Ochtodene Derivatives from the Red Alga *Carpopeltis crispata*

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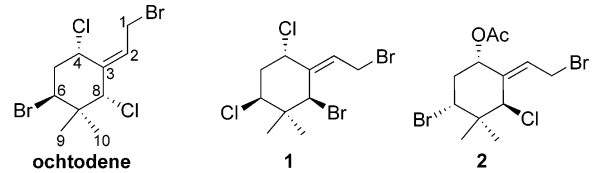
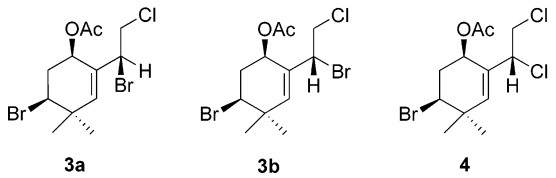
Received September 16, 2004

Red algae are well known as a source of halogenated monoterpenes such as derivatives of ochtodene. From *Carpopeltis crispata,* we have isolated four new ochtodene derivatives: dibromodichloro-, dibromochloro-, and bromodichlorocyclomonoterpenes. The structures of these monoterpenes were confirmed by NMR and mass spectroscopy and compared with spectral data in the literature.

The first report of polyhalogenated monoterpene constituents of marine organisms implicated the digestive gland of the sea hare *Apliysia californiа*.[1] Sea hares are macrophagous herbivores, and hence it was soon afterward that reports appeared of the isolation of these and other monoterpenes from their algal diet, in this case the red alga *Plocamium coccineum*.[2] Two families of red algae, the Plocamiaceae and the Rhizophyllidaceae, are known to contain large amounts of both acyclic and cyclic polyhalogenated monoterpenes.[3−7]

We have isolated four new ochtodene derivatives (**1**−**4**) from *Carpopeltis crispata* Kawaguchi (family Halymeniaceae). They are regioisomers or stereoisomers of known ochtodene derivatives from the genus *Ochtodes* of the family Rhizophyllidaceae.[8,9]





Air-dried algae were extracted in MeOH/$CHCl_3$ (1:2 v/v) for 1 day. After the removal of residue, the concentrate was partitioned between *n*-hexane and H_2O. The ^1H NMR spectrum of the *n*-hexane extract showed signals characteristic of halogenated monoterpenes. Column chromatography over silica gel (20% EtOAc in *n*-hexane), followed by repeated HPLC with 4% EtOAc in *n*-hexane, gave four new ochtodene derivatives (**1**−**4**).

Detailed examination of GC-MS spectra of crude **1** gave one main peak and a few small peaks having essentially the same parent ion at *m/z* 362 and the same fragments with two bromo and two chloro atoms.

Chromatographically repurified **1** revealed the molecular formula $C_{10}H_{14}{}^{79}Br_2{}^{35}Cl_2$, as determined by the HREIMS ($[M]^+$ *m/z* 361.8850, Δ +1.1 mmu). The ^1H and ^{13}C NMR spectra showed characteristic signals at δ 4.13 and 4.18 (δ_C 37.5, C-1), 5.41 (55.1, C-4), 4.23 (57.1, C-6), and 4.91 (53.2, C-8). The ^1H and ^{13}C signals were connected via HMQC and all these protons attached to carbons bearing halogens. The spectra of **1** were similar to the spectra of ochtodene, except that the ^1H and ^{13}C chemical shifts at the 4, 6, and 8 positions were different from those in ochtodene (Table 1).[8] The ^{13}C chemical shifts of halogenated cyclic monoterpenes can be used to differentiate bromine from chlorine on tertiary carbons, because the bromine-bearing carbons are shifted to higher field than the chlorine-bearing ones.[10,11]

The reaction of octodene with Zn in acetic acid afforded the 1,3-diene derivative, while the same reaction of **1** forms the 1,8-diene derivative (**5**) that has characteristic signals: a singlet at δ 5.66 (H-8) and double doublets at δ 4.71 (H-4). From these data, **1** was identified to be 1,8-dibromo-4,6-dichloroochtodene, in which the halogen atoms at C-6 and C-8 in ochtodene were interchanged.

The stereochemistry of **1** was determined by its coupling constants and by NOE measurements. The H-4 coupling constants of the cyclohexane rings in **1** show double doublets with $J = 11.9$ and 4.6 Hz. The coupling constants of $H_{5ax,6}$ and $H_{5eq,6}$ were 3.8 and 3.2 Hz, respectively. This demonstrates that H-4 and H-6 were a quasiaxial and a quasiequatorial proton, respectively, as in ochtodene. Irradiation at δ 2.72 ($H\text{-}5_{eq}$) in **1** increased the peak intensity at δ 5.41 (H-4) and 4.23 (H-6). Also, irradiation at δ 2.57 ($H\text{-}5_{ax}$) increased the methyl peak intensity at δ 1.24 (C-9: axial methyl). Hence, the configurations of C-4 and C-6 in **1** are both *S**. The C-8 stereochemistry is proposed as *S**, because NOE correlation of H-8 more strongly indicates a quasiaxial methyl group (C-9) than a quasiequatorial methyl group (C-10), as well as allyl and homoallyl couplings between H-2 and H-8 and between H-1 and H-8. As an NOE correlation occurred between H-1 and H-8, it is proposed that the geometry of the double bond is *Z* orientation.

