Pergamon

Tetrahedron 58 (2002) 8539–8542

TETRAHEDRON

New halogenated monoterpenes from the red alga *Plocamium cartilagineum*

Ana R. Díaz-Marrero,[a] Mercedes Cueto,[a] Enrique Dorta,[a] Juana Rovirosa,[b] Aurelio San-Martín[b] and José Darias[a,*]

[a]*Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofísico F. Sánchez 3, Apdo. 195, 38206 La Laguna, Tenerife, Spain*
[b]*Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago de Chile, Chile*

Received 13 June 2002; revised 5 August 2002; accepted 14 August 2002

Abstract—Three new halogenated monoterpenes, **1**–**3**, have been isolated from *Plocamium cartilagineum*. The structure and relative stereochemistry of these compounds were determined by spectroscopic data. Also the relative stereochemistry at C-7 for furoplocamioids A–C has been assigned.

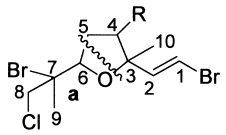
1. Introduction

Red algae of genera *Plocamium*,[1,2] *Ochtodes*,[3] *Chondrococcus*,[4] *Gelidium*,[5] and *Portiera*[6] have been a source of polyhalogenated monoterpenes,[7] whereas only the green alga *Cymopolia barbata* is able to produce brominated monoterpenes of mixed biogenesis.[8,9] The genus *Plocamium* has been the most widely investigated,[10] probably due to its widespread distribution throughout the world and as these studies were performed with material collected at high latitudes[11] an unusual level of oxygen incorporation in the monoterpene skeleton was appreciated, giving rise to new structural type of metabolites containing a tetrahydrofuran[12] or a tetrahydropyran[13] ring, some of them exhibiting an interesting 1,2-bromochloro vinyl functionality.[14] These findings prompted us to undertake investigation to trace the natural formation of these oxane ring-containing monoterpenes. Thus, *P. cartilagineum* is now being studied for its minor constituents.

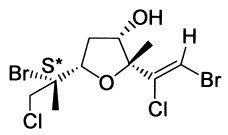
Recently we have described[14] the furoplocamioids A–C, three tetrahydrofuran derivatives with an unusual vicinal vinyl dihalide. In this paper we report the structure elucidation of two new related tetrahydrofuran halogenated monoterpenes **1**–**2**, and a new acyclic polyhalogenated monoterpene, **3**, which appears to be the biogenetic precursor of the oxane ring of compounds **1** and **2**.

2. Results and discussion

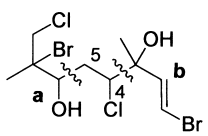
P. cartilagineum (L.) Dixon (Plocamiaceae) was collected at El Quisco (Chile). From the crude extract compounds **1**–**3** were obtained after flash chromatography followed by gel filtration and successive HPLC.



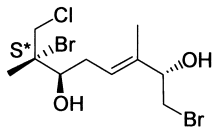
1, R= Cl
2, R= Br



Furoplocamioid C (4)



3



Plocamenol A (5)

Compound **1** was obtained as an oil whose HREIMS $[M]^+$ 379.869 corresponds to a molecular formula of $C_{10}H_{14}OBr_2Cl_2$, indicating two degrees of unsaturation. The ^{13}C NMR spectrum of **1** (Table 1), together with the information from a DEPT spectrum, showed the presence of 10 carbon signals assigned to $2CH_3$, $2CH_2$, 4CH (two olefinic, and two geminal to heteroatom) and two quaternary carbons bearing heteroatom.

The ^1H NMR showed signals for two protons of a *trans* disubstituted olefin at δ 6.56 (d, J=13.5 Hz) and 6.38 (d, J=13.5 Hz) and for protons geminal to heteroatom at δ 4.17 (m) and δ 3.99 (dd, J=5.3, 10.0 Hz). Methylene multiplets appeared at δ 2.32 and δ 2.51 and two signals at δ 4.18 (d, J=10.5 Hz) and δ 3.86 (d, J=10.5 Hz) were assigned to a

* Corresponding author. Tel.: +34-922-252144; fax: +34-922-260135; e-mail: jdarias@ipna.csic.es

