

## GRACIOSIN AND GRACIOSALLENE, TWO BROMOETHERS FROM *LAURENCIA OBTUSA*

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**Abstract**—Two new bromo ethers possessing a bromo propargylic and bromo allenic moieties, graciosin and graciosallene, were isolated from the red alga *Laurencia obtusa* and their structures determined by X-ray crystallography and chemical methods

### INTRODUCTION

The secondary metabolites elaborated by the marine red algae of the genus *Laurencia* include a group of  $C_{15}$  acetylenic cyclic ethers with a remarkable structural variation [1]. In our continuing studies on samples of this genus collected in the Canary Islands, we have reported the isolation and structural determination of several  $C_{15}$  acetylenes [2–4]. As a part of a further investigation on the constituents of this species, we examined a specimen of *Laurencia obtusa* collected at La Graciosa (Canary Islands) in September 1985. In this paper we wish to report the structures, including the absolute configurations, of two new bromo ethers possessing a bromo propargylic and bromo allenic moieties, respectively.

### RESULTS AND DISCUSSION

Graciosin (**1**) [ $\alpha_D^{25} = -14.5^\circ$  ( $CHCl_3$ ,  $c$  0.62)] was previously reported as **2** [4], without absolute configuration. However, by using 2D NMR techniques, the structure was modified to **1** and the chemical shifts were reassigned (Table 1). In addition, the absolute configuration was determined on the alcohol **3** as follows. Alkaline hydrolysis at room temperature of graciosin (**1**) with potassium carbonate in acetone gave a compound containing a 2,6-dioxabicyclo(3,3,0)octane ring system [4], but when potassium carbonate-methanol at  $-25^\circ$  was used, the alcohol **3** was obtained. This compound yielded a crystal suitable for X-ray analysis by crystallization from *n*-hexane and the crystallographic study was carried out. Crystals were orthorhombic, space group  $P2_12_12_1$ ,  $a = 7.309(1)$ ,  $b = 8.880(1)$ ,  $c = 27.196(3)$  Å,  $D_c = 1.54$  g/cm<sup>3</sup>,  $z = 4$ . The intensity of 1080 unique reflexions was measured using graphite-monochromated  $Cu-K_\alpha$  radiation ( $\lambda = 1.5418$  Å) and  $\omega$   $\theta$  scan in Siemens AED4 four circle computer controlled diffractometer. 1057 reflexions with  $I > 3\sigma(I)$  were judged observed after correction for Lorentz and polarization. The structure was solved by MULTAN 80 [5]. Full-matrix least squares refinement anisotropic temperature factors for bromine and oxygen, isotropic for carbon and fixed isotropic contribution for hydrogens atoms converged at  $R = 0.094$ . The absolute configuration was fixed by using the 10 more relevant Bijvoet

