Pergamon

Tetrahedron, Vol. 52, No. 16, pp. 5899-5906, 1996

0040-4020/96 $15.00 + 0.00

S0040-4020(96)00220-7

Uncommon Tetrahydrofuran Monoterpenes from Antarctic *Pantoneura plocamioides*

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Abstract: The participation of oxygen in the biogenesis of marine monoterpenes allowed us to isolate, from the Antarctic alga *Pantoneura plocamioides*, a variety of new monoterpenes that have a tetrahydrofuran ring. The structure and relative stereochemistry of these six compounds, pantofuranoids A-F (**1-6**), were established by spectroscopic methods. A biogenetic route for those compounds has also been proposed. Copyright © 1996 Elsevier Science Ltd

Pantoneura plocamioides (Delessereaceae, Ceramiales) is a red alga endemic to the Antarctic. In this chemical study of *P. plocamioides* we have isolated six new oxygenated monoterpenes denominated pantofuranoids A-F (**1-6**). The unusual participation of oxygen in the biogenesis of marine monoterpenes[1] led to alternative ways of cyclization of a common precursor to give for the first time monoterpenes containing a tetrahydrofuran ring. The pantofuranoids D-F (**4-6**) are also characterized by the fact that the number of oxygen atoms they contain exceeds that of the halogen substituents, suggesting a direct relationship between the oxygen content of marine monoterpene metabolites and latitude.

RESULTS AND DISCUSSION

From two different fractions, hexane: EtOAc (4:1) and (1:1), of the crude extract of *Pantoneura plocamioides*, six compounds named pantofuranoids A-C (**1-3**) and D-F (**4-6**), respectively, were isolated after successive chromatographies on silica gel followed by RHPLC (Recycling-HPLC).

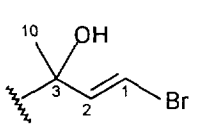
Pantofuranoid A (**1**) was a colorless oil $[\alpha]^{25}_{D}$ -63° (c, 0.22, $CHCl_3$). The CI-MS gave the molecular ion at m/z 327/329/331 (M^+ +1) for $C_{10}H_{16}Br_2O_2$. The EI-MS showed a characteristic[2] fragment **I** at m/z 149/151. Absorption for alcohol and double bond at 3565 and 1618 cm^{-1} respectively were detected by the IR spectrum.

The ^1H NMR spectrum showed signals for two trans-disubstituted olefinic protons at δ 6.40 (1H, d, *J*=13.6 Hz) and δ 6.28 (1H, d, *J*=13.6 Hz); two double doublets at δ 4.10 (1H, dd, *J*=6.7, 9.5 Hz) and δ 3.80 (1H, dd, *J*=6.0, 9.7 Hz) assignable to protons geminal to heteroatoms. At both δ 2.46 and δ 2.43 multiplets appeared (1H, ddd, *J*=6.6, 6.3, 12.9 Hz) and (1H, ddd, *J*=9.6, 9.6, 12.9 Hz) respectively that should correspond to a methylene on a ring. At δ 1.22 (3H, s); 1.34 (3H, s) and δ 1.36 (3H, s) signals for three methyl groups geminal to oxygen were located.

The ten carbon atoms of the monoterpene appeared on the ^{13}C NMR spectrum as follows: two disubstituted olefinic carbons at δ 106.71 (d) and δ 141.97 (d); two methines at δ 81.41 (d) and δ 54.08 (d); a methylene at δ 36.72 (t); two quaternary carbons at δ 73.94 (s) and δ 82.88 (s) and three methyl groups at δ 26.18 (q); 26.81(q) and δ 23.20 (q). The disubstitute nature of the olefinic carbons and also the presence of a methyl group geminal to oxygen are in agreement with fragment **I** shown by the EI-MS spectrum. Given that we have three methyl groups and only two quaternary carbon atoms, two of the methyls must be in the shape of gem-dimethyl, fragment **II**. The chemical shift for the methine carbons (δ 54.08 and δ 81.14) indicates that they were substituted by bromine and oxygen respectively and the ^1H-^1H-COSY NMR make the coupling of these methine protons (δ 4.10 and δ 3.80) with the methylene protons (δ 2.46 and δ 2.34) evident, determining the presence of fragment **III** in the molecule.

Fragments **I**, **II** and **III** contain the ten carbon atoms of the skeleton. The connectivities of these fragments were determined from the combination of the 2D-HMQC and HMBC NMR experiments.