Compound **2** failed to show a molecular ion peak under high-resolution conditions, but showed a peak due to $C_{12}H_{17}{}^{79}Br^{35}ClO_2$ (*m/z* 307.0104 $[M - Br]^+$, Δ +0.3 mmu). From 1D (^1H, ^{13}C) and 2D (COSY, HMQC, HMBC) NMR spectra, **2** was proven to be an ochtodene derivative having an acetyl group at the 4 position instead of a chlorine atom in ochtodene.

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10.1021/np0496946 CCC: $30.25
Published on Web 03/17/2005

Table 1. NMR Spectral Data of **1** and **2**

	1					**2**				
C#	δ_C	δ_H		*J* (Hz)	HMBC (H→C)	δ_C	δ_H		*J* (Hz)	HMBC (H→C)
1	37.5	4.13	ddd	11.9, 7.3, 1.4	2, 3	37.6	4.08	dd	11.9, 7.3, 1.8	2
		4.18	dd	11.9, 9.2	2, 3		4.15	dd	11.9, 9.7	3
2	123.5	6.24	ddd	9.2, 7.3, 1.4	4, 8	120.6	5.83	ddd	9.7, 7.3, 1.8	
3	140.1					137.8				
4	55.1	5.41	dd[a]	11.9, 4.6		68.3	5.72	ddt	12.4, 5.8, 1.8	
5ax	43.3	2.57	ddd	14.6, 11.9, 3.8	3, 4, 6	39.1	2.04	ddd	12.4, 12.4, 12.4	4, 6
5eq		2.72	ddd	14.6, 4.6, 3.2	3, 4, 6		2.63	ddd	12.4, 5.8, 4.3	4
6	57.1	4.23	ddd[a]	3.8, 3.2, 1.4	4	53.5	4.50	dd	12.4, 4.3	5, 7
7	40.1					41.7				
8	53.2	4.91	d[a]	1.4	3, 4	63.2	4.85	s		2, 3, 4, 6
9ax	27.9	1.24	s		6, 7, 8	21.0	1.11	s		6, 7, 8
10eq	29.8	1.45	s		6, 7, 8	27.0	1.30	s		6, 7, 8
11						169.3				
12						20.9	2.15	s		11

[a] The peaks were broadened because of long-range couplings (allyl, homoallyl, or *W* couplings).

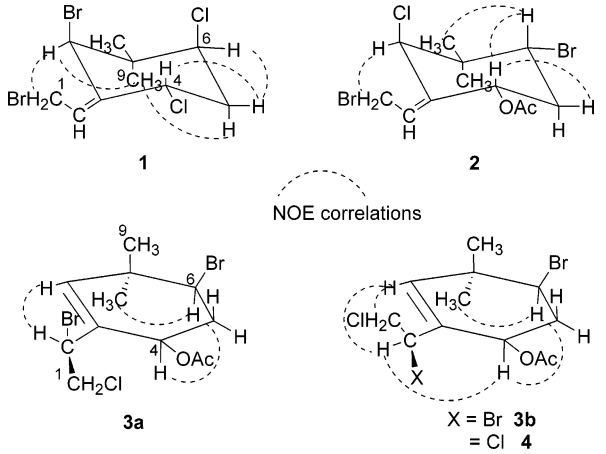

Figure 1. Configurations of ochtodene derivatives.

The stereochemistry of **2** was also determined by coupling constants and NOE measurements. The H-6 coupling constants with $J = 12.4$ and 4.3 Hz in **2** were different from those in ochtodene, consistent with H-6 having a quasiaxial conformation. This fact was supported by NOE correlation between H-4 and H-6 in **2**, whereas there was no correlation between those protons in **1**, as shown Figure 1.

The molecular formula of **3a**, $C_{12}H_{17}{}^{79}Br_2{}^{35}ClO_2$ (*m/z* 385.9295 $[M]^+$ Δ +1.1 mmu) was determined by HREIMS. The ^1H and ^{13}C NMR spectra of **3a** were very similar to those of a known ochtodene derivative (2-chloro-1,6(*S**)-dibromo-3(8)(*Z*)-ochtoden-4(*R**)-acetate) with the exception of δ_C 47.5 (C-1) and 51.6 (C-2) in **3a** compared with δ_C 40.5 (C-1) and 58.0 (C-2), respectively, in the known derivative, and with the exception of double doublets of H-1 at δ 3.94 and 3.88 instead of a doublet at δ 3.70.[9] It was proven that the primary halogen at C-1 is bromine and secondary halogen at C-2 is chlorine on the basis of ^{13}C NMR shifts.[11,12] As **3b** had similar MS fragmentation and IR spectra, and the NMR spectral characteristics of C-1 and C-2 were slightly different, **3b** was identified as the C-2 stereoisomer of **3a** (Experimental Section and Figure 1).

