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Oxachamigrenes, New Halogenated Sesquiterpenes from *Laurencia obtusa*

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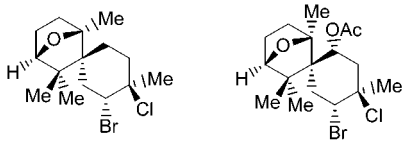
Two new sesquiterpenes belonging to a novel oxacyclic structural type of chamigrene skeleton, oxachamigrene (**1**) and 5-acetoxyoxachamigrene (**2**), have been isolated from the red alga *Laurencia obtusa*. The structures of the compounds were determined on the basis of spectroscopic evidence. A biogenetic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

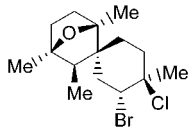
Species of algae from the genus *Laurencia* (Ceramiales, Rhodomelaceae) have been a subject of intensive research since an earlier study of marine natural products.[1] Most of the halogenated sesquiterpenes discovered occur in various species of *Laurencia*,[2] and although diterpenes, triterpenes, and 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

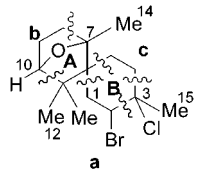
2



3

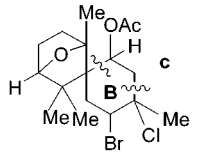
Vacuum flash chromatography of the dichloromethane extract of *L. obtusa* gave a fraction (90:10 hexane−ethyl acetate) from which oxachamigrene (**1**) and 5-acetoxyoxachamigrene (**2**) were obtained by standard chromatographic procedures, Si gel chromatography, and recycling-HPLC.

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-

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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

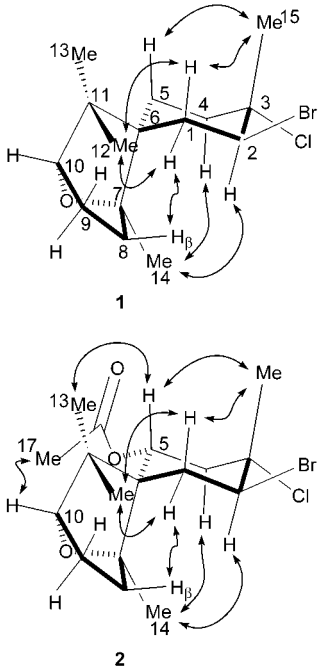

Figure 2. Selected NOEs and stereochemistry of oxachamigrenes.

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

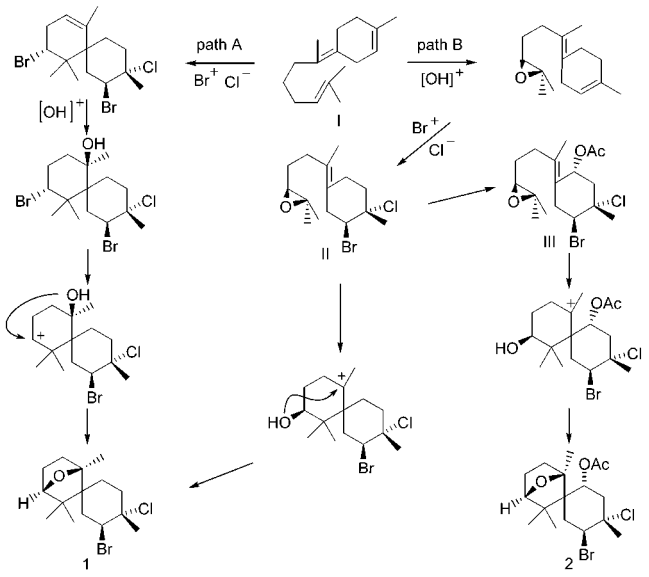

Figure 3. Possible biogenetic pathway of oxachamigrene derivatives.

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).

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

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