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Tetrahydropyran Monoterpenes from *Plocamium cartilagineum* and *Pantoneura plocamioides*

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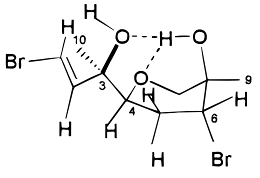
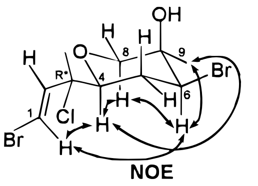
Four new marine monoterpenes have been isolated from two species of marine red algae, *Plocamium cartilagineum* and *Pantoneura plocamioides*. The structures and relative stereochemistry of these compounds were determined on the basis of spectroscopic evidence and suggest a relationship between *P. cartilagineum* and *P. plocamioides*.

Most of the known polyhalogenated monoterpenes come from red algae of the Plocamiaceae and Rhizophyllidaceae families[1] (order Gigartinales). *Plocamium cartilagineum* Dixon (Plocamiaceae) is a widely distributed alga, and a large variety of halogenated monoterpenes have been isolated from it.[2] From *Pantoneura plocamioides* Kylin, an Antarctic endemic that belongs to the Delesseriaceae family (order Ceramiales), we have isolated[3,4] a number of unusual oxane derivative monoterpenes with a high degree of oxygenation.

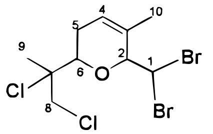
In this work we describe the structures of four new closely related monoterpenes, two of which (**1** and **2)** were isolated from *P. cartilagineum* collected off the Chilean coast, while the other two (**3** and **4)** were isolated from *P. plocamioides*.



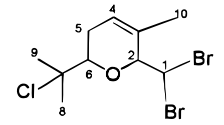
Pantopyranoid A



Plocamiopyranoid (1)



2



Pantoneurines A-B (3-4)

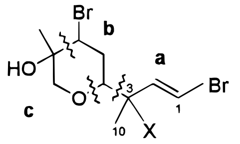
Results and Discussion

Plocamiopyranoid **1** was isolated from the fraction of the crude extract of *P. cartilagineum* eluted with hexane−EtOAc (85:15). The EIMS spectrum showed peaks at *m*/*z* 343, 345, 347, with relative intensities suggestive of two bromine atoms and one chlorine atom, which correspond to the empirical formula $C_{10}H_{14}OBr_2Cl$ [M^+-OH] (HREIMS). A hydroxyl group was observed at 3550 cm^{-1} in the IR spectrum.

The ^{13}C NMR (Table 1) showed signals for 10 carbons. Multiplicities of the carbon signals were determined from the DEPT spectrum: two methyls, two methylenes (one bearing oxygen) and four methines (two olefinic, one bearing oxygen and the other bromine), and two nonprotonated carbons.

The ^1H NMR spectrum of **1** (Table 1) showed signals corresponding to two trans-disubstituted olefinic protons at δ 6.61 (1H, d, $J = 13.5$ Hz) and 6.42 (1H, d, $J = 13.5$ Hz) and two doublets at δ 4.41 (1H, dd, $J = 2.2, 10.3$ Hz) and 4.68 (1H, dd, $J = 2.2, 10.3$ Hz), corresponding to methine protons geminal to heteroatoms. A methylene multiplet appeared at δ 2.68, while two doublets corresponding to the protons of the methylene geminal to oxygen appeared at δ 4.04 and 3.82 (1H, d, $J = 12.3$ Hz) each. At high field, the signals at δ 1.64 (3H, s) and 1.83 (3H, s) correspond to methyl groups geminal to oxygen and halogen, respectively.

Chemical shift arguments and $^1H-^1H$ COSY correlations supported by MS data allowed the assignment of fragments **a**−**c** as shown (**1**), resembling those of pantopyranoid A,[4] with the difference being the chlorine substituent at C-3 instead of the hydroxyl group of pantopyranoid A. This was confirmed by the mass spectrum that showed an ion at *m*/*z* 167, 169, 171, characteristic[5] of fragment **a**. The combination of the data from HMQC and HMBC was in agreement with the proposed structure.



