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Pantoneurotriols: Probable Biogenetic Precursors of Oxygenated Monoterpenes from Antarctic *Pantoneura plocamioides*

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Abstract: Pantoneurotriols **1a** and **2a** are acyclic polyoxygenated monoterpenes isolated from the Antarctic endemism *Pantoneura plocamioides* together with compounds **3** and **4**. The stereochemistries of these compounds were established by spectroscopic methods and pantoneurotriols have been proposed as biogenetic precursors of pantofuranoids, uncommon monoterpenes isolated from the same species. It was interesting to find compounds **4** and **5**, metabolites first isolated from *Plocamium cartilagineum*, in a species belonging to a different order such as *P. plocamioides*.

Marine monoterpenes are characterized by the high number of halogens they incorporate in their biogenesis. Most compounds contain five or even six halogen substituents in a ten carbon atoms[1] structure. From the first chemical study of *Pantoneura plocamioides*, an endemic red alga from the Antarctic, we isolated[2,3] a number of unusual oxane derivative monoterpene metabolites with a high oxygen content. Here we describe two acyclic polyhydroxylated monoterpenes, pantoneurotriols **1a** and **2a**, as the most probable biogenetic precursors of these oxygenated metabolites as well as the structure and stereochemistry of compounds **3** and **4**.

RESULTS AND DISCUSSION

The polar material obtained from flash chromatography of the crude alga extract after filtration on Sephadex LH-20 and successive chromatographies on silica gel yielded a mixture of two unstable compounds **1a** and **2a** which were separated as the monoacetate derivatives **1b** and **2b** by RHPLC (recycling-HPLC) after acetylation of the mixture with acetic anhydride and pyridine.

Compound **1b** was a colourless oil $[\alpha]^{25}_{D}$= -26° (c, 0.9, $CHCl_3$). The EI-MS spectrum showed the molecular ion (M^+–2) at m/z 304/306 with a relative intensity for the bromine atom in accordance with the empirical formula $C_{12}H_{19}BrO_4$ (HR-MS). The IR spectrum showed absorption for the hydroxyl and carbonyl

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group at 3582 cm^{-1} and 1736 cm^{-1}, respectively. The ^{13}C NMR spectrum of **1b** (table 1) gave signals for twelve carbon atoms (two carbons having the same chemical shift). Multiplicities of the carbon signals were determined from the DEPT spectrum: four methyl groups, one methine, four olefinic carbons (one of them bearing bromine), two quaternary carbons and one carbonyl group. The ^1H NMR spectrum (table 2) showed signals for two *trans*-disubstituted olefins at δ 6.25 (1H, d, *J*=13.4 Hz) and δ 6.41 (1H, d, *J*=13.4 Hz) and at δ 5.95 (1H, d, *J*=15.6 Hz) and δ 5.65 (1H, dd, *J*=7.4, 15.8 Hz), a doublet at δ 5.15 (1H, d, *J*=7.4 Hz) assignable to a proton geminal to the acetate group, the acetate methyl group at δ 2.09 and the three methyl groups geminal to alcohol at δ 1.26 (3H, s) and δ 1.33 (6H, s), completing all the protons of the molecule.

Chemical shift arguments and ^1H-^1H-COSY correlations supported by MS data allowed the assignments of fragments **a-c** as indicated (**1b**). The two olefins and the carbonyl group unsaturations are in keeping with the three degrees of unsaturation required by the molecular formula, indicating that the other two oxygen atoms must be alcoholic. The disubstituted nature of the olefinic carbon atoms as well as the carbinolic moiety of fragment **a** suggested by ^1H and ^{13}C NMR data are in agreement with the EI-MS spectrum that gave the ion at m/z 149/151 characteristic of the fragment **a** present in halogenated monoterpenes[2-4]. The presence of a hydroxylated isopropyl group, fragment **c**, was also supported by the MS spectrum which gave a peak at m/z 59 consistent with a C_3H_7O fragment. HMQC and HMBC data were used to confirm fragments **a-c** and establish the connectivity between them. The C-3 and C-4 carbons must be linked due to the correlations of C-4 with Me-10. The C-6/C-7 linkage was established by correlations of C-6 with Me-8 and Me-9.

