Oxachamigrenes, New Halogenated Sesquiterpenes from *Laurencia obtusa*

Inmaculada Brito,[†] Mercedes Cueto,[†] Ana R. Díaz-Marrero,[†] José Darias,*[,†] and Aurelio San Martín[‡]

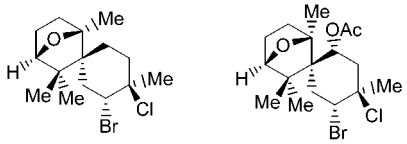
Instituto de Productos Naturales y Agrobiología del CSIC, Avenida Astrofísico F. Sánchez 3, Apartado 195, 38206 La Laguna, Tenerife, Spain, and Departamento de Química, Facultad de Ciencias, Casilla 653, Universidad de Chile, Santiago de Chile, Chile

Received November 21, 2001

Two new sesquiterpenes belonging to a novel oxacyclic structural type of chamigrene skeleton, oxachamigrene (**1**) and 5-acetoxyoxachamigrene (**2**), have been isolated from the red alga *Laurencia obtusa*. The structures of the compounds were determined on the basis of spectroscopic evidence. A biogenetic route for these metabolites has been proposed.

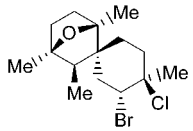
Species of algae from the genus *Laurencia* (Ceramiales, Rhodomelaceae) have been a subject of intensive research since an earlier study of marine natural products.[1] Most of the halogenated sesquiterpenes discovered occur in various species of *Laurencia*,[2] and although diterpenes, triterpenes, and especially C-15 acetogenins have also been found,[3,4] the sesquiterpene metabolites with a chamigrene skeleton appear to be the most generalized in the genus and could be a taxonomical marker for some of them. Other sesquiterpenes from *Laurencia* species with a monocyclofarnesane skeleton such as snyderols[6] and dactyloxenes[7] or having a bisabolane skeleton such as caespitol[8] and related[9] compounds or rearranged chamigrenes such as derivatives of cuparane,[10] laurane,[11] cyclolaurane,[12] and others[3,5] are less common.

Our interest[2] in the chemical analysis of species of the genus *Laurencia* led us to study the chemical content of *Laurencia obtusa* (Huds.) Lamoroux from Cuba, and we report now two minor interesting sesquiterpenes, **1** and **2**, isolated, together with nidificene[13] and acetoxyintrincatol,[14] from *L. obtusa* collected in Cayo Coco belonging to a novel oxacyclic structural class with a chamigrene skeleton. Recently a related rearranged chamigrene derivative, **3**, isolated from Malaysian *L. panosa*, has been reported.[15]



1

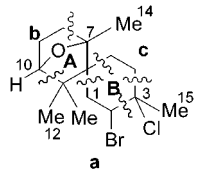
2



3

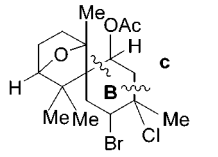
Vacuum flash chromatography of the dichloromethane extract of *L. obtusa* gave a fraction (90:10 hexane−ethyl acetate) from which oxachamigrene (**1**) and 5-acetoxyoxachamigrene (**2**) were obtained by standard chromatographic procedures, Si gel chromatography, and recycling-HPLC.

Compound **1** was a colorless oil. The EIMS showed peaks at *m/z* 334/336/338 $[M]^+$, with relative intensities suggestive of one bromine and one chlorine atom that correspond to the empirical formula $C_{15}H_{24}OBrCl$ $[M]^+$ (HRMS). The IR data revealed the absence of absorptions for hydroxyl group or unsaturation, suggesting that the oxygen is involved in ether linkages and that the molecule is tricyclic. The ^{13}C NMR spectrum of **1** (Table 1) showed signals for 15 carbons. Multiplicities of the carbon signals were determined from the DEPT spectrum: four methyls, five methylenes, two methines (bearing heteroatoms), and four nonprotonated carbons.



1

Oxachamigrene



2

5-Acetoxy-oxachamigrene

Figure 1. Significant fragments of oxachamigrenes.