Fragments **I** and **III** are connected by C-3/C-4, due to the correlation of C-4 (δ 81.41) with Me-10 (δ 1.22). The linkage C-6/C-7 was established by the correlation between C-6 (δ 54.08) and Me-8 (δ 1.36) and Me-9 (δ 1.34). Because the natural compound must have a ring, the tetrahydrofuran structure shown in figure 1 was obtained for pantofuranoid A (**1**).



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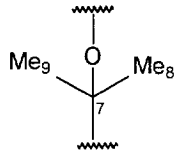

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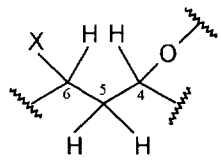
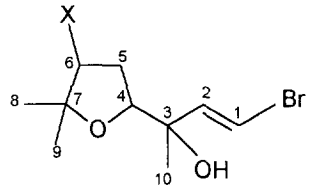


III X=Br

III' X=O



Pantofuranoid A-C (1-3) X=Br

Pantofuranoid D-F (4-6) X=OH

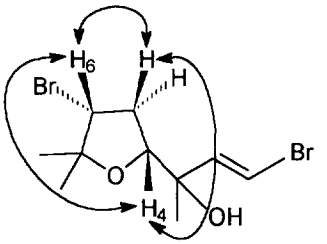
Figure 1

Pantofuranoid B (**2**) and C (**3**) gave ^1H NMR (table 1), ^{13}C NMR (table 2) and MS spectra similar to that observed for pantofuranoid A (**1**), suggesting that the compounds must be differentiated in the stereochemistry of the chiral centers.

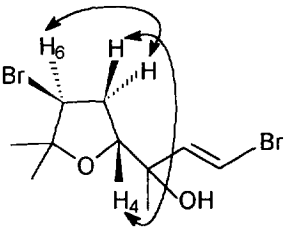
By comparing the spectroscopic values of each of these compounds, small variations are significant to aid in the study of their stereochemical differences. The 2D-ROESY NMR experiments were particularly illustrative in this sense.

In pantofuranoids A (**1**) and B (**2**) figure 2, a NOE effect was observed between H-4 and H-6, H-4 and H_5-β and also between H-6 and H_5-β, indicating that H-4 and H-6 are on the same side of the plane of the ring thus establishing the relative stereochemistry of the C-4 and C-6 chiral centers.

For pantofuranoid C (**3**) the only possibility is that H-4 and H-6 are on different sides of the plane. In the ROESY experiment a direct observation of a possible interaction between H-4 and H-6 protons was not possible due to the fact that the corresponding ^{1}H NMR signals were overlapping (even when the spectrum was run in C_6D_6). A NOE effect between the signal at δ 3.90 (corresponding to H-4 and H-6) and H_5-α and H_5-β was observed however and this indirectly confirms the relative trans-orientation of the methine protons.



1-2



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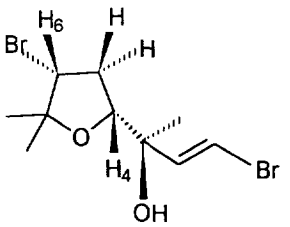
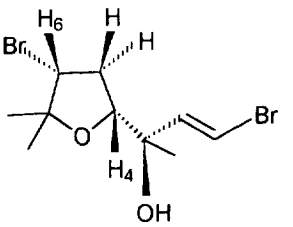


1



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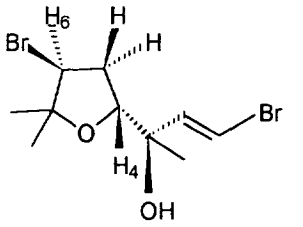


3

Figure 2. NOE effects and relative stereochemistry for Pantofuranoid A-C (**1-3**)

The relative stereochemistry of C-3 was established on the basis of the relative chemical shift of the Me-10 of each compound. In compounds **1** and **3** the chemical shift of the Me-10 was sufficiently differentiated and consequently easily assigned (HMQC and HMBC). All three methyl signals of compound **2** however, were too close to each other to make assignations. In tables 1 and 2 it can be observed that each of the ^{1}H and ^{13}C NMR signals for the methyls of compound **2** did not have chemical shifts similar to those of compound **3**. By observing the data of compound **1** and making comparisons with that of compounds **2** and **3**, we found the same values for the chemical shift of two of the three angular methyl groups (δ 1.36, and δ 1.34 ppm in all three compounds), while the chemical shift of the third methyl is the same in compounds **2** and **3** (δ 1.32 ppm). The shift corresponding to compound **1** however, shows differences (δ 1.22 ppm; Δδ = 0.1 ppm) and this was assigned to the Me-10. These correlations also apply to and are more significant for the ^{13}C NMR values. One of the methyls of compound **1** has a variation in the chemical shift of 2 ppm with respect to that corresponding to compounds **2** and **3**. This means that compounds **2** and **3** have the same relative stereochemistry on C-3