Plocamiopyranoid 1 X=Cl
Pantopyranoid A X=OH

The relative stereochemistry of the three chiral centers of the ring was deduced by a study of the coupling constants of the H-4 and H-6 protons and by 2D ROESY NMR experiments. In the 2D ROESY experiments, the H-4 and H-6 protons showed a NOE with the same proton of the methylene at C-8, and the C-9 methyl group showed a NOE with H-4 and H-6; all these effects are in accord with the *J* values observed and indicate the stereochemistry represented in the figure.

The relative stereochemistry at C-3 was deduced by 2D ROESY NMR and by molecular mechanics calculations using PCMODEL.[6] In the 2D ROESY experiment, H-1 showed a NOE with H-4 and H-6. After minimization of the structure, the energetically favorable conformation for the compound with an *R** stereochemistry at C-3 was in agreement with the observed NOEs, while the resulting

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Table 1. ^{1}H and ^{13}C Data of Compounds **1**−**4**, and HMBC of Compounds **1**−**3** [500 MHz, δ ppm, (J) Hz]

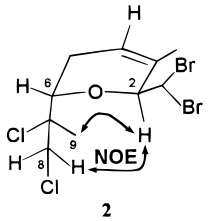
	1			**2**			**3**			**4**	
no	H^a	C^a	HMBC	H^a	C^a	HMBC	H^b	C^b	HMBC	H^b	C^b
1	6.61 d (13.5)	109.78	H2	5.67 d (9.4)	45.19	H2	5.26 d (9.3)	45.44	H2,	5.28 d (9.3)	45.29
2	6.42 d (13.5)	139.95	Me-10	4.79 d (9.4)	73.49		4.58 d (9.3)	73.18	H4,	4.60 d (9.3)	73.37
3		72.01	H1, H2, Me-10		134.85	H2, H5, Me-10		133.69	H2, H5, Me-10		133.59
4	4.41 dd (2.2, 10.3)	60.28	H5, Me-10	5.82 t (7.0)	129.46	H2, H5, Me-10	5.42 t (7.1)	130.29	H2, H6, H5, Me-10	5.34 t (7.6)	130.42
5	2.68 m	38.15	H4, H6	2.47 m	32.24	H4	2.33 m 2.88 ddd (1.1, 7.0, 15.0)	32.84	H4, H6	2.33 m 2.84 ddd (1.0, 6.1,15.5)	33.05
6	4.68 dd (2.2, 10.3)	56.70	H4, H5, H8, Me-9	3.48 br s	72.54	H8, Me-9	3.84 dd (2.2, 10.9)	63.50	H4, H5, Me-8, Me-9	3.76 dd (2.1, 10.9)	63.56
7		75.92	H6, Me-9		72.90			71.21	H6, Me-8, Me-9		71.15
8	β 3.82 d (12.3) α 4.04 d (12.3)	70.53	Me-9	4.28 d (10.8) 3.81 d (10.8)	51.13	H9, H6	1.56 s	32.52	Me-9, H-6	1.55	32.47
9	1.64 s	21.17	H6, H8(	1.82 s	25.95	H8	1.42 s	26.37	H6, Me-8	1.43	26.50
10	1.83 s	25.65	H4	1.74 s	11.18	H4, H2	1.44 s	10.62	H1, H2	1.45	10.62

[a] $CHCl_3$-*d*. [b] C_6H_6-*d*.

more stable conformation for an S^* stereochemistry at C-3 is unlikely to give the observed NOEs. Thus, the relative stereochemistry for **1** was established as that represented in the figure.

It is interesting to point out the influence of the substituent at C-3 on the conformation of the tetrahydropyran ring. In pantopyranoid A the hydroxyl group at C-3 induced an H-bond interaction of the C-7−OH proton with both the C-3 and ring oxygens, forcing the ring to adopt a twisted-boat conformation, in accordance with the observed J values for the protons H-4 and H-6. However, the absence of the H-bond interaction in plocamiopyranoid (**1**) gave the expected chair conformation for the compound, in agreement with the observed J values.