The ^1H NMR spectrum of **1** (Table 1) displayed signals corresponding to protons which are in the vicinity of heteroatoms at δ 4.38 (1H, dd, $J = 3.6, 13.7$) and 3.70 (1H, d, $J = 5.6$). At high field, the signals corresponding to the four tertiary methyl groups appeared at δ 1.69 (3H, s), 1.56 (3H, s), methyls geminal to halogen and oxygen, respectively, 0.99 (3H, s), and 0.85 (3H, s). Chemical shift arguments and $^1H-^1H$ COSY correlations supported by MS data allowed the assignment of fragments **a**−**c** as shown in Figure 1.

From the $^1H-^1H$ COSY NMR spectrum it was possible to differentiate three discrete spin systems. The coupling between the proton on carbon bearing halogen at δ 4.38 and the methylene protons at δ 1.90 and 2.19 established the connectivity of the H-1−H-2 fragment **a**. One of the protons at δ 1.67 of a methylene (δ 1.67 and 1.82) is coupled with both the methine at δ 3.70 and a methylene protons at δ 1.35 and 1.98, indicating the connectivity of the H-8−H-10 fragment **b**. A third fragment **c** was defined by the coupling of the respective protons of two methylene groups at δ 2.45, 2.23 and δ 1.82, 1.41. HMQC NMR data established the position of the heteroatoms.

HMQC and HMBC NMR data were used to confirm the fragments **a**−**c** and establish the connectivity between them. As the geminal to oxygen methyl group H_3-14 at δ 1.56 correlated with both the quaternary carbon bearing oxygen and the methylene (δ_{C-7} 89.4 and δ_{C-8} 33.9) and as a gem-dimethyl group (H_3-12, H_3-13) and the aforemen-

* To whom correspondence should be addressed. Tel: +34 922 252144. Fax: +34 922 260135. E-mail: jdarias@ipna.csic.es.
[†] Instituto de Productos Naturales y Agrobiología del CSIC.
[‡] Universidad de Chile.

10.1021/np010580t CCC: $22.00
Published on Web 05/24/2002

Table 1. ^{1}H, ^{13}C, and HMBC NMR Data of Compounds **1** and **2** [500 MHz, δ ppm, (J) Hz, Chloroform-d]

	1			**2**		
position	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	α: 2.19 dt (3.6, 14.7) β: 1.90 t (13.8)	42.2	C-2, C-3, C-5, C-6, C-7	α: 2.13 dd (3.2, 15.0) β: 1.80 t (13.9)	41.5	C-2, C-3, C-5, C-6, C-7
2	4.38 dd (3.6, 13.7)	62.0	C-3	4.30 dd (3.2, 13.9)	59.6	C-3
3		71.3			68.9	
4	α: 2.45 dt (4.6, 14.3) β: 2.23 ddd (2.5, 3.6, 13.7)	40.5	C-2, C-3, C-5, C-6, Me-15	2.54 m	44.4	C-2, C-3, C-5, C-6, Me-15
5	α: 1.82 m β: 1.41 dt (5.1, 14.7)	27.5	C-3, C-4, C-6, C-7	4.74 dd (6.4, 10.7)	71.0	C-4, C-6, C-7, C-11, C=O
6		49.9			53.5	
7		89.4			88.8	
8	α: 1.35 dt (5.6, 12.7) β: 1.98 ddd (3.8, 9.0, 12.2)	33.9	C-6, C-7, Me-14	α: 1.36 dt (5.9, 12.3) β: 1.95 ddd (3.2, 8.7, 12.3)	35.9	C-6, C-7, Me-14
9	a: 1.82 m b: 1.67 m	25.5	C-8, C-10, C-11	1.74 m	24.8	
10	3.70 d (5.6)	85.9	C-6, C-7, C-8, C-9, C-11	3.73 d (5.9)	85.1	C-6, C-7, C-8, C-9, Me-13
11		48.4			49.7	
12	0.85 s	20.8	C-6, C-10, C-11, Me-13	0.86 s	21.2	C-6, C-10, C-11, Me-13
13	0.99 s	26.2	C-6, C-10, C-11, Me-12	1.00 s	25.7	C-6, C-10, C-11, Me-12
14	1.56 s	23.3	C-6, C-7, C-8	1.78 s	23.6	C-6, C-7, C-8
15	1.69 s	23.6	C-2, C-3, C-4	1.76 s	24.5	C-2, C-3, C-4
16					169.9	
17				2.04 s	21.6	C=O