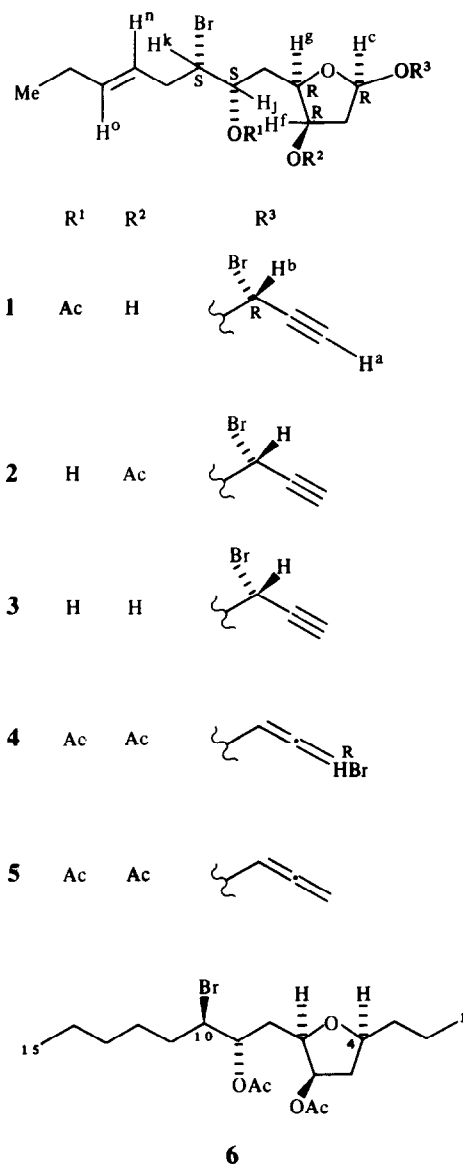


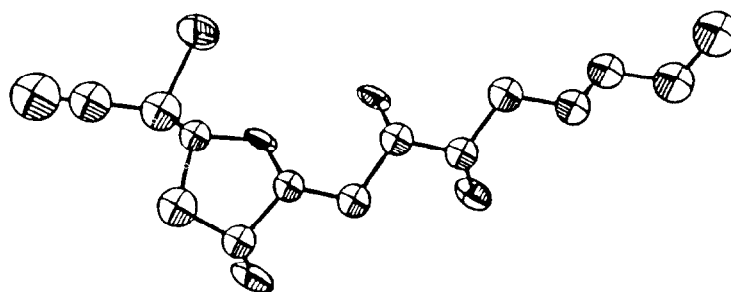
Table 1  $^1\text{H}$  NMR spectra of graciosin (**1**) and graciosallene (**4**) showing chemical shifts and multiplicities\*†

1				4	
H-1	H <sup>a</sup>	2.69	<i>d</i> , $J_{ab}=2.2$	6.06	<i>dd</i> , $J_{ab}=5.7$ ; $J_{ac}=1.6$
H-2	-	-	-	-	-
H-3	H <sup>b</sup>	4.58	<i>dd</i> , $J_{ba}=2.2$ , $J_{bc}=6.1$	5.47	<i>dd</i> , $J_{ba}=5.7$ , $J_{bc}=7$
H-4	H <sup>c</sup>	4.17	<i>ddd</i> , $J_{cb}=6.1$ , $J_{cd}=4.4$ , $J_{ce}=9$	4.48	<i>dddd</i> , $J_{ca}=1.6$ , $J_{cb}=7$ , $J_{cd}=3.8$ , $J_{ce}=15.8$
H <sub>2</sub> -5	H <sup>d</sup>	2.45	<i>m</i>	2.03	<i>m</i>
	H <sup>e</sup>	2.05	<i>m</i>	2.50	<i>m</i>
H-6	H <sup>f</sup>	3.75	<i>ddd</i> , $J_{fc}=3.4$ , $J_{fd}=7.4$ , $J_{fg}=3.4$	5.27	<i>ddd</i> , $J_{fg}=4.2$ , $J_{fd}=2$ , $J_{fe}=8$
H-7	H <sup>g</sup>	4.27	<i>ddd</i> , $J_{gf}=3.4$ , $J_{gh}=1.4$ , $J_{gi}=4.5$	3.86	<i>ddd</i> , $J_{gf}=4.2$ , $J_{gh}=3$ , $J_{gi}=9$
H <sub>2</sub> -8	H <sup>h</sup>	2.00	<i>m</i>	2.1	<i>m</i>
	H <sup>i</sup>	2.00	<i>m</i>	2.1	<i>m</i>
H-9	H <sup>j</sup>	5.15	<i>ddd</i> , $J_{jk}=3.8$ , $J_{ji}=1.2$ , $J_{jh}=10.2$	5.12	<i>ddd</i> , $J_{jk}=3.2$ , $J_{jh}=4.5$ , $J_{ji}=8$
H-10	H <sup>k</sup>	4.10	<i>ddd</i> , $J_{kj}=3.8$ , $J_{km}=5.5$ , $J_{kl}=6.1$	4.02	<i>ddd</i> , $J_{kj}=3.2$ , $J_{kl}=5.2$ , $J_{km}=8.5$
H <sub>2</sub> -11	H <sup>l</sup>	2.5	<i>m</i>	2.06	<i>m</i>
	H <sup>m</sup>	2.5	<i>m</i>	2.53	<i>m</i>
H-12	H <sup>n</sup>	5.42	<i>ddd</i> , $J_{nl}=5.4$ , $J_{no}=15.5$ , $J_{nm}=5.4$	5.38	<i>ddd</i> , $J_{no}=15.2$ , $J_{nm}=6$ , $J_{nl}=6$
H-13	H <sup>o</sup>	5.54	<i>ddd</i> , $J_{on}=15.5$ , $J_{op}=7.1$ , $J_{oq}=6.2$	5.55	<i>ddd</i> , $J_{on}=15.2$ , $J_{op}=7$ , $J_{oq}=7$
H <sub>2</sub> -14	H <sup>p</sup>	2.02	<i>m</i>	2.05	<i>m</i>
	H <sup>q</sup>	2.02	<i>m</i>	2.05	<i>m</i>
H <sub>3</sub> -15	H <sup>r</sup>	0.96	<i>t</i> , $J_{rp}=7.5$ , $J_{rq}=7.5$	0.95	<i>t</i> , $J_{rq}=7.4$ , $J_{rp}=7.4$
-OC(O)Me		2.09	<i>s</i> (3H)	2.07	<i>s</i> (6H)