Table 1. ^{1}H NMR Data of **1-6** [400 MHz, $CDCl_3$, δ ppm, (pattern, *J*, Hz)]

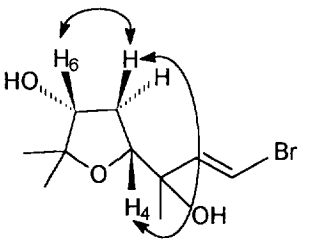
Proton	1	2	3	4	5	6
H-1	6.40 (d, 13.6)	6.43 (d, 13.5)	6.41 (d, 13.5)	6.43 (d, 13.5)	6.41 (d, 13.6)	6.38 (d, 13.5)
H-2	6.28 (d, 13.6)	6.15 (d, 13.5)	6.13 (d, 13.5)	6.13 (d, 13.5)	6.37 (d, 13.6)	6.28 (d, 13.5)
H-4	4.10 (dd, 6.7, 9.5)	4.08 (dd, 6.8, 9.2)	3.90 (m)	3.88 (dd, 3.8, 9.4)	3.91 (dd, 5.0, 8.8)	4.03 (dd, 6.7, 8.6)
H5-β	2.46 (ddd, 6.6, 6.3, 12.9)	2.40 (ddd, 6.5, 6.6, 13.1)	2.20 (m)	2.38 (ddd, 5.4, 9.3, 14.6)	2.42 (ddd, 5.8, 8.6, 14.3)	2.11 (ddd, 6.0, 8.7, 13.3)
H5-α	2.34 (ddd, 9.6, 9.6, 12.9)	2.26 (ddd, 9.5, 9.6, 13.2)	2.40 (m)	1.85 (dd, 3.2, 14.5)	1.93 (ddd, 2.3, 5.0, 14.2)	1.78 (ddd, 3.7, 6.6, 13.2)
H-6	3.80 (dd, 6.0, 9.7)	3.81 (dd, 6.2, 9.6)	3.90 (m)	3.74 (d, 5.1)	3.87 (dd, 2.2, 5.8)	3.94 (dd, 3.7, 6.0)
Me-8	1.36 (s)	1.36 (s)	1.36 (s)	1.29 (s)	1.28 (s)	1.21 (s)
Me-9	1.34 (s)	1.34 (s)	1.34(s)	1.13 (s)	1.15 (s)	1.25 (s)
Me-10	**1.22 (s)**	**1.32 (s)**	**1.32 (s)**	**1.38 (s)**	**1.22 (s)**	**1.30 (s)**

Table 2. ^{13}C NMR Data of **1-6** (100 MHz, $CDCl_3$, δ ppm)

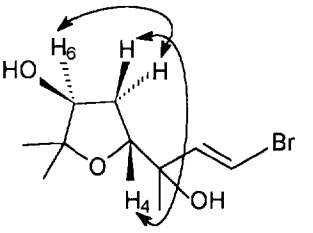
Carbon	1	2	3	4	5	6
C-1	106.71 (d)	107.31 (d)	107.27 (d)	107.31 (d)	106.82 (d)	106.77 (d)
C-2	141.97 (d)	139 .07 (d)	139.16 (d)	140.6 (d)	142.47 (d)	139.51 (d)
C-3	73.94 (s)	74.14 (s)	73.94 (s)	75.79 (s)	74.80 (s)	74.59 (s)
C-4	81.41 (d)	81.36 (d)	81.10 (d)	80.86 (d)	81.19 (d)	81.65 (d)
C-5	36.72 (t)	36.95 (t)	36.81 (t)	35.36 (t)	35.52 (t)	35.09 (t)
C-6	54.08 (d)	54.26 (d)	54.81 (d)	76.63 (d)	76.89 (d)	77.99 (d)
C-7	82.88 (s)	no obs.	83.65 (s)	85.37 (s)	84.57 (s)	83.67 (s)
C-8	26.18 (q)	26.16 (q)	25.20 (q)	22.31 (q)	22.28 (q)	21.39 (q)
C-9	26.81 (q)	26.79 (q)	26.61 (q)	25.54 (q)	25.33 (q)	27.62 (q)
C-10	**23.20 (q)**	**25.45 (q)**	**24.37 (q)**	**26.00 (q)**	**24.41 (q)**	**25.41 (q)**