Compound **2** was a colorless oil. The EIMS spectrum showed the molecular ion at m/z 378, 380, 382, 384, with relative intensities suggesting two bromine atoms and two chlorine atoms. The molecular ion was confirmed by HRMS to correspond to $C_{10}H_{14}OBr_2Cl_2$, indicating two degrees of unsaturation.



2

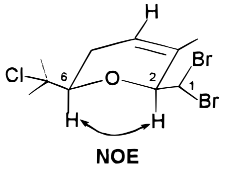
The ^{13}C NMR spectrum of **2** (Table 1) showed signals for 10 carbons. Multiplicities of the carbon signals were determined from the DEPT spectrum: two methyls (one vinylic and the other geminal to halogen), two methylenes (one bearing halogen), four methines (one olefinic, two bearing oxygen), and two quaternary carbons (one olefinic). The ^{1}H NMR spectrum (Table 1) showed signals for an olefinic proton [δ 5.82 (1H, t, J = 7.0 Hz)] and three methines each geminal to a heteroatom δ 5.67 (1H, d, J = 9.4 Hz), 4.79 (1H, d, J = 9.4 Hz), and 3.48 (1H, br s)]. The protons of one methylene appeared at δ 2.47 (2H, m), while two doublets corresponding to the protons of the methylene geminal to heteroatoms appeared at δ 4.28 (1H, d, J = 10.8 Hz) and 3.81 (1H, d, J = 10.8 Hz). The presence of only two methyl groups at δ 1.82 (3H, s) and 1.74 (3H, s) suggested that the third methyl group, corresponding to a monoterpene skeleton, was oxidized.

A ^{1}H−^{1}H COSY experiment established the connectivity of the H-4−H-6 fragment. The coupling constants and ^{1}H−^{1}H COSY established the connectivity of the H-2−H-1 fragment and the presence of a dibromo-substituted carbon; this finding was supported by the mass spectrum of **2**, which gave a peak at m/z 183, 185, 187 consistent with the fragment $CHBr_2$.

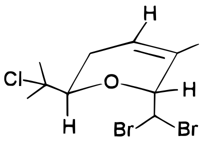
Proton−carbon chemical shift correlations for all the carbons directly bonded to protons were established by HMQC, and correlation of C-4 with H-2 in a HMBC experiment established the C-2/C-3 linkage. The C-8−C-7−C-9 linkage was established by the correlation of C-9 with H-8 and C-8 with Me-9, and the correlation of C-8 with H-6 established the overall structure **2**, with the requisite two degrees of unsaturation.

The relative stereochemistry of the chiral centers of the ring was determined by a 2D ROESY ($CDCl_3$) experiment that showed NOE of H-2 with Me-9 and a NOEDIFF experiment, which gave interaction between H-2 and a proton on the methylene C-8, indicating that the H-2 and H-6 are trans. Compound **2** is closely related to costatone.[7,8]

From the less polar fraction of the acetone extract of *P. plocamioides* we obtained material that gave an apparently homogeneous compound by TLC. Analysis of the ^{1}H NMR spectrum (C_6D_6) indicated that the material was a 1:1 mixture of two compounds that were purified by recycling-HPLC and named pantoneurines A (**3**) and B (**4**).



Pantoneurine A (3)



Pantoneurine B (4)

The CIMS spectrum of compound **3** showed the molecular ion [M^{+}+1] at m/z 345, 347, 349, 351 corresponding to $C_{10}H_{15}OClBr_2$ and indicating two degrees of unsaturation. The EIMS spectrum showed a fragment ion at m/z 327, 329, 331, 333 [M^{+}−OH].

The ^{13}C NMR (C_6D_6) spectrum of **3** (Table 1), together with the information from a DEPT spectrum, showed the presence of 10 carbon signals assigned to $3CH_3$, $1CH_2$, and 4CH (one olefinic and three geminal to the heteroatom) and two nonprotonated carbon atoms (one olefinic and the other bearing a heteroatom).