\* The spectra were recorded at 200 MHz in  $\text{CDCl}_3$  solution

Chemical shifts are reported in ppm relative to TMS (0)

† COSY 2D NMR data support the proton assignments

Fig. 1 ORTEP drawing of graciosin (**1**) showing the right enantiomer

pairs with  $\Delta F_c > 1.3$  which are in the ranges of:  $0.2 \leq \sin \theta / \lambda \leq 0.5$ ,  $15 \leq F_o \leq 60$  and  $F_o = 10\sigma(F_o)$ . The averaged Bijvoet differences are 0.62 for the right enantiomer vs 0.54 for the wrong one [6]. Figure 1 shows the structure with the right enantiomer. Atomic co-ordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

Graciosallene (**4**) was isolated in small amount as an oil,  $[\alpha]_D^{25} = -83.3^\circ$  ( $\text{CHCl}_3$ ,  $c$  0.018). In the IR spectrum a band at  $1950\text{ cm}^{-1}$  first suggested the presence of an allene function. The  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ )  $\delta$  5.47 (*dd*,  $J=7.08$  and  $5.76\text{ Hz}$ ), 6.06 (*dd*,  $J=1.65$  and  $5.76\text{ Hz}$ ) (Table 1) and the fragment ions in the mass spectrum at  $m/z$  375, 377  $[\text{M}-\text{C}_3\text{H}_2\text{Br}]^+$  showed the presence of a bromoallenic side chain. The structure and absolute configuration of **4** were established by the following chemical method. Treatment of **3** with zinc-acetic acid in ethanol at  $0^\circ$  yielded compound **5**. This compound was treated with acetic anhydride-pyridine at room temp

and hydrogenated over  $\text{PtO}_2$  in diethyl ether to give **6**. These results indicated that the stereochemistry at C-4, C-6, C-7, C-9 and C-10 in **4** should be *R*, *R*, *R*, *S* and *S*-configurations, respectively. Finally, in view of the strong negative rotation of graciosallene, the absolute configuration of the bromoallene moiety in **4** could be assigned as *R* by the application of Lowe's rule [7].

#### EXPERIMENTAL

Mps uncorr.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at 200 MHz.

Collection, extraction and chromatographic separation. *Laurencia obtusa* was collected in Sept 1985 using SCUBA ( $-3$  to  $-10\text{ m}$ ) in Graciosa Island. A voucher specimen was deposited in the Departamento de Biología Vegetal (Universidad de La Laguna, Tenerife, Spain). The alga was air-dried and ground in a Wiley mill to a 1 mm particle size. The dried alga (4 kg) was extracted with  $\text{Et}_2\text{O}$  (2 l) and  $\text{EtOAc}$  (2 l). The combined extracts were evapd and the crude extract chromatographed on a silica

gel column using a mixture of *n*-hexane-EtOAc of increasing polarity as eluent. The *n*-hexane-EtOAc (3:2) eluate yielded a dark green oil which was chromatographed on a Sephadex LH-20 column using *n*-hexane-CHCl<sub>3</sub>-MeOH (2:1:1) as eluent, yielding a colourless oil which contained a mixture of graciosin (1) and graciosallene (4). Prep. TLC (*n*-hexane-EtOAc, 4:1) gave 350 mg of pure 1 and 30 mg of pure 4.

**Graciosin (1)** Oil,  $[\alpha]_D^{25} = -14.5^\circ$  (CHCl<sub>3</sub>, *c* 0.62). The physical and spectroscopical data (TLC, GLC, IR, NMR, MS) were identical with those reported previously [4].