while in compound **1** the opposite is true and that the chemical shift for the Me-10 of compound **2** is δ 25.45. In compound **1** the chemical shift of the Me-10 to highfield indicates a bigger steric effect between Me-10 and the ring than is the case with compounds **2** and **3**. The structures and relative stereochemistry for compounds **1-3** therefore remain as shown in figure 2, C-3 showing an R* configuration for pantofuranoid A (**1**) and an S* for pantofuranoids B (**2**) and C (**3**).

From the more polar fraction, hexane: EtOAc (1:1), the diols named pantofuranoids D-F (**4-6**) were obtained. The structural differences of these compounds with respect to pantofuranoids A-C (**1-3**) lie in the nature of the substituent on C-6, which is hydroxy function instead of the bromine found in pantofuranoids D-F (**4-6**). All three compounds have the same empirical formula ($C_{10}H_{17}BrO_3$) and the analysis of their spectroscopic data (tables 1 and 2) was similar to that of pantofuranoids A-C (**1-3**), and gave fragments **I**, **II**, and **III'** which were connected, according to the HMBC experiments, C-3/C-4 linkage due to the correlation of C-4 (δ 80.86) with Me-10 (δ 1.38). The linkage C-6/C-7 was established by the correlation between C-6 (δ 76.63) and Me-8 (δ 1.29) and Me-9 (δ 1.13). Pantofuranoids D-F (**4-6**) gave acetyl derivatives corresponding to a secondary hydroxy group on each. In addition to the secondary alcohol, one of the three oxygens of the empirical formula must be tertiary by virtue of the characteristic fragment **I**, and the last one should form a part of a ring corresponding to the degree of unsaturation. The combination of these data for pantofuranoids D-F (**4-6**) gave the structure shown in figure 1, the differences between them being of a stereochemical nature. The relative stereochemistry of these compounds was determined by 2D-ROESY experiments.



4-5



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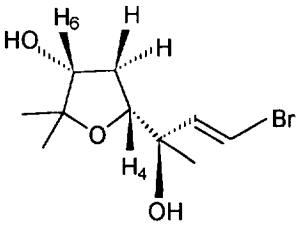

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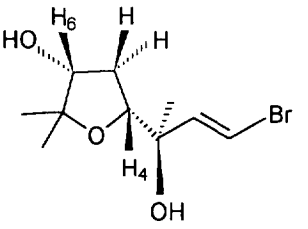

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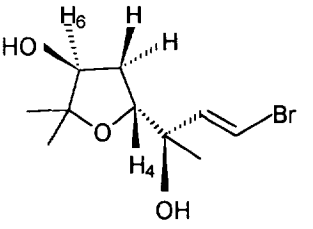

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Figure 3. NOE effects and relative stereochemistry for Pantofuranoids D-F (**4-6**)

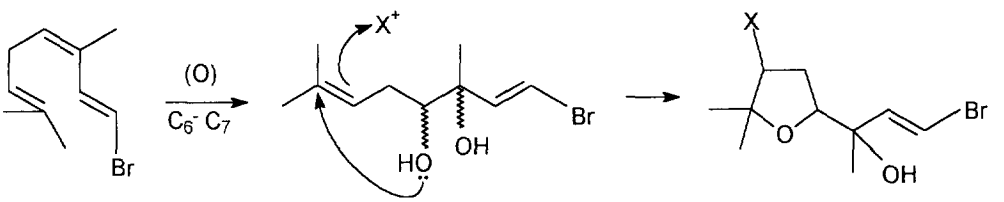
In compound **4** a NOE effect was observed between H-6 and H_5-β and H-4 with H_5-β suggesting that H-4 and H-6 are on the same side of the plane of the ring. In compound **5** the same correlation for H-4, H-6 with

H_5-β was observed thus establishing that compounds **4** and **5** must have the same stereochemistry on C-6 and C-4 carbons and that the differences between them lie in the stereochemistry of C-3. In compound **6** however a NOE effect was not observed between H-6 and H-4 protons but rather between H-6 and H_5-α and also between H-4 and H_5-β, showing that H-4 and H-6 were on different sides of the plane.

