for H-9 (*Z* form, 7.42, *E* form, 7.01 ppm). This assignment was supported by the absence of a NOE between H-9 and any proton nearby and by the absence of any coupling by H-9 in the ^1H NMR spectrum. The aldehyde proton (H-1, δ 9.55 d, $J_{1,3} = 2.0$ Hz) was coupled to the vinylic proton (H-3). The sole allylic proton (H-5) occurred at lowfield (δ 4.54 dd), as one of the chlorine atoms was joined to C-5. The proton was coupled to the vinyl proton, H-4 ($J_{5,4} = 8.8$ Hz), and also showed a long-range coupling to the vinylic proton, H-3 ($J_{5,3} = 0.8$ Hz).¹⁷ A gNOESY experiment revealed interactions between H-1 and H-3, H-3 and H-4, H-4 and H-5, and H-7 and H-8 as shown.

Chiral centers at C-5 and C-6 of **1** were assigned the relative stereochemistry ($5R^*$, $6R^*$) by applying the empirical rules of Mynderse and Faulkner¹⁴ and Crews¹⁷ to the proton and carbon chemical shifts of the methyl group (H-10, 1.82, C-10, 28.0 ppm). The ^{13}C NMR methyl shift (C-10) difference of 3 ppm between the (*R,S*) and (*R,R*) configurations was observed and is much larger than the ^1H NMR methyl shift (H-10) difference of 0.06–0.08 ppm.^{11,13,14,17} Compound **1**, which had a positive optical rotation, $[\alpha]_D +50.8^\circ$ (c 0.128, CH_2Cl_2), was thus shown to be a 5,6-*threo* compound.

To our knowledge, compound **1** is the third acyclic halogenated monoterpene aldehyde isolated from the *Plocamium* genus. Interestingly, all three are restricted to *P. cartilagineum*. The other aldehydes are cartilagineal (**5**)¹⁷ and (3*Z*,7*E*)-5, 8-dibromo-2,6-dichloro-2,6-dimethylocta-3,7-dien-1-al (**6**).¹³

Compound **2** was obtained as a viscous pale yellow oil. The EIMS showed peaks at m/z 428, 430, 432, 434, 436, with relative intensities for two bromine and four chlorine atoms, which correspond to the molecular formula of $\text{C}_{10}\text{H}_{10}\text{Br}_2\text{Cl}_4$ [M^+] (HREIMS), indicating three degrees of unsaturation. ^{13}C NMR data of **2** (Table 1) showed signals for 10 carbons, which, using the DEPT spectrum, were assigned as being one methyl, seven methines (four olefinic, two with attached chlorine, and one with an attached bromine and chlorine), and two nonprotonated carbons. All C–H one-bond connections in **2** were confirmed by gHMBC. These two new compounds (**1**, **2**) reported in this study have the same subunit (C-3 to C-8) as 11 known compounds (**3**, **7**–**16**).^{11,13,15} Five of them had the same stereochemistry as **1** and **2**. Like compound **1**, the chemical shift of **2** at 69.2 ppm showed that C-5 had an attached chlorine atom, while the signal at 110.9 ppm was due to a vinylic carbon atom (C-8) bearing a bromine.^{3,11,13,15–17}



Determination of the remainder of the structure of **2** was similar to that for **1**. All the connectivity was established by ^1H – ^1H gCOSY, gHMBC, and gHMBC experiments. The vinylic methine carbon (C-1, δ 124.6 ppm) must have an attached chlorine, rather than bromine.^{3,11} The remaining unassigned chlorine atom and bromine atom must thus be attached to the methine carbon (sp^3 , C-9, δ 69.6 ppm). The ^1H NMR spectrum of **2** (Table 1) showed signals corresponding to three double bonds, two of which were assigned as trans on the basis of coupling constants ($J_{3,4} = 16.1$ and $J_{7,8} = 13.3$ Hz). The stereochemistry of the remaining double bond (C-1 and C-2) was allocated a *Z* geometry on the basis of a NOE between H-1 and H-3. The gNOESY experiment of **2** established interactions between H-1 and H-3, H-3 and H-4, H-4 and H-5, and H-7 and H-8 as shown.