The ^{1}H NMR (C_6D_6) spectrum (Table 1) showed one olefinic proton at δ 5.42 (1H, t, J = 7.1 Hz). Two doublets at δ 5.26 (1H, d, J = 9.3 Hz) and 4.58 (1H, d, J = 9.3 Hz) were assigned to protons geminal to a halogen and an oxygen, respectively. The signal at δ 3.84 (1H, dd, $J = 2.2$, 10.9) was assigned to a proton geminal to oxygen. A methylene multiplet appeared at δ 2.88 (1H, ddd, $J = 1.1$, 7.0, 15.0 Hz) and 2.33 (1H, m), and three methyl groups, at δ 1.56, 1.44, and 1.42 (3H, s, each).

The presence of a gem dimethyl group bearing chlorine was supported by the mass spectrum of **3**, which gave a base peak at *m/z* 77, 79, consistent with fragment C_3H_6Cl. A $^1H-^1H$ COSY experiment established the connectivity of the H-4−H-6 fragment and the coupling constants, and $^1H-^1H$ COSY established the connectivity of H-2−H-1.

From the HMBC experiment the C-2/C-3 linkage was established by the correlation of C-4 with H-2. The linkage C-6/C-7 was confirmed by the correlation of C-8 and H-6 and the correlation of C-8 and Me-9. These assignments led to the overall structure **3**, with the requisite two degrees of unsaturation.

The ^1H and ^{13}C NMR spectra of pantoneurine B (**4**) were very similar to those of pantoneurine A (**3**) (Table 1). The stereochemistry for compound **3** was given by a NOEDIFF experiment, which showed a NOE between H-6 and H-2. In **4** the absence of the NOE between H-6 and H-2 suggested that both compounds must be epimers, despite the fact that the H-2 and H-6 coupling constants for both compounds have practically the same values. Molecular mechanics calculations using PCMODEL corroborated this. After minimization of compounds **3** and **4**, the coupling constants for H-2 and H-6 measured by the program are in good agreement with the observed *J* values for these compounds.

The close structural relationship between plocamiopyranoid (**1**) and pantopyranoid A and between compound **1** and pantoneurines A and B (**3**, **4**) is quite surprising because *P. cartilagineum* and *P. plocamioides* belong to different orders, Gigartinales and Ceramiales, respectively. This points out that *P. cartilagineum* and *P. plocamioides*, although belonging to different orders, are similar at the biochemical level or that a taxonomic revision is required.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin−Elmer model 241 polarimeter using a Na lamp at 25 m in $CHCl_3$ solutions. EIMS and HRMS spectra were taken on a Micromass Autospect spectrometer. CIMS spectra were determined with a Hewlett−Packard 5998 using methane as reactive gas. ^1H and ^{13}C NMR, HMQC, HMBC, 2D ROESY, and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^1H NMR and at 125 MHz for ^{13}C NMR, using TMS as internal standard. Two-dimensional spectra were obtained with the standard Bruker software. Recycling-HPLC separations were performed with a Japan Analytical LC-908. The gel filtration column (Sephadex LH-20) used hexane−MeOH−$CHCl_3$ (2:1:1 or 7:1:1) as solvent. Merck Si gels 7734 and 7729 were used on column chromatography. The spray reagent for TLC was H_2SO_4−H_2O−AcOH (1:4:20).

Plant Material. *P. cartilagineum* was collected in El Yeco (33° 18′S, 71° 38′ W; V Región, Chile), and *P. plocamioides*, off King George Island (South Shetland, Antarctic) by scuba diving. Voucher specimens have been deposited at the Museo de Historia Natural, Santiago de Chile (nos. R23P135Plc and R23D18Ppl, respectively).

