Apakaochtodenes A and B: Two Tetrahalogenated Monoterpenes from the Red Marine Alga *Portieria hornemannii*

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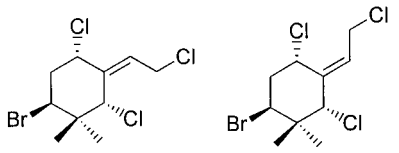
The structure of apakaochtodene A, the minor isomer of two tetrahalogenated ochtodene monoterpenes, isolated from the red marine alga *Portieria hornemannii* (Lyngbye) Silva has been identified as 6(*S**)-bromo-1,4(*S**),8(*R**)-trichloro-2(*Z*)-ochtodene (**1**) by NMR spectral and X-ray crystallographic analysis. Its geometrical isomer, apakaochtodene B (**2**), which could not be separated from **1** and thus characterized as a 95:5 mixture of **2**:**1** had ^{1}H and ^{13}C NMR spectral characteristics similar to previously known ochtodene (**3**) and the related tetrahalogenated monoterpene **4**.

The natural products chemistry of marine red algae (Rhodophyta) has been studied extensively over the past two decades. Many species of red algae have the ability to synthesize organic halogen-containing compounds that incorporate bromine, chlorine and occasionally iodine from seawater. Many novel halogenated terpenes are known from red algae, including species of the genera *Laurencia*, *Plocamium*, *Portieria*, and *Ochtodes*.[1] Among the red seaweeds, the genera *Plocamium*, *Portieria*, and *Ochtodes* are known to contain large amounts of both acyclic and cyclic polyhalogenated monoterpenes.[2] Continued studies of the natural products chemistry of these seaweeds have recently been stimulated by the discovery of the acyclic monoterpene, halomon [6(*R*)-bromo-3(*S*)-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene], which exhibits selective antitumor activity in the National Cancer Institute's human tumor and disease oriented in vitro screen.[3] Halomon was first isolated as a pure compound from a sample of the branching red alga *Portieria hornemannii* (Lyngbye) Silva (Order Gigartinales, Family Rhizophyllidaceae; formerly *Chondrococcus hornemannii*, *Desmia hornemannii*)[4] collected in Chanaryan, Batan Island, Philippines in April 1992.[3] To date, halomon has only been isolated from two other collections of *P. hornemannii*, as a mixture in 1975 collection in Hawaii[5] and in a recollection from Chanaryan in April 1992.[6] Multiple other collections from Chanaryan and a variety of locations in the Pacific have not yielded this compound in detectable amounts; instead an array of related halogenated acyclic and cyclic monterpenes have been reported.[3,6]

Portieria hornemannii exhibits notable site-to-site variation in secondary metabolite production.[6−9] This remarkable chemical variation has been documented for collections at various sites in Hawaii,[6] Guam,[7] Australia,[8,9] Japan,[10] and the Philippines.[6] Different sets of major secondary metabolites have been isolated from samples collected from sites separated by as few as 10 km.[9,11] *P. hornemannii* can be found in a variety of habitats in Guam, on reef flats and on the reef slope from 3 to 35 m. Preliminary studies of these populations of *P. hornemannii* suggested that ochtodene (**3**) is the major secondary metabolite produced by this alga in Guam with several acyclic monoterpenes as minor metabolites.[7] Feeding assays with different herbivorous reef fishes in Guam and in the Caribbean have shown ochtodene to be an effective feeding deterrent toward herbivores.[7] Ochtodene in *P. hornemannii* from Guam also showed selective solid tumor activity in cellular in vitro assays, but neither toxicity nor antitumor activity was observed in vivo.[12] Site-to-site chemical variation and the influence of nutrient availability on secondary metabolite production have also been examined in populations of *P. hornemannii* in Guam.[13] In this paper we wish to describe the structures of two new regioisomeric tetrahalogenated monoterpenes which we named apakaochtodene A (**1**) and B (**2**) from *P. hornemannii* collected at various reef sites around the island of Guam. It was interesting to note that the major isomer **2** had ^{1}H and ^{13}C NMR data identical with those reported for ochtodene (**3**)[14] and another recently isolated tetrahalogenated monoterpene **4**.[6]



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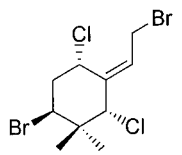

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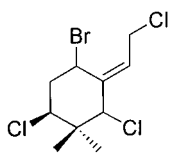