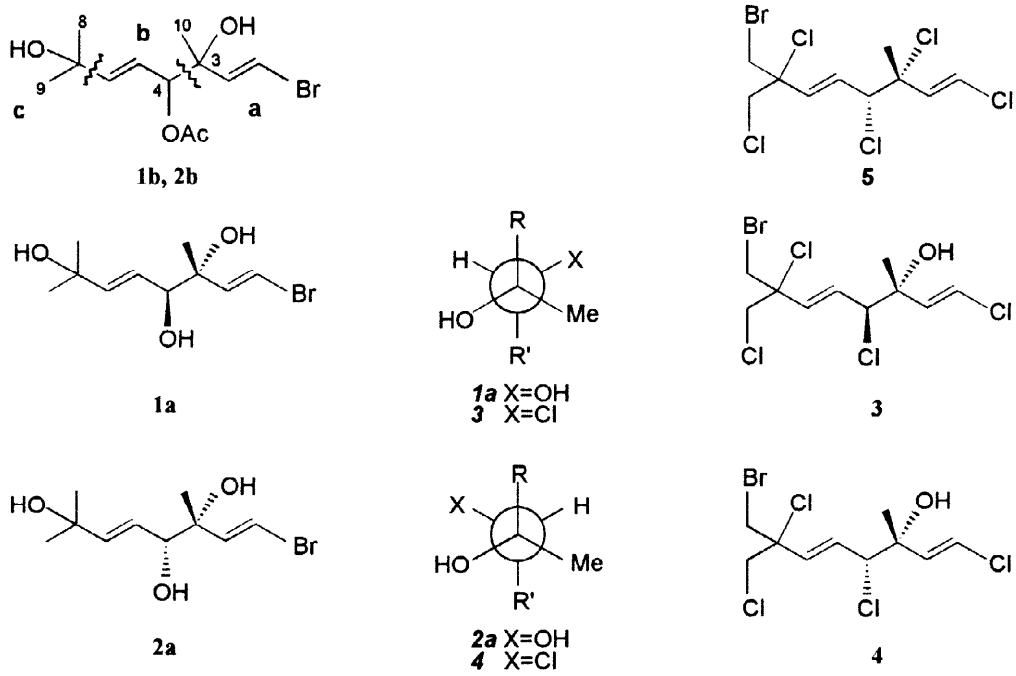


Figure 1

Table 1. ^{13}C NMR Data (50 MHz, $CDCl_3$, δ ppm)

Carbon	1a	2a	1b	1b-HMBC	2b	3	4
1	107.21 (d)	107.32 (d)	107.10 (d)	H-2	107.60 (d)	120.93 (d)	121.14 (d)
2	141.38 (d)	139.70 (d)	140.60 (d)	Me-10	139.00(d)	133.05 (d)	132.68 (d)
3	76.34(s)	no obs.	75.81 (s)	H-1, H-2, H-4, Me-10	75.58 (s)	75.00 (s)	75.05 (s)
4	77.61 (d)	78.40 (d)	78.77 (d)	H-6, Me-10	78.95 (d)	69.00 (d)	69.26 (d)
5	123.65 (d)	124.10 (d)	120.00 (d)	H-4	120.40 (d)	130.84 (d)	131.14 (d)
6	142.82 (d)	142.50 (d)	144.80 (d)	H-4, Me-8, Me-9	144.00 (d)	135.73(d)	134.45 (d)
7	70.71 (s)	no obs.	70.68 (s)	H-5, H-6, Me-8, Me-9	70.64 (s)	69.00 (s)	69.26 (s)
8	29.85 (q)	29.89 (q)	29.73 (q)	Me-9	29.60 (q)	37.42 (t)	37.14 (t)
9	29.76 (q)	29.79 (q)	29.73 (q)	Me-8	29.60 (q)	49.66 (t)	49.64 (t)
10	22.71 (q)	24.24 (q)	24.06 (q)	H-4, Me-12	24.41 (q)	24.64 (q)	25.61 (q)
11	---	---	170.18 (s)		170.23 (s)	---	---
12	---	---	2.09 (q)		2.11 (q)	---	---

