Halogenated Monoterpenes from *Plocamium costatum* and Their Biological Activity[1]

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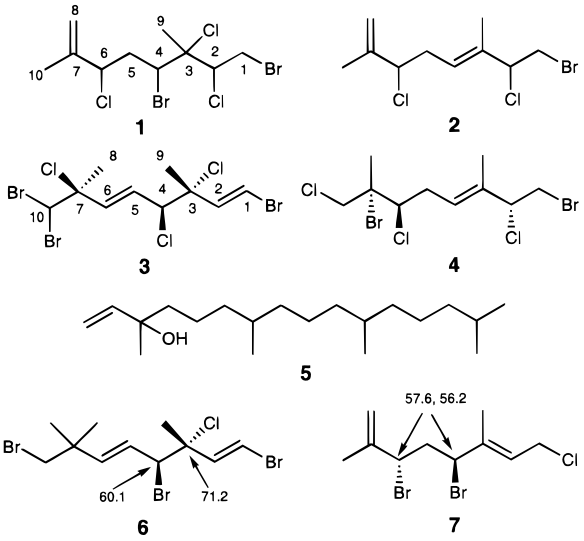
From the dichloromethane solubles of the temperate red alga *Plocamium costatum*, one new [1,4-dibromo-2,3,6-trichloro-3,7-dimethyl-7-octene] (**1**) and three previously reported polyhalogenated monoterpenes (**2**−**4**), and the known phytol derivative 3,7,11,15-tetramethylhexadec-1-en-3-ol (1-phyten-3-ol, **5**) were isolated. The structure of **1** was deduced from its spectroscopic data. For compound **3**, complete ^{1}H and ^{13}C NMR data are reported for the first time. The CH_2Cl_2 extract and compounds **3** and **5** deterred settlement of barnacle larvae, suggesting a potential ecological role of these isolates.

Plocamium species are known to yield numerous polyhalogenated monoterpenes.[2−4] The ecological role these molecules play is as yet unclear, although such compounds appear to have considerable activity in the natural environment of these plants.[5] Similar compounds to those found in Australian *Plocamium* species have also proven to be active in pharmaceutical and agrochemical testing.[6,7] Recently, interest in plants belonging to this genus has been revived as a result of some metabolites being shown to have considerable cytotoxicity.[8,9] The current examination of a sample of *P. costatum* (C. Agardh) Hooker and Harvey (Gigartinales, Plocamiaceae), collected from Deep Glen Bay, Eaglehawke Neck, Tasmania, Australia, was undertaken in order to identify the compounds responsible for the biological activity of the CH_2Cl_2 extract and because no previous studies on the secondary metabolite chemistry of Tasmanian samples of *Plocamium* spp. have been reported. One new (**1**), and four previously isolated metabolites (**2**−**5**), are reported from this new collection; compounds **3** and **5** causing some deterrence of barnacle settlement.

Compound **1** was obtained as a clear oil and, by mass spectral analysis, was found to have the molecular formula $C_{10}H_{15}Cl_3Br_2$. As the molecule contained only one multiple bond, a carbon−carbon double bond [117.3 (t), 141.2 (s) ppm], it had to be acyclic. After association of all protons with their directly bonded carbon atoms via a ^{1}H−^{13}C one-bond shift-correlated 2D NMR (HMQC) measurement (see Table 1), it was then possible from the ^{1}H−^{1}H COSY spectrum to discern three discrete spin systems, $-C(CH_3)=CH_2$, $-CHX-CH_2-CHX-CX(CH_3)$, and $CH_2X-CHX-$, which accounted for all of the carbon and hydrogen atoms contained in **1**. Correlations observed in the ^{1}H−^{13}C multiple-bond shift-correlated 2D NMR (HMBC) spectrum (see Table 1) allowed these three fragments to be linked to generate the planar hydrocarbon structure of **1** as $CH_2=C(CH_3)-CHX-CH_2-CHX-CX(CH_3)$-$CHX-CH_2X$. The five halogen atoms were positioned by ^{13}C NMR chemical shift comparisons made between the data for **1** and those for **2**[10] and **4**.[11] This comparison indicated one bromo function to be at C-1 and the chloro functions to be at C-2 and C-6, leaving one bromo and one chloro substituent to be positioned. Further ^{13}C NMR chemical shift comparisons, this time with the data for compounds **6**[12] and **7**,[12] for C-3 and C-4 show the chloro function to be at C-3 and the bromo function to reside at C-4. Compound **1** is thus assigned as 1,4-dibromo-2,3,6-trichloro-3,7-dimethyl-7-octene and, as such, is the 3-chloro-4-bromo derivative of **2**.