Table 2. Brine Shrimp Bioassay Results for the Third Dry-Column Flash Chromatography Fraction of *P. cartilagineum*

concentrated ($\mu\text{g/mL}$)	percent deaths at 15 h ^a
control	10
12.3	60 ^b
30.8	77 ^b
61.7	93 ^b
92.5	100
123.4	100
1542	100
3084	100
30 840	100

^a Average of 3 replicates. ^b 100% death after 20.5 h.

Chiral centers at C-5 and C-6 of **2** were assigned the relative stereochemistry (5*R**,6*R**) by using the methyl chemical shift rule (H-10, 1.80 and C-10, 28.0 ppm). Compound **2**, which had a positive optical rotation, $[\alpha]_{\text{D}} +49.1^{\circ}$ (*c* 1.120, CH₂Cl₂), was thus shown to be a 5,6-*threo* compound, the same as compound **1**. The chiral center at C-9 remains unassigned.

Comparison of the two collections revealed that both contained the same metabolites, the two new (**1**, **2**) and two known (**3**, **4**) compounds. No significant decomposition occurred in the second collection, obtained from a beach shortly after a storm. The only detectable difference in the latter collection was that it also contained elemental sulfur (isolated as crystals). It is quite likely that the sulfur was produced by anaerobic bacteria acting on the seaweed as it lay in heaps on the beach. Sulfur is a known metabolite of chemosynthetic bacteria, for example, *Thiobacillus thioparus*, if aerobic conditions occur, or by photosynthetic bacteria under continued anaerobiosis.¹⁹ Although *P. cartilagineum* has a worldwide distribution (although restricted to temperate seas), it is of interest to note that this Tasmanian collection afforded new metabolites and provides yet another example of chemical variability of marine natural products.

The brine shrimp (*Artemia salina*) bioassay²⁰ was performed on the third dry-column flash chromatography fraction (the one consisting mainly of the halogenated terpenes) of *P. cartilagineum*. One hundred percent mortality was obtained at concentrations of 92.5 $\mu\text{g/mL}$ or greater after 15 h (see Table 2). This bioactivity is considerably higher than the ones previously reported (e.g., 500 $\mu\text{g/mL}$) for acyclic halogenated terpenes from *Plocamium* species toward brine shrimp.^{15,16}

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a P20 polarimeter, Bellingham & Stanley Ltd., using CH₂Cl₂ as solvent. IR spectra were measured on a Perkin-Elmer FT-IR spectrometer Paragon 1000. UV spectra were recorded in CH₂Cl₂ on a Shimadzu UV-vis recording spectrophotometer. ¹H NMR spectra of **1** and **2** were measured at 200 and 400 MHz on Varian Gemini and Varian Inova wide-bore spectrometers, respectively. ¹³C NMR and DEPT of both **1** and **2** were measured at 100 MHz on a Varian Inova wide-bore spectrometer. All 2D NMR gradient NOESY phase sensitive, gradient COSY, gradient HMQC phase sensitive, gradient HMBC spectra were recorded at 400 MHz on a Varian Inova wide bore spectrometer, using CDCl₃ as solvent with TMS as internal standard. CIMS were recorded with a Kratos Concept & ISQ spectrometer using the desorption CI technique (NH₃ as reagent gas). HREIMS were also recorded on the Kratos concept & ISQ. HPLC was carried out with a Waters

600 multisolvent delivery system connected to a UV Waters 486 tunable absorbance detector using a Dynamax-60A Si gel preparative column. The MPLC system was equipped with UA-6 UV-vis ISCO detector. Merck Si gels of 70–230 mesh and 230–400 mesh were used for dry-column flash chromatography and MPLC, respectively. Merck Si gels 70–230 mesh with 60 F₂₅₄ indicator were used for preparative TLC. Aluminum-backed sheets coated with silica 60 F₂₅₄ 0.20 mm thick were used for TLC.

Plant Material. The algal material was (a) collected at Mayfield Bay, near the old jetty (42° 15' S, 148° 0.9' E) by scuba diving in November 1997, and (b) gathered on Schouten Beach, Swansea (42° 7.7' S, 148° 5' E), in April 1998. Both samples were freeze-dried. Voucher specimens have been deposited at the Tasmanian State Herbarium, reference HO 445478 and HO 444898, respectively.