The molecular formula, $C_{12}H_{17}{}^{79}Br^{35}Cl_2O_2$, of **4** was determined by HREIMS ($[M]^+$ *m/z* 341.9803, Δ +1.4 mmu). The ^1H and ^{13}C NMR spectra of **4** were shown to be very similar to those of **3a** except the C-2 chemical shift is at δ_C 57.4 instead of at 51.6 as in **3a**. Thus, **4** differed from **3a** and **3b** by having a chlorine atom at C-2 instead of a bromine atom.

The NMR spectra of **3a** show peaks for H-4 and H-6 as double triplets with coupling constants $J = 9.2$, 6.4, and 1.6 Hz and double doublets with $J = 13.0$ and 3.3 Hz, respectively. Thus, H-4 and H-6 were both quasiaxial as in the known derivative mentioned above.[9] This fact was supported by NOE correlation between H-4 and H-6. Also, the hexene ring of **3b** and **4** had almost the same chemical shifts and coupling constants as those of **3a**, indicating that they had the same configuration, but the chirality of C-2 might be different from that of **3a**. Irradiation of H-8 in **3a** increased the peak intensity of H-2, whereas irradiation

Table 2. NMR Spectral Data of **3a**, **3b**, and **4**

	3a					**3b**					**4**				
C#	δ_C	δ_H		*J* (Hz)	HMBC (H→C)	δ_C	δ_H		*J* (Hz)	HMBC (H→C)	δ_C	δ_H		*J* (Hz)	HMBC (H→C)
1	47.5	3.88	dd	11.0, 6.2	2, 3	44.3	3.96	d	7.8	2, 3	45.0	3.81	dd	11.5, 6.5	2, 3
		3.94	dd	11.0, 9.4								3.88	dd	11.5, 7.6	2, 3
2	51.6	4.49	dd	9.4, 6.2	4, 8	47.2	4.65	dd	7.8, 7.6	1, 3	57.4	4.53	br dd	7.6, 6.5	1, 3, 4, 8
3	130.0					131.4					131.0				
4	70.3	5.64	ddd	9.2, 6.4, 1.6		68.9	5.64	dd	8.0, 8.0		68.7	5.60	br ddd	9.0, 6.9, 0.9	3, 5, 8
5ax	36.5	2.24	ddd	13.0, 12.8, 9.2	4, 6	36.2	2.21	ddd	13.3, 12.3, 9.2	4	36.3	2.20	ddd	13.3, 12.8, 9.0	3, 4, 6, 7
5eq		2.77	ddd	12.8, 6.4, 3.3	4, 6		2.80	ddd	12.3, 6.9, 3.2	4, 6		2.78	ddd	12.8, 6.9, 3.2	3, 4, 6, 7
6	55.8	4.08	dd	13.0, 3.3		55.7	4.08	dd	13.3, 3.2		55.7	4.08	dd	13.3, 3.2	4, 5, 7, 9
7	38.0					37.9					37.8				
8	141.0	5.89	d	1.6	2, 4	138.8	5.89	s		2, 3, 4, 6, 7	139	5.87	br s		2, 3, 4, 6, 7
9ax	23.8	1.20	s		6, 7, 8	23.6	1.21	s		6, 7, 8	23.8	1.20	s		6, 7, 8
10eq	28.4	1.17	s		6, 7, 8	28.4	1.17	s		6, 7, 8	28.5	1.18	s		6, 7, 8
11	170.6					170.1					170.0				
12	21.2	2.11	s		11	21.1	2.11	s		11	21.1	2.10	s		

of H-8 in **3b** and **4** increased the peak intensity of both H-1 and H-2. Also the irradiation of H-4 in **3a** increased the peak intensity of H-6, and the same position in **4** was correlated with H-2 and H-6. From these results and the Dreiding model, C-2 in **3a** had the *S** configuration and C-2 in **3b** and **4** was *R** configured, as shown Figure 1.

Unfortunately, these halogenated monoterpenes do not inhibit *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*.[12]

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer 341 digital polarimeter. IR spectra were run on a Shimadzu FTIR 8200A. NMR spectra were measured in $CDCl_3$ solution on a JEOL ECP-500 spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Mass spectra were obtained with a JEOL MS-700 mass spectrometer. HPLC was performed on a Shimadzu LC-10 apparatus equipped with RI or UV detector using a GL Science column (Inertsil prep-SIL, Inertsil prep-ODS, 10 × 250 mm or 20 × 250 mm). The solvents were distilled prior to use.

