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Chamigrenelactone, a polyoxygenated sesquiterpene with a novel structural type and devoid of halogen from *Laurencia obtusa*

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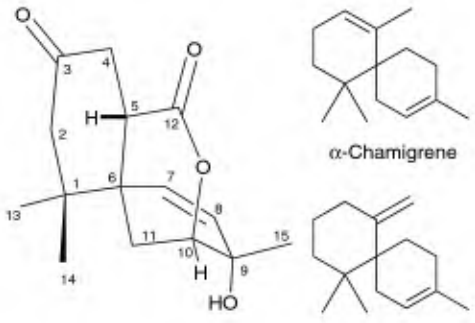
Abstract—A biogenetically interesting halogen-devoid metabolite chamigrenelactone **1**, with a high oxygen-content, has been isolated from *Laurencia obtusa* from Isla Grande (Caribbean Panama). Its structure and stereochemistry were determined on the basis of spectral studies.

Algae from genus *Laurencia* (Ceramiales, Rhodomelaceae) have been the subjects of intensive research since earlier studies of marine natural products.[1] Nevertheless, new chemical studies of *Laurencia* species from different latitudes always result in the discovery of interesting and novel structures[2–4] as well as biologically active metabolites.[5,6] The widespread and prolific production of secondary metabolites observed in *Laurencia* spp. may be interpreted as an ecological adaptive response.

Sesquiterpenes with a chamigrene skeleton appear to be the most generalized metabolites in *Laurencia* and most of these are characterized by the predominant incorporation of chlorine and bromine atoms in their structures.[7] Over 110 chamigrenes have been isolated from *Laurencia* species and sea hares grazing on them. Curiously, all these metabolites are α- or β-chamigrene derivatives in a ratio of approximately 1:1. Studies of the natural products from *Laurencia* indicate that specific compounds are only produced by some species therefore conferring a chemical signature to these.[8]



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We collected *L. obtusa* from the intertidal rocky shore at Isla Grande, Caribbean Panama and here we report on a biogenetically interesting minor sesquiterpene **1** belonging to a novel structural class of chamigrene, inasmuch as: (a) the compound is a polyoxygenated metabolite that does not incorporate a halogen atom in its structure; (b) one of its isoprenic methyl groups was oxidized to a carboxylic group, which is an unusual functionality in chamigrene metabolites; and (c) since C-5 is a hydrogenated sp^3 carbon it is impossible to establish if **1** is an α- or a β-chamigrene skeleton derivative.

Keywords: Sesquiterpene; Chamigrene; *Laurencia obtusa*; Biogenesis.
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Vacuum flash chromatography of the dichloromethane extract of *L. obtusa* gave a fraction (1:1 hexane–ethyl acetate) from which the tricyclic keto-lactone **1** was obtained by standard chromatographic procedures involving gel filtration, Si gel chromatography, and HPLC.[9] From the fraction (9:1 hexane–ethyl acetate) obtusol[10] and dechloroelatol[11] were obtained.

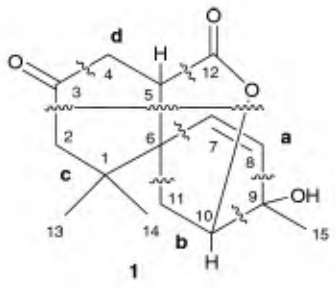
Chamigrenelactone, **1**, was a colorless oil.[12] The EIMS spectrum showed a peak at *m/z* 264 $[M]^+$, that corresponds to the empirical formula $C_{15}H_{20}O_4$ $[M]^+$ (HRMS). The ^{13}C NMR spectrum of **1** (Table 1) showed signals for 15 carbons. Multiplicities of the carbon signals were determined from the DEPT spectrum: three methyl groups, three methylenes, four methines (two olefinic, one bearing oxygen), and five non-protonated carbons (two carbonyl groups).

The nice ^1H NMR spectrum of **1** displayed signals due to olefinic protons at δ 6.20 (1H, dd, $J = 2.1$, 10.3) and δ 5.86 (1H, dd $J = 1.8$, 10.3). A doublet of doubles at 4.49 (1H, dd, $J = 1.6$, 5.0) was attributed to a proton geminal to oxygen, whereas seven well-resolved signals, each attributed to one of the remaining aliphatic protons, appeared between 2 and 3 ppm. At high field, three methyl singlets appeared at δ 1.48 (3H, s), methyl geminal to oxygen, δ 1.01 (3H, s), and δ 1.00 (3H, s), Table 1.