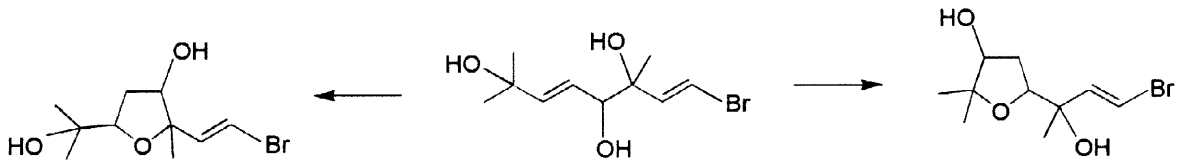
Table 2. ^{1}H NMR Data [200 MHz, $CDCl_3$, δ ppm, (pattern, *J*, Hz)]

Proton	1a	2a	1b	2b	3	4
1	6.43 (d, 13.6)	6.39 (d, 13.6)	6.41 (d, 13.4)	6.39 (d, 13.6)	6.38 (d, 13.2)	6.36 (d, 13.4)
2	6.27 (d, 13.6)	6.24 (d, 13.6)	6.25 (d, 13.4)	6.22 (d, 14.0)	6.05 (m)	5.96 (d, 13.0)
4	3.97 (d, 6.7)	3.97 (d, 6.8)	5.15 (d, 7.4)	5.14 (d, 7.6)	4.34 (d, 7.2)	4.37 (d, 7.6)
5	5.68 (dd, 6.7, 15.6)	5.68 (dd, 6.7, 15.6)	5.65 (dd, 7.4, 15.8)	5.61 (dd, 7.6, 15.4)	6.05(m)	6.08 (dd, 7.8, 15.4)
6	5.94 (d, 16.0)	5.93 (d, 16.4)	5.95 (d, 15.6)	5.89 (d, 15.6)	6.00 (d, 15.2)	5.98 (d, 15.2)
8	1.34 (s)	1.33 (s)	1.33 (s)	1.31 (s)	3.84 (dd, 11.0, 18.5)	3.81 (dd, 10.8, 17.7)
9	1.34 (s)	1.33 (s)	1.33 (s)	1.31 (s)	3.96 (dd, 11.6, 14.6)	3.93 (dd, 12.0, 14.0)
10	1.23 (s)	1.29 (s)	1.26 (s)	1.28 (s)	1.39 (s)	1.44 (s)
12	---	---	2.09 (s)	2.11 (s)	---	---

Compound **2b** was a colourless oil $[\alpha]^{25}_D$= -3.93° (c, 0.23, $CHCl_3$). The IR, ^1H and ^{13}C NMR spectra of **2b** were very similar to those of compound **1b** suggesting that the differences were in the stereochemistry at the C-3, C-4 chiral centres. It has been established[5] that the large shift difference observed in the ^{13}C chemical shift of the Me-10 is a good method for assigning relative stereochemistries at C-3/C-4, but we were unable to make any stereochemical assignments due to the little differences on the values of the chemical shifts of Me-10 of both compounds (table 1). However, when the acetate group of **1b** and **2b** were removed (potassium carbonate in methanol at 0°) the ^{13}C NMR data of the C-10 methyl group of the corresponding alcohols **1a** and **2a** showed a chemical shift difference of 1.53 ppm. Assuming that the lowest δ_C value for C-10 corresponds to the most hindered methyl group[5], it can be seen from a Newman projection of the C-3/C-4 chiral centres of both compounds (figure 1) that in the most stable conformations ***2a***, the C-10 methyl group is less hindered and then shifted downfield than the C-10 methyl group of ***1a***. This observation, in addition to the fact that the compound **1a** is 1.82 Kcal/mol. less stable[6] than the corresponding epimer **2a**, allows us to propose the relative stereochemistries for C-3/C-4 in **1a** and **2a** as (3S*, 4R*) and (3R*, 4R*) respectively, as shown in figure 1.