**Compound 3** To a soln of 30 mg of graciosin (1) in 10 ml of MeOH cooled at  $-25^\circ$  was added excess of K<sub>2</sub>CO<sub>3</sub> (15 mg) and the mixture stirred for 6 hr. The mixture was filtered off, the solvent evapd and the residue crystallized from *n*-hexane to give 24 mg of 3, colourless crystals, mp  $96^\circ$ , IR  $\nu_{\max} \text{ cm}^{-1}$ : 3500, 3300, 2950, 2100, 1435, 1370, 1240 and 1100. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.91 (3H, *t*, *J* = 7.3 Hz), 2.63 (1H, *d*, *J* = 2.4 Hz), 3.8–4.03 (3H, *m*); 4.19 (2H, *m*), 4.57 (1H, *dd*, *J* = 2.4 and 6.4 Hz), 5.36 (1H, *ddd*, *J* = 5.4, 5.4 and 15 Hz), 5.53 (1H, *ddd*, *J* = 6.2, 7 and 15 Hz). MS *m/z* (rel int.): 329, 331 [M–Br]<sup>+</sup> (4), 311, 313 [M–Br–H<sub>2</sub>O] (15); 273, 275 [M–C<sub>3</sub>H<sub>2</sub>Br–H<sub>2</sub>O]<sup>+</sup> (3).

**Graciosallene (4)** Oil,  $[\alpha]_D^{25} = -83.3^\circ$  (CHCl<sub>3</sub>; *c* 0.018). IR  $\nu_{\max} \text{ cm}^{-1}$ : 3000, 1950, 1735, 1730 and 1370. <sup>1</sup>H NMR (See Table 1), MS *m/z* (rel. int.): 375, 377 [M–C<sub>3</sub>H<sub>2</sub>Br] (2), 353, 355 [M–HOAc–Br]<sup>+</sup> (1), 255, 257 [M–C<sub>3</sub>H<sub>2</sub>Br–2 HOAc]<sup>+</sup> (7).

**Compound 5** To a soln of 10 mg of 3 in 1 ml of EtOH at  $0^\circ$ , was added 10 mg of Zn powder and 50  $\mu$ l of HOAc. The mixture was stirred for 1 hr, filtered off and the solvent evapd to give 6 mg of 5: oil,  $[\alpha]_D^{25} = -5.5^\circ$  (CHCl<sub>3</sub>, *c* 0.4), IR  $\nu_{\max} \text{ cm}^{-1}$ : 3660, 3565, 1950, 1600, 1570 and 1260. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.98 (3H, *t*, *J* = 7.4 Hz); 4.3 (1H, *m*); 4.5 (1H, *m*), 4.8 (1H, *ddd*, *J* = 1.6, 2.7 and 6.6 Hz), 5.3 (1H, *ddd*, *J* = 6.55, 6.57 and 12.3 Hz), 5.42 (1H, *ddd*, *J* = 5.4, 5.4 and 15.5 Hz), 5.54 (1H, *ddd*, *J* = 6.2, 7 and 15.5 Hz). MS at *m/z* (rel int.): 273, 275 [M–C<sub>3</sub>H<sub>3</sub>]<sup>+</sup>, 255, 257 [M–C<sub>3</sub>H<sub>3</sub>–H<sub>2</sub>O]<sup>+</sup>.

**Compound 6** Compound 5 (5 mg) was treated with

Ac<sub>2</sub>O–pyridine at room temp to give the diacetate. The resulting crude compound was dissolved in 1 ml of dry Et<sub>2</sub>O and a catalytic amount of PtO<sub>2</sub> was added. The mixture was stirred under H<sub>2</sub> for 2 hr, the soln filtered and the Et<sub>2</sub>O evapd to give, after chromatography on silica gel, the pure compound 6, oil,  $[\alpha]_D^{25} = -18.6^\circ$  (CHCl<sub>3</sub>, *c* 0.5). IR  $\nu_{\max} \text{ cm}^{-1}$ : 1735, 1730. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.89 (3H, *t*, *J* = 6.7 Hz), 0.92 (3H, *t*, *J* = 7.1 Hz), 2.03 (3H, *s*), 2.05 (3H, *s*), 3.73 (2H, *m*), 4.07 (1H, *ddd*, *J* = 4.3, 3.4 and 8.7 Hz), 5.15 (1H, *m*), 5.21 (1H, *m*). MS *m/z* (rel int.): 341 [M–Br] (2), 300, 302 [M–2 HOAc] (7), 257, 259 [M–2 HOAc–C<sub>3</sub>H<sub>7</sub>] (7), 221 [M–2 HOAc–Br] (100).

**Catalytic hydrogenation of graciosallene (4)** Hydrogenation of graciosallene 4 in the same manner as described above for 5 gave in 90% yield a compound which was identified as 6 by comparison of the  $[\alpha]_D$  and spectroscopical data with those of an authentic sample.

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