0040–4020/02/$ - see front matter
PII: S0040-4020(02)01019-0

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**–**5** (500 MHz, δ ppm, (J) Hz, $CDCl_3$)

#	**1**		**2**		Furoplocamioid C (**4**)		**3**		Plocamenol A (**5**)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	6.56 d (13.5)	108.2	6.47 d (13.5)	106.7	7.08 s	106.2	6.50 d (13.5)	108.0	3.52 dd (4.3, 10.3); 3.46 dd (7.8, 10.3)	37.7
2	6.38 d (13.5)	138.4	6.32 d (13.5)	139.7		141.0	6.37 d (13.5)	139.9	4.28 m	76.2
3		85.3		90.0		89.5		76.5		137.1
4	4.17 m	62.9	4.21 m	51.5	4.49 dd (3.6, 7.0)	75.7	4.19 dd (2.0, 11.1)	67.1	5.70 t (7.1)	123.9
5	2.32 m	38.8	2.51 m	38.9	1.95 ddd (2.6, 9.8, 13.0) 2.26 ddd (1.7, 7.0, 13.0)	36.5	1.88 m; 2.16 m	36.5	2.44 m	31.7
6	3.99 dd (5.3, 10.0) 2.51 m	77.7	3.94 dd (5.4, 10.3)	78.5	4.18 dd (6.1, 10.2)	78.6	3.91 d (8.4)	70.9	3.63 dt (8.3, 3.8)	72.9
7		67.8		68.0		69.5		73.8		73.3
8	3.86 d (10.5); 4.18 d (10.5)	50.9	3.84 d (10.8); 4.19 d (10.8)	50.9	3.84 d (10.7); 4.18 d (10.7)	50.9	3.82 d (10.9); 4.24 d (10.9)	51.0	3.80 d (10.6); 4.28d (10.6)	51.2
9	1.74 s	26.1	1.72 s	25.7	1.72 s	25.9	1.81 s	25.7	1.82 s	25.9
10	1.41 s	25.1	1.47 s	25.9	1.46 s	19.8	1.45 s	25.3	1.60 s	12.4

methylene geminal to heteroatom. The upfield singlets at δ 1.74 and δ 1.41 correspond to two methyl groups geminal to halogen and oxygen, respectively. The molecular formula implies two degrees of unsaturation and the absence of any signal for hydroxyl, carbonyl groups or other unsaturations in the IR spectrum indicates that the oxygen of the molecule must form part of an oxane ring.

From comparison of the ^{1}H and ^{13}C NMR spectra of **1** with that of furoplocamioid C, **4**, we observed that these compounds possess an identical C-6–C-9 fragment **a**, as can be seen by the similarities of the NMR signal of both proton and carbon (Table 1).

A ^{1}H–^{1}H COSY experiment established the connectivities H-1–H-2, and H-4–H-6. From the HMBC spectrum the linkage C-2/C-3 was secured by the correlation between H-10 with C-2 and C-3. The linkage C-6/C-7 was confirmed by the correlation of H-8 with C-6 and the correlation of H-8 with C-9. The chemical shift of 62.9 ppm of C-4 and 50.9 ppm of C-8 indicates that the substituent on those carbons must be chlorine. All these data support the structure proposed for **1**.

2D NOESY experiments established the relative configurations of C-3, C-4 and C-6. A NOE effect was observed between Me-10 with H-4 and H-6, indicating that they are on the same side of the molecule. Thus, the relative stereochemistry of compound **1** as shown in Fig. 1 is coincident with that of the furoplocamioid C, **4**.

The NMR data for compound **2** are very similar to those of compound **1** (Table 1). The most significant difference was the carbon chemical shift of the C-4 methine. This was observed at δ 62.9 for **1** and appeared at δ 51.5 in compound **2**. This variation can be rationalised by the substitution of the chlorine by a bromine atom.

The relative stereochemistries of the C-3, C-4 and C-6 chiral centres of compound **2** were assigned on the basis of 2D NOESY experiments in C_6D_6 (see Section 3), because ^{1}H NMR signals for H-5α and H-5β were overlapped in $CDCl_3$. A NOE effect was observed between H-5β with H-4 and H-6, which implies that H-4 and H-6 are on the same side of the molecule. Also there was a correlation between H-4 and H-2, and between H-5α and Me-10. These data allowed the methyl group C-10 to be placed on the same face as the bromine on C-4, Fig.1.