Also isolated from this algal species were the previously reported metabolites **2**,[10] **3**,[13] **4**,[11] and **5**.[14] As there are no ^{13}C NMR data published for compound **3** and as the ^{1}H NMR data were incomplete, they were recorded and assigned (see Table 1). This is the first report of these metabolites from this species, and of **5** from the order Gigartinales.

An antifouling assay using cypris larvae of the cosmopolitan barnacle, *Balanus amphitrite*,[15] was performed using the CH_2Cl_2 extract of the alga and compounds **2**, **3**, and **5** (see Table 2). The CH_2Cl_2 extract deterred settlement of cypris larvae at the 100 and 10 μg cm^{-2} levels but not at lower concentrations. Of the three purified metabolites tested, only **3** and **5** were significantly deterrent at concentrations of both 10 and 1 μg cm^{-2}. These levels of activity suggest a possible role as natural antifoulants; however, further investigation of the presentation of these com-

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Table 1. ^{1}H [$CDCl_3$, 600 MHz (**1**), 300 MHz (**3**)][a] and ^{13}C [$CDCl_3$, 150.9 MHz (**1**), 75.5 MHz (**3**)] NMR Data (ppm) for Compounds **1** and **3**

position	^{13}C (**1**)	^{1}H (**1**)	^{1}H−^{1}H COSY (**1**)	HMBC[b] (**1**)	^{13}C (**3**)	^{1}H (**3**)
1	33.5 t[c]	3.96 dd (J = 2.7, 11.4 Hz)	2		110.2 d	6.57 d (J = 13.6 Hz)
		3.66 dd (J = 8.9, 11.4 Hz)		2		
2	69.3 d	4.61 dd (J = 2.7, 8.9 Hz)	1	1	138.9 d	6.44 d (J = 13.6 Hz)
3	74.7 s				71.1 s	
4	56.9 d	4.21 dd (J = 1.2, 11.6 Hz)	5, 9	2, 3, 5, 6, 9	59.8 d	4.65 d (J = 9.3 Hz)
5	39.7 t	2.85 ddd (J = 1.2, 11.4, 14.7 Hz)	4, 6	3, 6, 7	130.0 d	6.20 dd (J = 9.3, 15.1 Hz)
		2.30 ddd (J = 4.1, 11.6, 14.7 Hz)		4, 6, 7		
6	63.4 d	4.77 dd (J = 4.1, 11.4 Hz)	5	4, 5, 7, 10	135.1 d	6.07 d (J = 15.1 Hz)
7	141.2 s				71.4 s	
8	117.3 t	5.18 br s	10	6, 10	26.9 q	1.96 s
		5.07 br s		6, 7, 10,		
9	22.4 q	1.78 s			26.3 q	1.82 s
10	15.8 q	1.82 br s	8	6, 7, 8	52.7 d	5.75 s

[a] Residual solvent peaks were used as internal standards ^{1}H ($CDCl_3$, 7.26 ppm) and ^{13}C ($CDCl_3$, 77.0 ppm). [b] These cross-peaks are from proton to carbon. [c] Multiplicity by DEPT, C=s, CH=d, CH_2=t, CH_3=q.

Table 2. Results of Antifouling Assay Using Cypris Larvae (*Balanus amphitrite*) with the CH_2Cl_2 Extract of *Plocamium costatum* and Compounds **2**, **3** and **5**

test material	conc. (μg/cm^2)	average[a]	SD[b]	SE[c]
SW[f] control		83[d]	6.6	2.7
EtOH control[g]		75[d]	7.6	3.1
extract	100	0[e]	0	0
	10	39	4.4	2.5
	1	69	11.4	6.6
	0.1	76	16	9.2
	0.01	78	13.1	7.6
	0.001	68	15.8	9.1
SW[f] control		39[d]	17	6.9
EtOH control[g]		47[d]	19	7.7
2	10	58[e]	16	9.3
	1	53	30.3	17.5
	0.1	52	19.1	11
	0.01	57	10.4	6.0
3	10	2.2	2.3	1.4
	1	37	15.8	9.1
	0.1	54	10.1	5.9
	0.01	42	21.1	12.2
5	10	13	3.1	1.8
	1	23	7.5	4.3
	0.1	54	17	9.8
	0.01	43	7.6	4.4