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An extract of *P. hornemannii* collected in Apaka Point Beach Park in Guam was fractionated as described in the Experimental section to yield a homogeneous fraction as judged by HPLC whose ^{1}H NMR spectrum indicated it to be a 1:1 mixture of two isomeric compounds. All attempts to separate this mixture by chromatographic techniques [normal and reversed-phase HPLC using chiral and non-

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Table 1. Selected ^{1}H and ^{13}C and HMBC Spectral Data of **1** and **2**

	1			**2**		
position	δ_H (mult., J in Hz)	δ_C	HMBC	δ_H (mult., J in Hz)	δ_C	HMBC
1	4.02 (dd, 12.1, 6.6)	37.37 (t)	C-2, C-3	4.04 (dd, 12.2, 6.7)	37.62 (t)	C-2, C-3
	4.16 (dd, 12.1, 9.6)		C-2, C-3	4.18 (dd, 12.2, 9.5)		C-2, C-3
2	6.03 (dd, 9.6, 6.6)	131.67 (d)	C-4, C-8	5.95 (dd, 9.5, 6.7)	131.89 (d)	C-4, C-8
3		138.22 (s)			137.83 (s)	
4	4.65 (m)	59.58 (d)		4.97 (dt, 5.0, 1.8)	50.44 (d)	C-2, C-3
5	2.50 (ddd, 15.0, 4.6, 2.4)	41.80 (t)	C-6	2.53 (ddd, 15.3, 12.7, 5.0)	41.32 (t)	C-6
	2.65 (ddd, 15.0, 12.4, 2.4)		C-6	2.68 (ddd, 15.1, 4.1, 1.8)		
6	4.83 (dd, 12.5, 4.3)	52.72 (d)		4.83 (dd, 12.7, 4.1)	52.70 (d)	
7		41.03 (s)			41.36 (s)	
8	4.73 (d, 1.2)	60.65 (d)		4.38 (d, 1.2)	69.98 (d)	C-2, C-3
						C-4, C-6
9, 10	1.02 (s)	20.63 (q)		1.01 (s)	20.47 (q)	
	1.33 (s)	28.60 (q)		1.28 (s)	28.53 (q)	

chiral columns, GC, and supercritical fluid chromatography (SFC)] failed. However, low-temperature crystallization of this mixture from hexane over a long period of time afforded **1**, albeit in very poor recovery. Extracts derived from *P. hornemannii* collected from other locations of Guam when purified by chromatography yielded a mixture of the same two compounds as above, but in different ratios. The extracts richest in **2** were from specimens collected in Gun Beach, Double Reef, and Pago Bay, as these contained **1** and **2** in the ratio of ca. 5:95. Attempted purification of the mixture derived from the Gun Beach collection by chromatography and preferential crystallization failed. However, on SFC employing the recycling technique, two peaks with baseline separation and having an area ratio of 95:5 were observed, but a preparative scale purification of the major compound **2** was not possible. This required us to investigate the structure of **2** (in a ca. 95:5 mixture of **2**:**1**) without further separation. Knowing the spectral data for **1** it was possible to subtract signals due to this compound from the spectra of **2** contaminated with ca. 5% of **1**.

Compound **1** failed to show a molecular ion peak under high-resolution conditions. However, its LRMS gave a parent peak at *m/z* 318 with characteristic isotopic components for Cl_3Br.[15] The ^{1}H NMR spectrum of **1** exhibited an olefinic proton at δ 6.03 (dd, J = 9.6 and 6.6 Hz) coupled to a set of two geminally coupled protons attached to a carbon containing a halogen atom [δ 4.02 (1H, dd, J = 12.1 and 6.6 Hz) and 4.16 (1H, dd, J = 12.1 and 9.6 Hz)]. In addition it had three protons on methine carbons bearing halogens [δ 4.65 (m), 4.73 (d, J = 1.2 Hz) and 4.83 (dd, J = 12.5 and 4.3 Hz)], a methylene [δ 2.50 (ddd, J = 15.0, 4.6 and 2.4 Hz) and 2.65 (ddd, J = 15.0, 12.4 and 2.4 Hz)] and a gem dimethyl moiety [δ 1.02 (s) and 1.33 (s)]. The ^{13}C NMR spectrum had signals corresponding to all 10 carbons, two of which were olefinic [δ 138.22 (s) and 131.67 (d)] and three of which were secondary carbons [δ 60.65 (d), 59.58 (d), and 52.72 (d)], each bearing a halogen atom. The remaining halogen atom was located at C-1 based on its ^{1}H and ^{13}C NMR chemical shifts and HMBC correlations (Table 1). Although it was possible to assign most of the ^{1}H and ^{13}C NMR signals of **1** with the help of their chemical shifts, multiplicity, DQCOSY, HMQC, and HMBC data, confirmation of its halogenation pattern and the determination of stereochemical disposition of each halogen atom and the olefinic double bond was not attempted due to recently recognized problems in the application of NMR data for structure elucidation of polyhalogenated monoterpenes;[16] consequently several structures of this class of compounds have been revised after X-ray analysis.[5,11,17] An X-ray diffraction analysis of a single crystal of **1** was thus undertaken.