From the spectral data and the molecular formula it was deduced that the molecule is tricyclic and because no IR absorption for free acid was observed, two of the oxygens given by the molecular formula must be involved in a lactone ring, one of the remaining oxygens being a part of a methyl carbinol (H_3-15: δ 1.48; C-9: δ 70.0) and the other of a non-conjugated ketone (C-3, δ 207.0), which is consistent with the IR absorption at 3422 and 1714 cm^{-1} observed for the oxygenated functionalities. This suggested that one of the four methyl groups expected for a sesquiterpene skeleton was oxidized.

The absence of any carbon-substituted quaternary carbon atom around 50 ppm in the ^{13}C NMR spectrum, which is characteristic of the spiro carbon of a chamigrene skeleton, as well as the lack of a halogen functionality, which is common in chamigrene-derived metabolites, prompted us to undertake a careful spectral analysis of **1**.

All C–H correlations for **1** were detected in the HSQC spectrum. From the ^1H–^1H-COSY NMR spectrum it was possible to differentiate three discrete spin systems: **a**, **b**, and **d**. The HMBC long-range correlation between the methyl carbinol C-15 and one of the coupled protons of an isolated disubstituted olefin, H-8, as well as with the closing lactone ring proton H-10, which in turn is coupled with a methylene H_2-11, established the connectivity of fragments **a** and **b** through C-9. The mutual correlation of the methyls (H_3-13, H_3-14) of a geminal methyl group, and their long-range correlations with an isolated C-2 methylene and with a quaternary C-6 carbon defined fragment **c**.



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The connectivity of fragment **c** with both **a** and **b** fragments, through the spiro carbon C-6, was secured by the following HMBC correlations: H-7/C-6, C-11; H-11/C-6, C-7. The remaining spin system fragment **d** was linked to fragment **b** through C-6 and C-12 by the

Table 1. ^1H, ^{13}C NMR, HMBC, COSY, and NOESY data of compound **1** [500 MHz, δ ppm, (J) Hz, $CDCl_3$]

#	δ_H	δ_C	HMBC	COSY	NOESY
1		39.9			
2	α 2.67 d (15.1)	51.4	C-1, C-3, C-6, C-13		H-7
	β: 2.14 dd (2.2, 15.1)		C-1, C-3, C-4, C-6, C-14		H_3-14
3		207.0			
4	α: 2.34 dd (13.6, 15.1)	39.4	C-5, C-12	H-5	H-7
	β: 2.81 ddd (2.2, 4.6, 15.2)		C-3, C-5	H-5	
5	2.92 dd (4.6, 13.6)	46.7	C-1, C-4, C-6, C-11, C-12	H_2-4	H-11β, H_3-14
6		39.0			
7	6.20 dd (2.1, 10.3)	130.5	C-6, C-9, C-11	H-8	H-2α, H-4α
8	5.86 dd (1.8, 10.3)	133.1	C-6, C-10	H-7	H_3-15
9		70.0			
10	4.49 dd (1.6, 5.0)	80.5	C-9, C-12	H_2-11	H-11β
11	α: 2.48 d (13.2)	26.9	C-7, C-10	H-10	O*H*
	β: 1.99 ddd (2.1, 4.9, 13.5)		C-5, C-6	H-10	H-5, H-10, H_3-14
12		171.5			
13	1.01 s	24.6	C-1, C-2, C-6, C-14		
14	1.00 s	22.9	C-1, C-2, C-6, C-13		H-2β, H-5, H-11β
15	1.48 s	25.8	C-8, C-9, C-10		H-8
O*H*	1.98 s				H-11α

correlations H-5/C-1, C-6, C-11 and C-12/H-4, H-5, H-10, and was linked to fragment **c** through C-3 by the correlations H-2/C-3, C-4. Thus, the overall planar structure of a chamigrene skeleton for **1** with the requisite three degrees of unsaturation can be suggested.

The relative stereochemistry of **1** (Fig. 1) was assigned on the basis of the study of the coupling constants and NOESY experiments. The strong NOEs observed between H-5/H_3-14, H-11β; and H-11β/H_3-14 fixed the stereochemistry at C-5. The large *J* value (13.6 Hz) of H-5 with the coupled *trans* diaxial proton of the vicinal methylene, H_2-4, allowed us to identify H-4α. The strong NOEs observed between H_4-α and a H-7 olefinic proton, and between H-7 and one of the protons of the H_2-2 methylene determined H-2α. All this together secured the stereochemistry around the spiro carbon at C-6 as *S* (Fig. 1). A β-stereochemistry for the H_3-15 methyl group at C-9 was assigned by the NOE observed between OH-C-9 and H-11α, which is in agreement with their relative low field resonance (δ 1.48) of the H_3-15 caused by the neighboring effect of the lactone carbonyl. The proposed stereochemistry of **1** (as shown in Fig. 1) is in accord with additional NOEs observed (Table 1).