tioned methyl (H_3-14) and methine (H-10) correlated with the same quaternary carbon (C-6) a subunit **A** was established. The spiro nature of C-6 of subunit **A** was verified by a long-range correlation between the methyl (H_3-15) on carbon bearing halogen with the H-2 bromomethine-containing fragment **a** and a methylene (H_2-4) of fragment **c**, both of which in turn showed cross-peaks with the remaining quaternary C-6 of subunit **A**, accounting for all 15 carbons of the molecule. The ether function was verified by the long-range correlation between the methine (H-10) and the quaternary carbon bearing oxygen (C-7). Thus, the overall planar structure for **1** with the requisite three degree of unsaturation can be suggested.

Compound **2** was isolated as a colorless oil. The EIMS of the compound showed peaks at m/z 333/335/337 [M − OAc]$^+$ with relative intensities suggesting one bromine and one chlorine atom, and m/z 313/315 [M − Br]$^+$. The elemental composition of peaks at m/z 333 and 313 was confirmed by HREIMS, and the overall molecular formula was hence deduced to be $C_{17}H_{26}O_3BrCl$. The IR data showed absorption for a carbonyl group at 1740 cm^{-1}, and the ^{1}H NMR and ^{13}C NMR spectra indicate that the carbonyl is part of a secondary acetyl group (δ_H 4.74, δ_{3H} 2.04; δ_C 21.6, $\delta_{C=O}$ 169.9). In the absense of other unsaturation the molecule must be tricyclic.

Comparison of the ^{1}H and ^{13}C NMR spectra of **2** and **1** (Table 1) indicates similar spectral features for ring **A**. As in **1**, the ^{1}H−^{1}H COSY NMR spectrum of **2** showed identical spin systems for fragments **a** and **b**, whereas another spin system correlated H-5 (δ 4.74) geminal to an acetate group with the protons of the methylene at δ 2.54. HSQC and HMBC NMR experiments confirmed that the acetyl group was in ring **B**, and it was linked to C-5 by the long-range correlation between H-5 and C-7 and C-11. Thus, compound **2** possesses the same planar structure as **1**, the difference between them being the degree of oxidation of ring **B**.

It was deduced from the carbon chemical shift of the sp^3 halogen-bearing carbon at 62.0 ppm in **1** and 59.6 ppm in **2** that this halogen atom was bromine[16,17] in both compounds. Therefore, the halogen regiochemistry is that

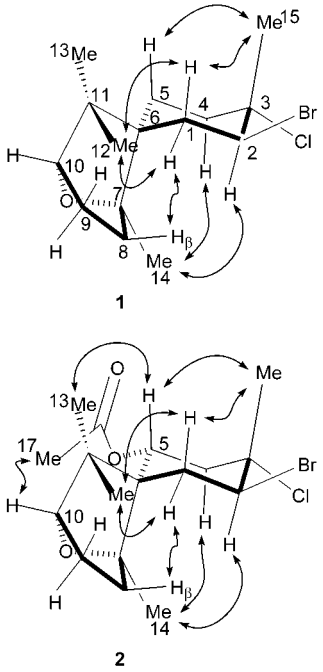


Figure 2. Selected NOEs and stereochemistry of oxachamigrenes.

shown in Figure 1 where Br is on C-2 and Cl is on C-3. Moreover, the spectral data for the chloro-bromo system of **1** and **2** are very similar to that recently reported[13] for **3** (H-2, δ 4.44 dd J = 4.4, 13.2; C-2 δ 62.7), (C-3 δ 71.6), (H_3-15, δ 1.69 s; C-15 δ 23.6), whose regiochemistry was assigned by the halogen-induced ^{13}C isotope shifts[18,19] in the ^{13}C NMR spectrum.