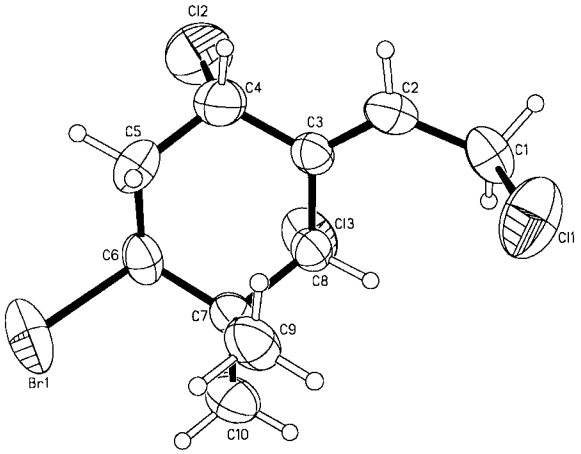

Figure 1. Perspective view of X-ray crystal structure of apakaochtodene A (**1**).

The crystal and molecular structures of **1** were determined from three-dimensional X-ray diffraction data[18] and the result is shown in Figure 1. The assignment of heteroatom types is based on a refinement of site occupancies which unambiguously distinguishes between chlorine and bromine atoms.[14] Resulting carbon−halogen bond distances are also consistent with these assignments. The assignment of absolute configuration, based on the refined absolute structure parameter, is consistent with other known compounds in this family.[14]

With the X-ray crystal structure of **1** in hand, we attempted to determine the structure of the major constituent **2**. ^{1}H and ^{13}C NMR spectral data of **2** showed a close resemblance to those of **1** (Table 1) except for the signals for the C-4 and C-8 positions. More interestingly these data for **2** are almost identical with those reported for ochtodene (**3**)[14] and the related polyhalogenated monoterpene **4**,[6] except for H-4. In **2**, H-4 appears as a dt (J = 5.0 and 1.8 Hz) whereas in **3** and **4** it appears as a dd (J = 4.8 and 1.8 Hz) and d (J = 4.9 Hz), respectively. This difference appears to be too minor to suggest that they have different structures. Comparison of ^{1}H and ^{13}C NMR data of **1** and **2**, while showing differences in chemical shifts for positions 4 and 8 (Table 1), also revealed an interesting trend. In going from **1** to **2**, $\Delta_{\delta(H-4)}$ and $\Delta_{\delta(C-4)}$ were found to be +0.32 and −9.14 ppm, respectively, and for position 8 $\Delta_{\delta(H-8)}$ and $\Delta_{\delta(C-8)}$ were −0.35 and +9.43 ppm, respectively. Since the chemical shifts of protons and carbons at all other positions of **1** and **2** were identical, the above differences can only be explained by postulating two isomeric (*Z* and *E*) structures for **1** and **2**. Such differences in chemical shifts of alicyclic carbons due to *E* and *Z*

orientations of substituted γ-alkyl groups have been observed previously for ochtodiene stereoisomers.[19]

Experimental Section

General Methods. For vacuum chromatography, 400−230 mesh Si gel 60 (E. Merck No. 9385) was employed. HPLC separations were carried out using Waters isocratic system on Altech Econosil column with RI detection. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Unity 400 spectrometer at 399.95 and 100.57 MHz, respectively. All NMR spectra were recorded in $CDCl_3$ using solvent (δ_c 77.0 ppm) or residual $CHCl_3$ (δ_H 7.26 ppm) as internal standards. Coupling constants are reported in Hz. ^{1}H−^{1}H COSY, DEPT, ^{1}H−^{13}C HETCOR, HMBC, and HMQC NMR experiments were performed on the same spectrometer, using standard Varian pulse sequences; in HMBC determinations a 9 Hz optimization was employed for the long-range coupling pathways. MS were taken on a VG 7070 E-HF mass spectrometer. X-ray crystallographic data were collected on an Enraf Nonius CAD4 diffractometer.