Extraction and Isolation. The freeze-dried samples (20.626 g and 2 kg dry wt from the first and second collection, respectively) were extracted with CH₂Cl₂ and MeOH to yield 0.158 and 52.2 g of a CH₂Cl₂-soluble material, respectively. GCMS suggested that the second collection contained sulfur, S₈, which did not appear in the first collection. Dry-column flash Si gel chromatography¹⁰ of the CH₂Cl₂ extract from the first collection (0.158 g) and a portion of the CH₂Cl₂ extract from the second collection (14.253 g), using petroleum ether with increasing proportions of EtOAc gradually as eluent, gave three main fractions. From the first collection, the first fraction (4 mg) contained nonhalogenated hydrocarbons. The second fraction (10 mg) contained unidentified volatile terpenes and the known compound **3**, identified by comparison of its mass spectral fragmentation obtained by GCMS with published data.¹¹ The last fraction (43 mg), which was further purified by preparative TLC (10% EtOAc–petroleum ether), gave **1** (5 mg, 0.024% of dry wt of the alga) and a mixture of the new compound **2** and the known compound **4** (20 mg), identified by comparison of its mass spectral fragmentation obtained by GCMS with literature values.¹² From the second collection, the first fraction (123 mg) contained nonhalogenated hydrocarbons and sulfur, which was isolated and recrystallized from petroleum ether as pale yellow crystals (7 mg, equivalent to 0.001% of dry wt). The second fraction (1.303 g), which was eluted with 25% EtOAc–petroleum ether, contained unidentified volatile terpenes and the known compound **3**, identified by comparison of its mass spectral fragmentation obtained by GCMS. The last fraction (6.301 g) was rechromatographed by MPLC (10% EtOAc–petroleum ether), and only two major interesting fractions were investigated further. The first fraction (0.127 g) contained the known compound **4**, identified by comparison of its mass spectral fragmentation obtained by GCMS with literature values.¹² The second fraction (4.498 g) was further purified by HPLC (0–10% EtOAc in petroleum ether, Si gel preparative column) and preparative TLC (10% EtOAc–petroleum ether) to yield **1** (32 mg, equivalent to 0.006% of dry wt) and **2** (637 mg, equivalent to 0.117% of dry wt).

(3*E*,7*E*)-8-Bromo-(2*E*)-chloromethylene-(5*R,6*R**)-dichloro-6-methyloctadien-1-ol (**1**):** oil; $[\alpha]_{\text{D}} +50.8^{\circ}$ (*c* 0.128, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} (log ϵ) 261 (3.98), 234 (3.98) nm; IR (Nujol) ν_{max} 1726, 1055, 939 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; CIMS *m/z* found 347.9310 [M + NH₄]⁺ (calcd for C₁₀H₁₄79Br³⁵Cl₃NO req 347.9324); EIMS *m/z* 51; 115, 117; 131, 133 [C₄H₄Br]⁺; 167, 169, 171 (BP) [C₄H₅BrCl]⁺; 180, 182 [M – HBr – 2Cl]⁺; 215, 217, 219 [M – HBr – Cl]⁺; 251, 253, 255 [M – Br]⁺; 295, 297, 299, 301 [M – Cl]⁺.

(1*Z*,3*E*,7*E*)-9-Bromo-(1*Z*,5*R,6*R**,9)-tetrachloro-6-methyloctatriene (**2**):** oil; $[\alpha]_{\text{D}} +49.1^{\circ}$ (*c* 1.120, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} (log ϵ) 249 (5.31) nm; ¹H and ¹³C NMR data, see Table 1; HREIMS *m/z* found 431.77949 [M]⁺ (calcd for C₁₀H₁₀Br₂Cl₄ req 431.78559, average of all isotopes, due to the lowest isotope peak being very weak); EIMS *m/z* 51; 75; 133; 147; 167, 169, 171 (BP) [C₄H₅BrCl]⁺; 197, 199, 201; 233, 235, 237; 269, 271, 273; 305, 307, 309; 384, 386, 388, 390; 428, 430, 432, 434, 436 [M]⁺.

Bioassay. The brine shrimp bioassay was performed as described.²⁰

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