[a] Average % of settled larvae. [b] Standard deviation % of settled larvae. [c] Standard error % of settled larvae. [d] Average of 6 replicates. [e] Average of 3 replicates. [f] Seawater. [g] 500 μL are added to each test well and allowed to evaporate in the same way as if it contained test compound.

pounds and their activity against ecologically relevant fouling organisms is necessary before ascribing such a role (e.g., de Nys et al. and Dworjanyn et al.[16,17]).

The antimicrobial and antialgal activities of **1**−**5** were investigated in agar diffusion tests,[18] which showed all of the compounds to have little or no effect. No compound demonstrated HIV-1 RT inhibitory activity.[19] In the assays performed with *Artemia salina* and *Caenorhabditis elegans*, compounds **4** and **5** had only weak effects, and only toward brine shrimp at a test concentration of 0.5 mg/mL.[20,21]

Experimental Section

General Experimental Procedures. These were performed as previously reported.[22]

Plant Material. The algal material was obtained in January 1997, from Deep Glen Bay, Eaglehawk Neck, Tasmania, Australia. Plants growing at 25−30 m depth were collected, deep frozen, and, on return to the laboratory, freeze-dried. A voucher specimen is deposited at the Herbarium of the Royal Botanical Gardens, Sydney (voucher number NSW 405114).

Extraction and Isolation. The dry algal tissue (218.7 g) was exhaustively extracted with CH_2Cl_2 (2 L) and MeOH (2 L), to yield 7.62 g of a CH_2Cl_2-soluble material. Vacuum−liquid chromatography (VLC) of the crude extract over Si gel, using hexane with increasing proportions of EtOAc as eluent, followed by MeOH, afforded eight fractions each of 90 mL. TLC and ^{1}H NMR examination of these fractions indicated VLC fractions 1-3 to be of further interest. Separation of combined VLC fractions 1-3, by VLC using hexane with increasing proportions of EtOAc as eluent yielded 13 fractions, each 65 mL.

HPLC separation of VLC fraction 1-3.2, using Si gel with hexane as eluent, afforded compounds **1** and **2**. HPLC separation of VLC fraction 1-3.4, using Si gel with hexane−Me_2CO (97:3) as eluent, afforded compounds **3** and **4**. HPLC separation of VLC fraction 1-3.7, using Si gel with hexane−Me_2CO (93:7) as eluent, afforded compound **5**.

1,4-Dibromo-2,3,6-trichloro-3,7-dimethyl-7-octene (1): a clear oil (1.3 mg, 0.0006%); $[\alpha]^{22}_D$ −33.1° (c 0.13, $CHCl_3$); IR ν_{max} (film) 2925, 1450, 1380, 1035, 914 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS m/z $[M]^+$ 404, 402, 400, 398 (1), $[M-Cl]^+$ 369, 367, 365, 363 (1), $[M-Br]^+$ 325 (2), 323 (6), 321 (10), 319 (6), $[M-Br-HCl]^+$ 287 (12), 285 (26), 283 (12), 251 (10), 249 (24), 169 (28), 167 (52), 115 (50), 91 (82), 90 (88), 89 (100); HREIMS m/z 401.855 (calcd for $C_{10}H_{15}{}^{35}Cl_2{}^{37}Cl{}^{79}Br{}^{80}Br$ 401.856).

Compound 2: (60.2 mg, 0.028%); with identical physical and spectroscopic properties with those previously reported.[10]

Compound 3: (17.9 mg, 0.0082%); ^{1}H and ^{13}C NMR data see Table 1; with remaining physical and spectroscopic properties identical to those previously reported.[13]

Compound 4: (2.3 mg, 0.0011%); with identical physical and spectroscopic properties with those previously reported.[11]

Compound 5: (3.0 mg, 0.0014%); with identical physical and spectroscopic properties with those previously reported.[14]

Bioassays. The cypris larvae assay was done according to the methods of de Nys et al.[15] The agar diffusion assays and ELISA based assays were performed as previously described.[18,19] The assays undertaken with *Artemia salina* and *Caenorhabditis elegans* were performed as reported.[20,21]

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