Plant Material. For this study, *P. hornemannii* was collected from a variety of reef sites on Guam and Mariana Islands over a period of several years.

Extraction and Isolation. The yields of organic extracts and the major metabolite **2** have been previously documented for each of six reef habitats.[13] Extract yields (weight of extract per gram of algal dry weight) varied from approximately 2.5−5.0% among most collection sites; however, the amounts of the major and minor metabolites (**2** and **1**, respectively) varied considerably among sites. Algae collected from the Agat Bay area of Guam (collection sites at Anae Island and Apaka Point Beach Park) had the highest levels of both compounds (ca. 0.5% of algal dry weight for each compound), and we focused on collections from Apaka Point, where the alga was abundant, for isolating monoterpene **1**.

P. hornemannii was collected from Apaka Point Beach Park, Guam on February 10, 1995. The alga was immediately extracted 4 times in 1:1 (v/v) CH_2Cl_2/MeOH. The resulting extract was partitioned between hexanes and water to yield 2.2 g of organic extract from 28.9 g extracted dry alga. Other collections of *P. hornemannii* from Apaka Point were made in April 1995 and processed similarly to yield an additional 6.0 g of organic extract. The combined extracts (8.2 g) were vacuum chromatographed over Si gel with a gradient of hexanes to EtOAc. The monoterpenes were eluted in the first five fractions (100% hexanes, 5%, 10%, 15%, and 20% EtOAc in hexanes). These fractions were recombined (3.7 g) and chromatographed over Sephadex LH-20 in 1:1 (v/v) CH_2Cl_2/MeOH. A series of 15 mL fractions were collected, and were combined based on their similarity by TLC analysis; fractions 20−26 contained most of the compounds **1** and **2**. Combined fractions 20−26 (2.8 g) were fractionated using Si gel Mega Bond-Elut column chromatography in a gradient of increasing polarity of hexanes and EtOAc. The combined fractions eluted with 100% hexanes and 5% EtOAc in hexanes (1.0 g), which contained **1** and **2**, were chromatographed in the same solvent system by semipreparative Si gel HPLC. HPLC fractions 7 and 8 (533 mg combined) contained a 1:1 mixture of compounds **1** and **2**, and the mixture was inseparable by HPLC.

Purification of compound **1** from this mixture was accomplished by crystallization from cold hexanes. The mixture of compounds **1** and **2** (above combined HPLC fractions 7 and 8; 533 mg) was dissolved in hexanes and stored in a 20 °C freezer until crystals formed. The supernatant was removed and crystals were washed with cold (−20 °C) hexanes. The crystals were then dissolved in hexanes at room temperature, and the procedure was repeated twice more to yield 5.3 mg of **1**. The supernatant from the first purification step was stored at −20 °C to get more crystals of **1** and the same procedure of recrystallization repeated to yield another crop (6.9 mg) of **1**.

The major constituent **2** was isolated by chromatography from several populations of *P. hornemannii* in Guam that did not contain major amounts of **1**. Collections from Double Reef, Gun Beach, and Pago Bay contained compounds **1** and **2** in the ratio of 5:95 (based on GC and ^{1}H NMR analysis), whereas collections of the alga from Apaka Point contained compounds **1** and **2** in the ratio of 2:3. Monoterpene **2** was isolated from the Double Reef and Pago Bay collection by the chromatographic methods described above for monoterpene **1**, but we were never able to eliminate the 5% contaminant of monoterpene **1** (by HPLC, fractional recrystallization or SFC).

Supporting Information Available: Copies of ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HETCOR, HMQC, and HMBC NMR, and mass spectra of compounds **1** and **2** (containing ca. 5% of **1**), X-ray crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Single-crystal X-ray structure determination: clear colorless prisms, monoclinic, $P2_12_12_1$, $a = 7.235(3)$ Å, $b = 12.842(2)$ Å, $c = 13.815(3)$ Å, $U = 1283.6(6)^3$, $Z = 4$, R1 (for 1308 data $I > 2\sigma(I)$) = 0.042, wR2 = 0.015, GOF = 1.077. The absolute structure parameter (Falck, H. D. *Acta Crystallogr.* **1985**, *A39*, 876−881) refined to 0.03 for the reported enantiomer.
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