Compound **3** was a colourless oil. Analysis of its ^{1}H and ^{13}C NMR spectra showed signals for two methyl groups, two methylenes (one bearing chlorine), four methines (two bearing heteroatom and two olefinic), and two quaternary carbons. The presence of two olefinic protons at δ 6.50 (d, J=13.5 Hz) and δ 6.37 (d, J=13.5 Hz) suggested that the double bond must be *trans* disubstituted. The IR spectrum gave absorption for a hydroxyl group at 3412 cm^{-1}.

Compound **3** contains the same C-6–C-9 fragment **a** as plocamenol A, **5**,[15] as can be deduced by comparison of both the respective NMR signals of proton and carbon (Table 1) as well as the peaks at m/z 183/185/187 corresponding to a fragment C_4H_5OBrCl in the HRMS, leading us to assign the signal at δ_C 70.9 to a carbon bearing oxygen. The base peaks at m/z 149/151 (100, 97) in the EIMS spectrum indicate[1,11] the presence of the C-1–C-3 fragment **b**. The connectivities between C-5 with C-6 and C-4 were established by a HMBC experiment. Finally, the chemical shift at 67.1 ppm of the methine carbon C-4

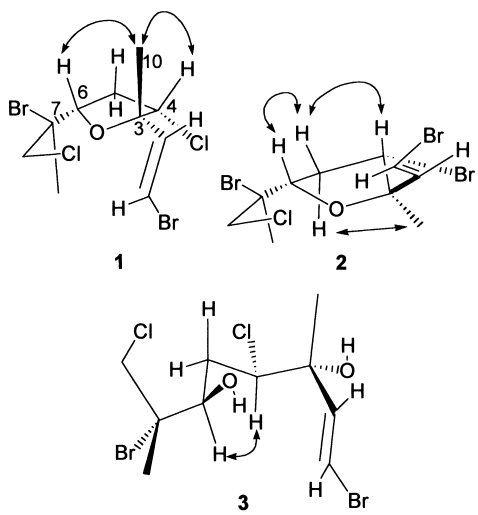
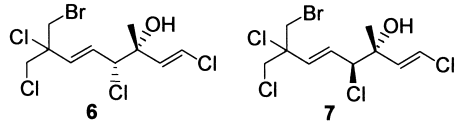


Figure 1. Selected NOE for compounds **1**–**3**.

indicated that the substituent must be chlorine. All these data support the structure proposed for **3**.

The proton and carbon chemical shifts of the C-10 methyl group (δ_C 25.3, δ_H 1.45) suggest a 3*R, 4*R stereochemistry, by comparison[16] with the proton and carbon chemical shifts of the respective methyl group of *threo* (δ_C 25.6, δ_H 1.44) and *erythro* (δ_C 24.6, δ_H 1.39) compounds **6** and **7**. This was reinforced by molecular mechanics (MM2) calculations[17] on the C-3–C-4 *erythro* and C-3–C-4 *threo* relative configurations of **3**. After minimisation the calculated ^3J coupling constants between the respective H-4 protons and the vicinal pair of methylene protons were H-4 (J=2.79, 4.26 Hz) for the *erythro* and H-4 (J=11.56, 3.61 Hz) for the *threo* configuration. The calculated values for the *threo* configuration are in good agreement with those observed for compound **3** (Table 1), suggesting that C-3–C-4 *threo* should be the configuration for compound **3**. On the other hand, the calculated ^3J coupling constants between H-6 and H_2-5 (1.19, 8.90 Hz) agree with the experimental J values obtained (Table 1) for the proposed *R configuration at C-6. This configuration is in agreement with the NOE effect observed between H-4 and H-6. The calculated ^3J coupling (J=2.18, 11.6 Hz) for a *S relative configuration differ significantly from the measured values.