The configuration of C-3 for each compound was established following the assumption that the steric interaction between Me-10 and the ring promoted differences in the chemical shift of the Me-10 to highfield. From the analysis of the HMQC and HMBC experiments the assignation of the Me-10 group for each compound was determined. It can be observed that the chemical shifts of the Me-10 are similar for compounds **4** and **6**, suggesting that these compounds have the same stereochemistry on C-3, while in compound **5** the highfield value for the Me-10 means that it is more hindered than in compounds **4** and **6**.

Thus an S* configuration for C-3 in pantofuranoids D (**4**) and F (**6**) and an R* configuration in pantofuranoid E (**5**) has been proposed, the structure and relative stereochemistry of the compounds being that represented in figure 3.

Biogenetically, compounds **1-6** can be explained beginning with the oxidation of a common precursor **7**, generating **8**, followed by the electrophylic attack of a bromonium or hydroxonium ion on the terminal isopropylene bond inducing a cyclization of the C_4-alcohol to give the skeleton of pantofuranoids, A-C (**1-3**) or D-F (**4-6**).



7 8 X=Br **Pantofuranoids A-C**

X=OH **Pantofuranoids D-F**

EXPERIMENTAL

Optical rotations were measured on a Perkin-Elmer model 241 polarimeter using a Na lamp at 25°. Ir spectra were obtained with a Perkin-Elmer 1650/FTIR spectrometer in $CHCl_3$ solutions. Eims spectra were taken on a Hewlett-Packard 5995; cims spectra were determined with a Hewlett-Packard 5998 using methane as the reactive gas and hrms spectra on a VG Micromass ZAB-2F spectrometer. ^1H NMR and ^{13}C NMR, HMQC, HMBC ROESY and COSY spectra were measured employing a Bruker AMX 400 instrument operating at 400 MHz for ^1H NMR and 100MHz for ^{13}C nmr, using TMS as internal. Two-dimensional spectra were obtained with the standard Bruker software. Recycling-HPLC separations were performed with a Japan Analitycal 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).

Plant material

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.

Extraction and Isolation

The dried alga (1.400 g) was extracted with acetone at room temperature and the acetone extract was concentrated to give a dark green residue (39 g). This extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane: EtOAc (4:1) (2.57 g) was chromatographed on a LH-20 column, affording a fraction (619.1 mg) that was further separated by silica gel chromatography to give a fraction (166 mg) of a complex mixture that was separated with RHPLC to give Pantofuranoid A (10 mg), Pantofuranoid B (3 mg) and Pantofuranoid C (3 mg). The fraction eluted with hexane-EtOAc (1:1) (4.58 g) was chromatographed on a Sephadex LH-20 column with hexane: MeOH-$CHCl_3$ (2:1:1) as eluent, affording a fraction (2.51 g) that was further separated by silica gel chromatography to give a fraction (60 mg) that contained a complex mixture that was separated with rhplc to give pantofuranoid D (**4**) (15 mg); pantofuranoid E (**5**) (8.1 mg) and pantofuranoid F (**6**) (11 mg).

Pantofuranoid A (1). Colorless oil; $[\alpha]^{25}_D$ -63° (c, 0.22, $CHCl_3$); IR ν max ($CHCl_3$) 3565; 1618 cm^{-1}; EI-MS m/z (%) 327/329/331 (M^+; 14/30/20); 247/249 (M^+-Br; 11/11); 177/175 (M^+- C_4H_6OBr; 100/100); 149/151 (C_4H_6OBr; 96/58); CI-MS 325/327/329 (M^+-1); 327/329/331 (M^++1); 355/357/359 (M^++29); 367/369/371 (M^++41); HR-MS calcd for $C_4H_6O^{81}Br$ 150.948, found 150.956

Pantofuranoid B (2). Colorless oil; $[\alpha]^{25}_D$ -108° (c, 0.12, $CHCl_3$); IR ν max ($CHCl_3$) 3582; 1618 cm^{-1}; EI-MS m/z (%) 327/329/331 (M^+; +1; 2, 3, 3); 247/249 (M^+-Br; 6, 5); 177/179 (M^+- C_4H_6OBr; 59, 59); 149/151 (C_4H_6OBr; 68, 61); CI-MS 325/327/329 (M^+-1); 327/329/331 (M^++1); 355/357/359 (M^++29); 367/369/371 (M^++41).