Extraction and Isolation of Monoterpenoids 1 and 2. *P. cartilagineum* (770 g, dry wt) was extracted with EtOAc−CH_2Cl_2−hexane (1:1:1) at room temperature. The extract was concentrated to give a dark-green residue (30 g) and chromatographed by flash chromatography on Si gel. The fraction eluted with hexane−EtOAc (85:15) (0.97 g) was further separated by filtration chromatography to give a fraction (371.2 mg) that was chromatographed on a Si gel column to obtain 8 mg of plocamiopyranoid (**1**) after purification using recycling-HPLC (Jaigel-sil column 20 × 250 mm). The fraction eluted with hexanes−EtOAc (95:5) (1.25 g) was chromatographed on a Sephadex LH-20 column with hexane−MeOH−$CHCl_3$ (7:1:1) as eluent, affording **2** (49 mg).

Plocamiopyranoid A (1): colorless oil; $[\alpha]^{25}_{D}$ −33° (*c*, 1.05, $CHCl_3$); IR ν_{max} 3550 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 343, 345, 347 [$M^+ - OH$; 1, 2, 1]; 167, 169, 171 [C_4H_5BrCl; 17, 19, 7]; 119 (58); 105 (20); 91 (50); 53 (100); HRMS [$M^+ - OH$] 344.908 (calcd for $C_{10}H_{14}O^{79}Br^{81}Br^{35}Cl$ 344.908).

Compound 2: colorless oil; $[\alpha]^{25}_{D}$ +14° (*c*, 0.01, $CHCl_3$); IR 3015; 2350; 1445; 1377; 1044 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 378, 380, 382, 384 [$C_{10}H_{14}OBr_2Cl_2$; 1, 2, 2, 1]; 359, 361, 363, 365 [$M^+ - OH - HBr$; 9, 20, 14, 2]; 315, 317, 319, 321 [$C_8H_{10}OBr_2Cl$; 7, 12, 5, 7]; 274, 276, 278, 280 ($C_5H_5OBr_2Cl$ 5, 11, 8, 1); 183, 185, 187 ($CHBr_2$ 30, 57, 30); 159,161 (C_6H_8Br 100, 60); 103, 105 (C_5H_8Cl 62, 39); HRMS [M^+ 381.876] (calcd for $C_{10}H_{14}O^{81}Br_2{}^{35}Cl_2$ 381.874).

Extraction and Isolation of 3. The dried alga (1.4 kg) was extracted with Me_2CO at room temperature, and the Me_2CO extract was concentrated to give a dark-green residue (39 g). This extract was chromatographed by flash chromatography on Si gel. The fraction eluted with hexane−EtOAc (95:5) (1.39 g) was further separated by Si gel chromatography followed by separation with recycling-HPLC to give 15.6 mg of pantoneurine A and 4.0 mg of pantoneurine B.

Pantoneurine A (3): IR 1602; 1146 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 327, 329, 331, 333 [$M^+ - OH$; 22, 50, 38, 7]; 273,375,377,379 [$C_5H_4OBr_2Cl$; 7, 12, 8, 2]; 247, 249, 251 [$M^+ - OH - Br$; 4, 9, 6]; 77,79 [C_3H_6Cl; 100, 80]; CIMS 343, 345, 347, 349 [$M^+ - 1$]; 345, 347, 349, 351 [$M^+ + 1$]; 373, 375, 377, 379 [$M^+ + 29$]; 385, 387, 389, 391 [$M^+ + 41$]; HRMS [$M^+ - OH$] 328.904 (calcd for $C_{10}H_{14}79Br_2{}^{37}Cl$ 328.912).

Pantoneurine B (4): IR 1602; 1140 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 327, 329, 331, 333 [$M^+ - OH$; 18, 39, 27, 5]; 273,375,377,379 [$C_5H_4OBr_2Cl$; 7, 13, 9, 3]; 247, 249, 251 [$M^+ - OH - Br$; 5, 9, 6]; 77,79 [C_3H_6Cl; 100, 79]; CIMS 343, 345, 347, 349 [$M^+ - 1$]; 345, 347, 349, 351 [$M^+ + 1$]; 373, 375, 377, 379 [$M^+ + 29$]; 385, 387, 389, 391 [$M^+ + 41$]; HRMS [$M^+ - OH$] 328.907 (calcd for $C_{10}H_{14}79Br_2{}^{37}Cl$ 328.912).

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