We propose an *S configuration for C-7 in compounds **1**–**3** (Fig. 1) on the basis of the similarities of the ^{13}C NMR chemical shifts of the C-9 methyl group (δ_C~25.7) compared with the data reported for C-7 (Me-9 δ_C~25.3) in a series of related monoterpenes whose stereochemistries have been determined[18,19] by X-ray crystallography. Also a *S configuration for the C-7 of furoplocamioids A–C[14] could be proposed in view of the similarities of both proton and carbon chemical shifts of the C-9 methyl group of these compounds with those of compounds **1**–**3** and plocamenols A–C.[15]

3. Experimental

3.1. General procedures

IR spectra were obtained with a Perkin–Elmer 1650/FTIR spectrometer in $CHCl_3$ solutions. ^1H and ^{13}C NMR, HMQC, HMBC 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. Two-dimensional NMR spectra were obtained with the standard Bruker software. EIMS and HRMS data were taken on a Micromass Autospec spectrometer. HPLC separations were performed with a Hewlett Packard 1050 (Jaigel-sil semipreparative column 10μ 20×250 mm) with hexane–EtOAc mixtures. The gel filtration column (Sephadex LH-20) used hexane–MeOH–CH_2Cl_2 (3:1:1) as solvent. Merck Si gels 7734 and 7729 were used in column chromatography. The spray reagent for TLC was H_2SO_4–H_2O–AcOH (1:4:20).

3.2. Plant material

P. cartilagineum was collected by SCUBA diving at El Quisco, V Region of Chile.

3.3. Extraction and isolation

Air-dried samples were extracted with a mixture of hexane/EtOAc/CH_2Cl_2/MeOH (1:4:4:1) at room temperature, and were concentrated to give a dark residue (18 g). The extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane/EtOAc (98:2) (381 mg) was chromatographed on a LH-20 column to give a complex mixture that was further separated on HPLC to give compounds **1** (1.9 mg), **2** (0.8 mg), and **3** (1.0 mg).

3.3.1. Compound 1. Colourless oil; $[\alpha]_D^{25}$=−153 (*c*, 0.02, $CHCl_3$); IR ν_{max} (film) 2929, 1448, 1378, 1075, 935 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 378/380/382/384 $[M]^+$ (<1, <1, <1, <1), 343/345/347/349 $[M-Cl]^+$ (8, 18, 13, 3), 299/301/303 $[M-Br]^+$ (37, 59, 27), 79 (100); HREIMS $[M]^+$ 379.8691 (calcd for $C_{10}H_{14}O_2^{79}Br_2^{35}Cl_2$, 379.8758), $[M-Cl]^+$ 342.9055 (calcd for $C_{10}H_{14}O^{79}Br_2^{37}Cl^{35}Cl$, 342.9099), $[M-Br]^+$ 298.9573 (calcd for $C_{10}H_{14}O^{79}Br^{35}Cl_2$, 298.9605).

3.3.2. Compound 2. Colourless oil; $[\alpha]_D^{25}$=−120 (*c*, 0.01, $CHCl_3$); IR ν_{max} (film) 2932, 1446, 1373, 1053, 934 cm^{-1}; ^1H and ^{13}C NMR in $CDCl_3$, see Table 1; ^1H NMR in C_6D_6: δ 1.14 (s, Me-10), 1.23 (s, Me-9), 1.62 (dd, J=5.5, 13.4 Hz, H-5β), 2.05 (q, J=10.5 Hz, H-5α), 3.22 (dd, J=5.2, 10.3 Hz, H-6), 3.27 (d, J=10.4 Hz, H-8a), 3.35 (dd, J=4.8, 10.8 Hz, H-4), 3.84 (d, J=10.9 Hz, H-8b), 6.02 (d, J=13.5 Hz, H-2), 6.29 (d, J=13.6 Hz, H-1); EIMS *m/z* 407/409/411/413/415 $[M-CH_3]^+$ (4, 15, 17, 9, 1), 343/345/347/349 $[M-Br]^+$ (18, 43, 28, 5), 79 (100); HREIMS $[M-CH_3]^+$ 406.8030 (calcd for $C_9H_{11}O^{79}Br_3^{35}Cl$, 406.8048), 342.9106 $[M-Br]^+$ (calcd for $C_{10}H_{14}O^{79}Br^{35}Cl_2$, 342.9099).