Pantofuranoid C (3). Colorless oil; $[\alpha]^{25}_D$ -100° (c, 0.2, $CHCl_3$); IR ν max ($CHCl_3$) 3570; 1617 cm^{-1}; EI-MS m/z (%) 326/328/330 (M^+; 10/15/15); 247/249 (M^+-Br; 12/12); 177/179 (M^+- C_4H_6OBr; 97/97); 149/151 (C_4H_6OBr; 86, 75); CI-MS 325/327/329 (M^+-1); 327/329/331 (M^++1); 355/357/359 (M^++29); 367/369/371 (M^++41).

Pantofuranoid D (4). Colorless oil; $[\alpha]^{25}_D$ -29° (c, 077, $CHCl_3$); IR ν max ($CHCl_3$) 3600; 1626 cm^{-1}; EI-MS m/z (%) 247/249 (M^+-OH; 16, 17); 229/231 (M^+-H_2O-OH; 6, 7); 149/151 (C_4H_6OBr; 17, 11); 133/135 (19, 17); 185 (M^+-Br; 5); 115 (M^+- C_4H_6OBr; 100); 71 (100); CI-MS 263/265 (M^+-1); 265/267 (M^++1); 293/295 (M^++29); 305/307 (M^++41).

Pantofuranoid E (5). Colorless oil; $[\alpha]^{25}_D$ -27° (c, 1, $CHCl_3$); IR ν max ($CHCl_3$) 3600; 1618 cm^{-1}; EI-MS m/z (%) 247/249 (M^+-OH; 20, 21); 229/231 (M^+-H_2O-OH; 11, 12); 185 (M^+-Br; 11); 149/151 (100/78; C_4H_6OBr); 133/135 (45/38); 115 (M^+- C_4H_6OBr; 87); 71 (97); CI-MS 263/265 (M^+-1); 265/267 (M^++1); 295/297 (M^++29); 205/207 (M^++41); HR-MS calcd for $C_6H_{11}O_2$ 115.076, found 115.075.

Pantofuranoid F (6). Colorless oil; $[\alpha]^{25}_{D}$ -48° (c, 0.33, $CHCl_3$); IR ν max ($CHCl_3$) 3580; 1624 cm^{-1}; EI-MS m/z (%) 247/249 (M^+-OH; 7, 7); 185 (M^+-Br; 3); 149/151 (C_4H_6OBr; 15, 13); 133/135 (4, 3); 115 ($C_6H_{11}O_2$; 77); 71 (100).

Acetylation of Pantofuranoid D (4). To a solution of **4** (7 mg) in dry C_5H_5N (1.5 ml) was treated with Ac_2O (1 ml) and stirred at room temperature for 2.5h and then it was poured into 10% aqueous HCl and extracted with $CHCl_3$. The organic layer was washed with H_2O and brine, dried (Na_2SO_4) and concentrated. The residue (4 mg) was identified by ^1H NMR. Acetate of pantofuranoid D Colorless oil; ^1H NMR δ 1.21 (3H, s); 1.23 (3H, s); 1.31 (3H, s); 1.89-1.82 (1H, m); 2.10 (3H, s); 2.41-2.22 (1H, m); 3.90 (1H, dd, *J*=6.9, 8.2 Hz); 4.96 (1H, dd, *J*=3, 6.6 Hz); 6.19 (1H, d, *J*=13.6 Hz); 6.41 (3H, d, *J*=13.6); EI-MS m/z (%) 229/231 (M^+-OAc-H_2O; 18, 18); 149/151 (C_4H_6OBr; 20, 12); 133/135 (16, 15); 115 (20); 97 (100); CI-MS 305/307 (M^+-1); 307/309 (M^+; +1); 347/349 (M^++41).

ACKNOWLEDGEMENTS

This work was supported by the Comisión Interministerial de Ciencia y Tecnología (CITYT) (ANT94-0047) and INACH (Instituto Antártico Chileno). M. Cueto acknowledges a fellowship from the Programa Nacional de la Antártida. We are grateful to Dr. Eliana Ramírez (Museo de Historia Natural de Chile) for the taxonomic classification of the alga.

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(*Received in UK* 31 *December* 1995; *revised* 20 *February* 1996; *accepted* 22 *February* 1996)