Plant Material. The red alga was collected in April 2002 and 2003 at Johgashima Island in Kanagawa Prefecture, Japan. It was identified by Y. Takahashi as *Carpopeltis crispata* Kawaguchi, and a voucher specimen has been deposited at the Department of Chemistry & Biological Science, Aoyama Gakuin University. The sample was kept refrigerated until use.

Extraction and Separation. An air-dried sample (880 g) was soaked in 1:2 (v/v, 1.8 L) MeOH/$CHCl_3$ for 1 day. The extract (66 g) was partitioned between *n*-hexane and H_2O (700 mL each), and the hexane extract (9.3 g) was subjected to SIL-flash column chromatography (Wakogel, 9.0 × 5.0 cm) using a gradient EtOAc/*n*-hexane solution. The 20% EtOAc in *n*-hexane fraction (1.89 g) was subjected to open column chromatography using 20% EtOAc in *n*-hexane. The roughly collected yellow band (R_f = 0.5; 20% EtOAc in *n*-hexane) was subjected to repeated SIL-HPLC using 4% EtOAc in *n*-hexane to afford new halogenated compounds **1** (46 mg, 5.2 × 10^{-3} %), **2** (0.8 mg, 9 × 10^{-5} %), **3a** (7.7 mg, 9 × 10^{-4} %), **3b** (0.6 mg, 7 × 10^{-5} %), and **4** (21 mg, 2.5 × 10^{-3} %).

Compound 1: colorless oil; $[\alpha]^{20}_D$ +280° (*c* 0.028, $CHCl_3$); IR (KBr) ν_{max} 3450, 1732, 1713, 1456, 1227, 1150, 737, 711 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS (glycerol matrix) *m/z* 361.8850 $[M]^+$ (calcd for $C_{10}H_{14}{}^{79}Br_2{}^{35}Cl_2$, 361.8839).

Compound 2: colorless oil; $[\alpha]^{20}_D$ −235° (*c* 0.049, $CHCl_3$); IR (KBr) ν_{max} 2981, 1749, 1489, 1367, 1230, 1055, 897, 775 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HREIMS *m/z* 307.0104 $[M - Br]^+$ (calcd for $C_{12}H_{17}{}^{79}Br^{35}ClO_2$, 307.0101).

Compound 3a: colorless oil; $[\alpha]^{20}_D$ −75° (*c* 0.030, $CHCl_3$); IR (KBr) ν_{max} 2962, 2927, 1747, 1228, 1024, 772, 669 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HREIMS *m/z* 385.9295 $[M]^+$ (calcd for $C_{12}H_{17}{}^{79}Br_2{}^{35}ClO_2$, 385.9284).

Compound 3b: colorless oil; $[\alpha]^{20}_D$ −87° (*c* 0.030, $CHCl_3$); IR (KBr) ν_{max} 2962, 2927, 1747, 1228, 1024, 772, 669 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HREIMS *m/z* 307.0078 $[M - Br]^+$ (calcd for $C_{12}H_{17}{}^{79}Br^{35}ClO_2$, 307.0101).

Compound 4: colorless oil; $[\alpha]^{20}_D$ −68° (*c* 0.058, $CHCl_3$); IR (KBr) ν_{max} 2966, 2929, 1747, 1638, 1231, 772 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HREIMS *m/z* 341.9803 $[M]^+$ (calcd for $C_{12}H_{17}{}^{79}Br^{35}Cl_2O_2$, 341.9789).

Diene 5. Zinc powder (0.025 g, 0.385 mmol) and **1** (0.025 g, 0.069 mmol) were stirred in 2 mL of dry acetic acid at room temperature. After stirring for 1 h, 10 mL of ether was added, the mixture was filtered, and the filtrate was washed with ether. The solvent was then removed in vacuo, and the residue was analyzed by NMR ($CDCl_3$) δ 6.18 (dd, J = 17.4, 11.0 Hz, H-2), 5.66 (s, H-8), 5.35 (d, J = 17.4 Hz, H-1_{trans}), 5.18 (d, J = 11.0 Hz, H-1_{cis}), 4.71 (dd, J = 3.0, 2.8 Hz, H-4), 4.58 (dd, J = 9.7, 6.4 Hz, H-6), 2.62 (m, H-5). Isolation of the resultant diene (**5**) by HPLC was attempted, but was unsuccessful because of polymerization.

Acknowledgment. The authors wish to thank K. Matsuura of JEOL for HRMS measurements. This work was supported in part by the Research Institute of Aoyama Gakuin University, and by a Grant in Aid (No. 12640527) for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

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NP0496946