From a less polar fraction of the crude alga extract of *Pantoneura plocamioides* the two epimeric alcohols **3** and **4** were separated by RHPLC. Compound **4**, a colourless oil $[\alpha]^{25}_D$= -28° (c, 0.3, $CHCl_3$), was previously isolated from *Plocamium cartilagineum* but the structure was reported[7] without stereochemistry. The ^1H and ^{13}C NMR spectra of **3** and **4** (tables 1 and 2) are very similar except in the chemical shift of the C-10 indicating that their differences must lie in the configuration at C-3 or C-4. Following the criterion of the C-10 chemical shift mentioned above it was possible to assign the relative stereochemistries for C-3/C-4 in **3** and **4** as (3S*, 4R*) and (3R*, 4R*) respectively, as shown in figure 1. Also from this fraction of the crude extract the known compound **5**, previously isolated[8] from an Antarctic *Plocamium* sp, was identified.

Biogenetically, the polyhydroxylated compounds **1a** and **2a** can be considered to be precursors of pantofuranoids D-F[2] and pantoisofuranoids A-C[3]. The occurrence in *Pantoneura plocamioides* (Ceramiales) of compounds **3-5** which are typical monoterpene structures from *Plocamium* (Gigartinales), is interesting because *P. plocamioides* belongs to a different order and this raises a question about a possible biogeographical evolution of the species.



Pantoisofuranoids A-C **Pantofuranoids D-F**

EXPERIMENTAL

General Methods

Optical rotations were measured on a Perkin-Elmer model 241 polarimeter using a Na lamp at 25°C. IR spectra were obtained with a Perkin-Elmer 1650/FTIR spectrometer in $CHCl_3$ solutions. EI-MS and HR-MS spectra were taken on a Micromass Autospect spectrometer. CI-MS spectra were determined with a Hewlett-Packard 5998 spectrometer using methane as the reactive gas. ^1H NMR and ^{13}C NMR and COSY spectra were measured employing a Bruker AMX 200 instrument operating at 200 MHz for ^1H NMR and at 50 MHz for ^{13}C NMR, using TMS as internal standard. Two-dimensional spectra HMQC, HMBC were obtained with a Bruker AMX 400 employing the standard Bruker software. RHPLC separations were performed with a Japan Analytical LC-908. The gel filtration column (Sephadex LH-20) used hexane-MeOH-$CHCl_3$ (2:1:1) as solvent. Merck silica gel 7734 and 7729 were used for column chromatography. The spray reagent for TLC was H_2SO_4: H_2O: AcOH (1:4:20).

Extraction and Isolation.

Pantoneura plocamioides was collected by a scuba diver off of King George Island (South Shetland, Antarctic) at -18 m. A voucher specimen has been deposited at the Museo de Historia Natural, Santiago de Chile. The dried alga (1400 g) was extracted with acetone at room temperature to give a dark residue (39 g). This extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane: EtOAc (95: 5) was further separated by filtration chromatography and Si-gel chromatography to yield **5** (763.3 mg). The fraction eluted with hexane: EtOAc (4:1) (2.57 g) was further separated by filtration chromatography to give 323.7 mg of a mixture that was chromatographed on a silica gel column and RHPLC until the separation of compounds **3** (5 mg) and **4** (7 mg). The fraction eluted with EtOAc (100%) (8.67 g) was further separated by filtration chromatography to give 1.032 g of an unstable mixture. This fraction was acetylated with Ac_2O in pyridine to obtain stable acetates of the mixture which was chromatographed on a silica gel column and RHPLC until the separation of compounds **1b** (17 mg) and **2b** (19 mg).