The relative stereochemistries for **1** and **2** (Figure 2) were assigned on the basis of a study of the coupling constants and NOESY experiments. The almost identical chemical shifts and coupling constants for the respective H-2 protons of **1** (J = 3.6, 13.7) and **2** (J = 3.2, 13.9), typical of an axial proton, suggested the same equatorial stereochemistry for the bromine atom in both compounds. The J values for H-5 (6.4, 10.7) of **2** indicate that the acetyl group was also

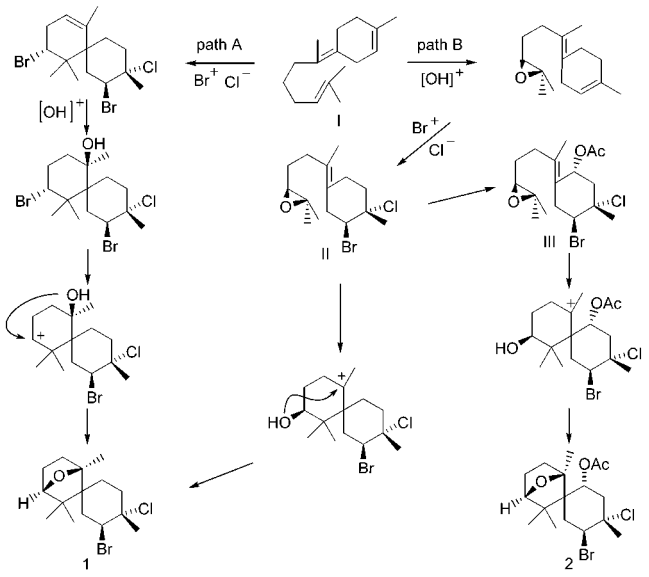


Figure 3. Possible biogenetic pathway of oxachamigrene derivatives.

equatorial. The conformation of ring **B** of **1** and **2** with a trans-diequatorial chloro-bromo system was assigned by the strong NOE observed between the respective H_3-14 methyl groups and both $H_{\alpha ax}$-4 and $H_{\alpha ax}$-2, as well by the NOE observed between $H_{\beta ax}$-1, $H_{\beta ax}$-5, and H_3-15. Furthermore, a NOE observed between H_β-8/$H_{\alpha eq}$-1 and Me-12 with $H_{\beta ax}$-1 and $H_{\alpha eq}$-1 in both **1** and **2** and, on the other hand, the NOEs between Me-13/$H_{\beta ax}$-5 and Me-17/H-10 established the stereochemistry around the spiro carbon at C-6 as shown in Figure 2.

A biogenetic route was proposed for a related rearranged chamigrene **3**.[15] However, although a similar route, Figure 3, for **1** and **2** involving a γ-bisabolene precursor I could also be considered for the oxetane ring formation (path A), the postulation of a secondary carbocation intermediate, generated by leaving bromine on C-10, seemed unlikely. Alternative path B appeared to be more plausible for these compounds. Terminal epoxide ring-opening of II inducing spiro-ring formation and subsequent nucleophilic trapping of the tertiary carbonium ion intermediate to form an oxetane ring is a suitable way to explain the formation of **1**. On the other hand, allylic oxidation of II to give III followed by similar spiro-ring and oxetane formation as previously described will give the acetoxy chamigrene **2**.

Experimental Section

General Experimental Procedures. 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 1605/FTIR spectrometer in $CHCl_3$ solutions. ^1H and ^{13}C NMR, HMQC, HMBC, NOESY, and $^1H-^1H$ 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. Recycling-HPLC separations were performed with a Japan Analytical LC-908. Merck Si gels 7734 and 7741 were used in column chromatography. The spray reagent for TLC was $H_2SO_4-H_2O-AcOH$ (1:4:20).

Plant Material. *L. obtusa* was collected off Cayo Coco by scuba diving. A voucher specimen has been deposited at the Department of Marine Biology, Universidad de La Laguna, Tenerife, Canary Islands, Spain (deposit number LoCu01-1).

Extraction and Isolation of Sesquiterpenoids 1 and 2. Air-dried *L. obtusa* (180.2 g, dry wt) was extracted with dichloromethane at room temperature. The extract was concentrated to give a residue (3.1 g), which was fractionated by flash chromatography on Si gel. Compounds **1** (3.8 mg) and **2** (5.7 mg) were obtained from the fraction eluted with hexane−EtOAc (90:10) (80 mg) after separation and purification on a Si gel column followed by recycling-HPLC using chlorofom as eluent.