Compound **1** is a chamigrene metabolite that possesses a hydrogen at C-5 instead of the olefinic unsaturation common to most naturally occurring α- and β-chamigrene derivatives. This characteristic is solely shared with **4**, isolated from *Aplysia dactylomela*.[13] Although pinnatifidone **2**[14] also exhibits a similar feature, the origin of its α-methyl ketone may have a straightforward explanation in the rearrangement of an epoxidized α-chamigrene such as **3**,[15] since both **2** and **3** were isolated from *Laurencia* species. However, the origin of the hydrogenated C-5 carbon of **1** and **4** is not apparent on these grounds (Fig. 2).

The current biogenetic hypothesis[7,10,16] for the biosynthesis of chamigrene metabolites from farnesyl pyrophosphate involves a stepwise cyclization to give: (a) a γ-bisabolene derivative **5** after enzymatic functionalization of a γ-bisabolonium ion and (b) cyclization of **5**

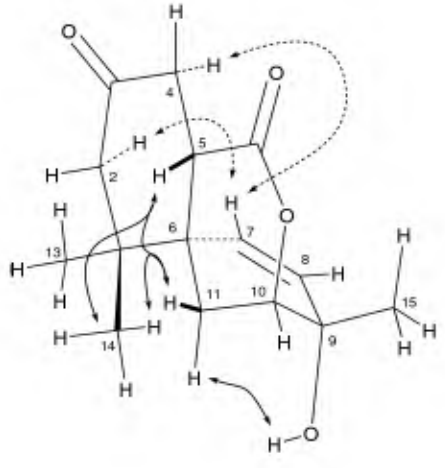

Figure 1. Selected NOEs of **1**.

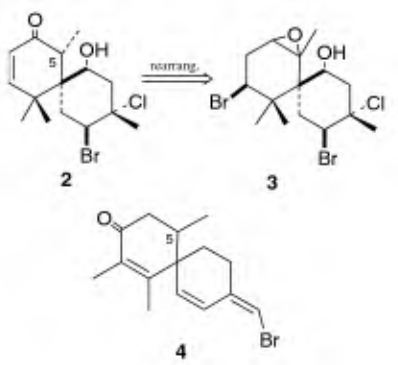

Figure 2. Chamigrene metabolites hydrogenated at C-5.

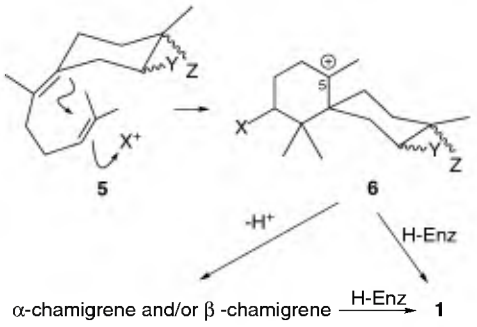

Figure 3. Possible precursors of **1** in the C-5 reductive stage.

to the chamigrene intermediate **6**, which collapses to the well-known α- or β-chamigrene skeletons. Assuming this hypothesis, an additional, perhaps enzymatically catalyzed, reductive step should be considered to account for the formation of **1** and **4** (Fig. 3). Therefore, the question arises of whether a discrete α- or β-chamigrene is involved in the reductive stage or if reduction takes place at the intermediate cationic level **6**.

The novel structural features of compound **1**, the unusually high degree of oxidation and oxygen content as well as the lack of a halogen, may reflect adaptive responses of the alga.

Acknowledgements

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12. $[\alpha]_D^{25}$ −150 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3422, 2344, 1714, 1076, 771 cm^{-1}; EIMS *m/z* 264 $[M]^+$ (19), 246 $[M-H_2O]^+$ (41), 165 (32), 151 (42), 118 (55), 100 (90), 98 (100), 91 (73), 77 (35), 55 (32); HREIMS 264.1345 (calcd for $C_{15}H_{20}O_4$, 264.1361); ^1H and ^{13}C NMR (see Table 1).
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