3.3.3. Compound 3. Colourless oil; $[\alpha]_D^{25}$=−143 (*c*, 0.03, $CHCl_3$); IR ν_{max} (film) 3412, 2933, 1378 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 317/319/321 $[M-Br]^+$ (<1, <1, <1), 301/303/305 $[(M-H_2O-Br]^+$ (<1, <1, <1), 183/185/187 $[C_4H_5OBrCl]^+$ (<l, <l, <1), 149/151 $[C_4H_6OBr]^+$ (100, 97); HREIMS $[M-Br]^+$ 318.9568 (calcd for $C_{10}H_{14}O^{79}Br^{37}Cl^{35}Cl$, 318.9681), $[M-H_2O-Br]^+$ 302.9456 (calcd for $C_{10}H_{14}O^{79}Br^{37}Cl_2$, 302.9546), $[C_4H_5OBrCl]^+$ 186.9207 (calcd for $C_4H_5O^{81}Br^{37}Cl$, 186.9162), $[C_4H_6OBr]^+$ 148.9590 (calcd for $C_{15}H_{14}O_2^{79}Br$, 148.9602).

Acknowledgements

This work was supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT), FEDER, MCYT (project PPQ2002-02494), Subdirección General de Cooperación Internacional and the Cooperation Programme between the Consejo Superior de Investigaciones Científicas (CSIC, Spain)-Universidad de Chile. A. R. D.-M. acknowledges a fellowship from the CICYT.

References

1. Faulkner, D. J.; Stallard, M. O. *Tetrahedron Lett.* **1973**, 1171–1174.
2. Crews, P.; Kho, E. *J. Org. Chem.* **1974**, *39*, 3303–3304.
3. McConnell, O. J.; Fenical, W. *J. Org. Chem.* **1978**, *43*, 4238–4241.
4. Coll, C. J.; Wright, A. D. *Aust. J. Chem.* **1987**, *42*, 1983–1993.
5. Aazizi, M. A.; Assef, G. M.; Faure, R. *J. Nat. Prod.* **1989**, *52*, 829–831.
6. Fuller, R. W.; Cardellina, II., J. H.; Hato, Y.; Brinen, L. S.; Clardy, J.; Snader, K. M.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 3007–3011.
7. Naylor, S.; Hanke, F. J.; Manes, L. V.; Crews, P. *Prog. Chem. Org. Nat. Prod.* **1983**, *44*, 189–241, and references therein.
8. Högberg, H.-E.; Thomson, R. H.; King, T. J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1696–1701.
9. Dorta, E.; Darias, J.; Rovirosa, J.; San-Martín, A.; Cueto, M. *J. Nat. Prod.* **2002**, *65*, 329–333, and references therein.
10. Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1–48, and references therein.
11. Cueto, M.; Darias, J. *Tetrahedron 52* **1996**, 5899–5906.
12. Cueto, M.; Darias, J.; San-Martín, A.; Rovirosa, J. *J. Nat. Prod.* **1998**, *61*, 17–21.
13. Cueto, M.; Darias, J.; Rovirosa, J.; San-Martín, A. *J. Nat Prod.* **1998**, *61*, 1466–1468.
14. Darias, J.; Rovirosa, J.; San-Martín, A.; Díaz, A. R.; Dorta, E.; Cueto, M. *J. Nat. Prod.* **2001**, *64*, 1383–1387.
15. Díaz-Marrero, A. R.; Rovirosa, J.; Darias, J.; San-Martín, A.; Cueto, M. *J. Nat. Prod.* **2002**, *65*, 585–588.
16. Cueto, M.; Darias, J.; Rovirosa, J.; San-Martín, A. *Tetrahedron* **1998**, *54*, 3575–3580.
17. *PCModel*, v. 7.0; Serena Software: Bloomington, IN, 47402-3076.
18. Blunt, J. W.; Bowman, N. J.; Munro, M. H. G.; Parsons, M. J.; Wright, G. J.; Kon, V. K. *Aust. J. Chem.* **1985**, *38*, 519–525.
19. Bates, P.; Blunt, J. W.; Hartshorn, M. P.; Jones, A. J.; Munro, M. H.; Robinson, W. T.; Yorke, S. C. *Aust. J. Chem.* **1979**, *32*, 2545–2554.