Oxachamigrene (1): colorless oil; $[\alpha]^{25}_D$ −10.2 (*c*, 0.3, $CHCl_3$); ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 334/336/338 $[M]^+$ (2.6, 2.9, 0.8), 316/318/320 $[M - H_2O]^+$ (5, 6, 2), 298/300 $[M - HCl]^+$ (3, 3), 255/257 $[M - Br]^+$ (1, 3), 91 $[C_7H_7]^+$ (100); HREIMS $[M]^+$ 334.0732 (calcd for $C_{15}H_{24}O^{79}Br^{35}Cl$, 334.0699).

5-Acetoxyoxachamigrene (2): colorless oil; $[\alpha]^{25}_D$ −4.3 (*c*, 0.2, $CHCl_3$); IR ν_{max} 1740 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 333/335/337 $[M - OAc]^+$ (2, 2, 1), 313/315 $[M - Br]^+$ (5, 2), 291 (3), 199 (55), 159 (75), 199 (47), 105 (58), 91 (8), 83 (100); HREIMS $[M - OAc]^+$ 333.0588 (calcd for $C_{15}H_{23}O^{79}Br^{35}Cl$, 333.0620), 313.1541 (calcd for $C_{17}H_{26}O_3{}^{35}Cl$, 313.1570).

Acknowledgment. This work was supported by Ministerio de Ciencia y Tecnología (MCYT), FEDER (project 1FD97-0348-C03-03), Subdirección General de Cooperación Internacional, Program of Cooperation between the Consejo Superior de Investigaciones Científicas (CSIC, Spain)-Universidad de Chile and the collaboration of CEBIMAR of Cuba. I.B. acknowledges a grant from the CICYT.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55, and references therein.
(2) Martin, J. D.; Darias, J. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. I, pp 125−174.
(3) Moore, R. E. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. I, pp 44−124.
(4) Gribble, G. W. *Prog. Chem. Org. Nat. Prod.* **1996**, *68*, 66−100.
(5) Howard, B. M.; Fenical, W. *Tetrahedron Lett.* **1976**, 41−44.
(6) Schmitz, F. J.; McDonald, F. J. *Tetrahedron Lett.* **1974**, 2541−2544.
(7) González, A. G.; Darias, J.; Martin, J. D.; Pérez, C. *Tetrahedron Lett.* **1974**, 1249−1250.
(8) González, A. G.; Darias, J.; Martín, J. D. *Tetrahedron Lett.* **1973**, 3625−3626.
(9) Irie, T.; Suzuki, M.; Hayakawa, Y. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 843−844.
(10) Irie, T.; Suzuki, M. *Tetrahedron Lett.* **1965**, 3619−3624.
(11) Ichiba, T.; Higa, T. *J. Org. Chem.* **1986**, *51*, 3364−3366.
(12) González, A. G.; Darias, J.; Díaz, A.; Fourneron, D. J.; Martín, J. D.; Pérez, C. *Tetrahedron Lett.* **1976**, 3051−3054.
(13) Waraszkiewicz, S. M.; Erickson, K. L. *Tetrahedron Lett.* **1974**, *23*, 2003−2006.
(14) McMillan, J. A.; Paul, I. C.; White, R. H.; Hager, L. P. *Tetrahedron Lett.* **1974**, *23*, 2039−2042.
(15) Suzuki, M.; Daitoh, M.; Vairappan, C. S.; Abe, T.; Masuda, M. *J. Nat. Prod.* **2001**, *64*, 597−602.
(16) Sims, J. J.; Rose, A. F.; Izac, R. R. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. I, Chapter 5.
(17) Crew, P.; Naylor, S.; Hanke, J.; Hogue, E. R.; Kho, E.; Braslau, R. *J. Org. Chem.* **1984**, *49*, 1371−1377.
(18) Raynes, W. T.; Sergeyev, N. M.; Sandor, P.; Grayson, M. *Magn. Reson. Chem.* **1997**, *35*, 141−143.
(19) Sergeyev, N. M.; Sandor, P.; Sergeyeva, N. D.; Raynes, W. T. *J. Magn. Reson. (Ser. A)* **1995**, *115*, 174−182.

NP010580T