1b. Colourless oil; $[\alpha]^{25}_D$= -26° (c, 0.9, $CHCl_3$); IR ν max ($CHCl_3$) 3582; 1736; 1618 cm^{-1}; EI-MS m/z (%) 304/306 (M^+-2; $C_{12}H_{16}O_4Br$; 7/4) 149/151 (C_4H_6OBr; 48/47); 140 (24); 115 (23); 59 (22); 98 (100); CI-MS 305/307 (M^+-1); 307/309 (M^++1); 335/337 (M^++29); 347/349 (M^++41); HR-MS calcd. for $C_{12}H_{21}O_4{}^{79}Br$ (M^++2) 308.0623, found 308.0632. ***2b.*** Colourless oil; $[\alpha]^{25}_D$= -3.93° (c, 0.23, $CHCl_3$); IR ν max ($CHCl_3$) 3595; 1736; 1621 cm^{-1}; CI-MS 305/307 (M^+-1); 307/309 (M^++1); 335/337 (M^++29); 347/349 (M^++41). EI-MS m/z (%) 304/306 (M^+-2; $C_{12}H_{16}O_4Br$; 9/7) 149/151 (C_4H_6OBr; 28/27); 140 (16); 115 (12); 59 (29); 98 (100); CI-MS 305/307 (M^+-1); 307/309 (M^++1); 335/337 (M^++29); 347/349 (M^++41); HR-MS calcd. for $C_{12}H_{18}O_4{}^{81}Br$ (M^+-1) 307.0367, found 307.0374. *Saponification of 1b.* To a solution of **1b** (8.6 mg) in MeOH (1ml) at 0°C was added an excess of K_2CO_3. The mixture was stirred at room temperature for 45 m and then it was poured into water and extracted with EtOAc. The organic layer was dried with $MgSO_4$

and concentrated. The crude of the reaction was chromatographed on RHLPC to give 3 mg of **1a**. *Saponification of 2b.* To a solution of **2b** (6.5 mg) in MeOH (1ml) at 0°C was added an excess of K_2CO_3. The mixture was stirred at room temperature for 45 m and then it was poured into water and extracted with EtOAc. The organic layer was dried with $MgSO_4$ and concentrated. The crude of the reaction was chromatographed on RHLPC to give 2 mg of **2a**.

Pantoneurotriol (1a). Colourless oil; $[\alpha]^{25}_D$= -90° (c, 0.21, $CHCl_3$); IR ν max ($CHCl_3$) 3590; 1623 cm^{-1}. EI-MS m/z (%) 149/151 (C_4H_6OBr; 46/42); 133/135 (13/15); 98 (100); 59 (46); HR-MS calcd. for $C_4H_6O^{79}Br$ 149.0238, found 149.0238. *Pantoneurotriol (2a).* Colourless oil; $[\alpha]^{25}_D$= -100° (c, 0.2, $CHCl_3$); IR ν max ($CHCl_3$) 3580; 1618 cm^{-1}. EI-MS m/z (%) 149/151 (C_4H_6OBr; 30/30); 133/135 (11/11); 98 (32); 59 (100); HR-MS calcd. for $C_4H_6O^{79}Br$ 148.9606, found 148.9602. *3.* Colourless oil; $[\alpha]^{25}_D$= -50.8° (c, 0.42, $CHCl_3$); IR ν max ($CHCl_3$) 3541; 1618 cm^{-1}; EI-MS m/z (%) 229 (9); 105/107 (C_4H_6OCl; 70/29); 149 (34); 113 (11); 91 (91) 71(57); 57 (100); CI-MS 367/369/371/373/375/377 (M^+-1); 369/371/373/375/377/379 (M^++1); 399/401/403/405/407/409 [M^+ +29]; 411/413/415/417/419/421 (M^++41); HR-MS calcd. for $C_4H_6O^{35}Cl$ 105.0107, found 105.0127. *4.* Colourless oil; $[\alpha]^{25}_D$= -28° (c, 0.30, $CHCl_3$); IR ν max ($CHCl_3$) 3525; 1617 cm^{-1}; EI-MS m/z (%) 105/107 (C_4H_6OCl; 34/11); 113 (10); 71(66); 57 (100); CI-MS 367/369/371/373/375/377 (M^+-1); 369/371/373/375/377/379 (M^++1); 399/401/403/405/407/409 (M^++29); 411/413/415/417/419/421 (M^++41); HR-MS calcd. for $C_4H_6O^{35}Cl$ 105.0107, found 105.0084.

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