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METABOLITES FROM *LAURENCIA OBTUSA*

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Key Word Index—*Laurencia obtusa*; Rhodomelaceae; Rhodophyta; marine natural products; chamigrene derivatives.

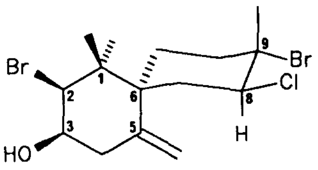
Abstract—*Laurencia obtusa*, collected in different places in the Canary Islands, afforded, in addition to known compounds, three new chamigrene derivatives. The structures were elucidated by spectroscopic methods and X-ray analysis.

INTRODUCTION

Marine algae from the genus *Laurencia* are widely distributed in the Canary Islands. These algae are a rich source of polyhalogenated sesquiterpenes with different types of skeleton. It has been repeatedly postulated that this structural variety owes its origin to particular rearrangements of common bisabolane or chamigrane precursors. However, the number of metabolites with a chamigrene skeleton isolated from *Laurencia* is smaller than that with a rearranged chamigrene skeleton [1–3]. We report here the isolation and structural elucidation of three new bromochlorinated sesquiterpenes (**2**–**4**) with a chamigrene skeleton which are biogenetically related to obtusol (**1**), previously reported [4].

RESULTS AND DISCUSSION

In connection with our continuing studies on the constituents of *Laurencia*, samples of *L. obtusa* were collected at Lanzarote and Gran Canaria (Canary Islands) in September 1984 and May 1986, respectively. Both collections contained the known compounds elatol [5], obtusol [4], isoobtusol and obtusane [6] together with other minor secondary metabolites. In the collection from Gran Canaria, these were the 2-debromo derivatives of elatol, obtusol and isoobtusol [7] and 5,12-epoxyobtusol (**2**), which has not been reported previously. On the other hand, in the collection from Lanzarote the minor secondary metabolites were the rearranged chamigrene derivatives rhodolaureol, rhodolaurediol [8], guimare-



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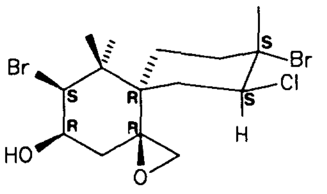

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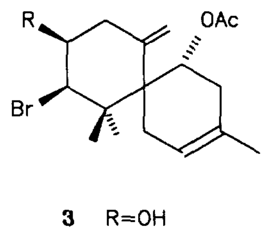

3 R=OH

4 R=OAc

diol [9] and two new chamigrane sesquiterpenes, compounds **3** and **4**. Extraction of the air-dried algae with acetone followed by chromatography on silica-gel and Sephadex LH-20 afforded the novel sesquiterpenes.

5,12-Epoxyobtusol (**2**), $[\alpha]_D^{25} = +5.9°$ ($CHCl_3$; *c* 1.69) was isolated as a crystalline solid, mp 103–105°. The high resolution mass spectrum gave a peak at *m/z* 350.0603 with a characteristic isotope pattern consistent with the presence of one chlorine and one bromine atom being analysed for $C_{15}H_{23}{}^{79}Br{}^{35}Cl{}^{16}O_2$ (calc. 350.0648). This peak arose from cleavage α to a bromine and established a molecular formula of $C_{15}H_{23}Br_2ClO_2$. The ^1H NMR spectrum showed signals for all the heteroatom-methine protons (δ4.23, 4.42 and 5.00) found in obtusol, but it lacked signals for the two H-12 vinyl protons. The presence of two resonances at δ3.10 and 2.74 in the ^1H NMR and also at δ58.39 and 50.00 in the ^{13}C NMR of this compound, suggested a disubstituted epoxy ring in the molecule. X-Ray analysis confirmed the structure as 5,12-epoxyobtusol and established the absolute configuration as 2(*S*), 3(*R*), 5(*R*), 6(*R*), 8(*S*), and 9(*S*). The compound crystallized from *n*-hexane–dichloromethane (1 : 1) in the orthorhombic system, space group $P2_12_12_1$, $a = 10.368$ (2), $b = 11.867$ (5), $c = 14.049$ (3) Å, $D_c = 1.65$ g/cm^3, $z = 4$. The intensity of 876 unique reflexions was measured using graphite-monochromated CuK_αradiation ($\lambda = 1.5418$ Å) and ω–θ scan in a four-cicle computer controlled diffractometer; 858 (98%) reflexions with $I > 3\sigma(I)$ were judged as observed after correction for Lorentz and polarization. The structure was solved by direct methods [10]. Most of the.hydrogen atoms were found on a difference synthesis map and the remainder placed in calculated positions. Full-matrix l.s. refinement, anisotropic temperature factors for bromine and chlorine, isotropic for oxygen and carbon and the fixed isotropic contribution for hydrogen atoms converged at $R = 0.064$. The absolute configuration was fixed by using 28 Bijvoet pairs with $F_o > 15\sigma(F_o)$ which are in the ranges of $0.2 < \sin\ \theta/\lambda\ < 0.5$ and $15\ < F_o < 50$. The averaged Bijvoet differences are 1.18 for the correct enantiomer as against 4.10 for the alternative one [11]. The structure of the enantiomer identified is shown in Fig. 1. Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

2-Bromo-3-hydroxy-11-acetoxy β-chamigrene (**3**) was isolated as an oil, $[\alpha]_D^{25} = +49.9°$ ($CHCl_3$; *c* 0.13). The high resolution mass spectrum of this compound showed a peak at *m/z* 316.0839 displaying a characteristic doublet indicative of the presence of a bromine atom and analysed for $C_{15}H_{23}{}^{81}Br{}^{16}O$ (calc. 316.0860). This ion originating from α-cleavage to the C=O bond, as well as the IR bands at 3520 and 1720 cm^{-1}, suggested that the compound contained an acetyl and hydroxyl groups. ^{13}C NMR supported these assignments with resonances at δ171.15 and 72.47. ^1H NMR showed the presence of three methyl groups (δ1.0, 1.03 and 1.56), three methines: α to a bromine (δ4.14), α to a hydroxyl group (δ4.60) and α to an acetoxy group (δ4.91) and three olefinic protons (δ4.74, 5.07 and 5.48). The COSY spectrum of this compound showed cross peaks correlating the signal at δ4.60 due to the protons at C-2 with H-3 at δ4.14, which was also correlated with the H-4 at δ2.52 and 2.88. Moreover, in the COSY map long-range correlations could be observed between the vinyl proton H-12 at δ4.74 and the H-4 protons. In addition to the above, the coupling constant values (Table 1) established that the cyclohexane ring had substitutions and relative stereochemistry identical to those of obtusol. The secondary acetoxy group was located in the cyclohexane ring at C-11, in accordance with the H-11 chemical shifts at δ4.91. The relative stereochemistry of this group was established as α in accordance with the coupling constant values $J_{11-10\beta} = 3$ Hz and $J_{11-10\alpha} = 10$ Hz. This stereochemistry agrees with the long-range correlations observed in the COSY map between H-12–H-11 and H-2–H-7α.

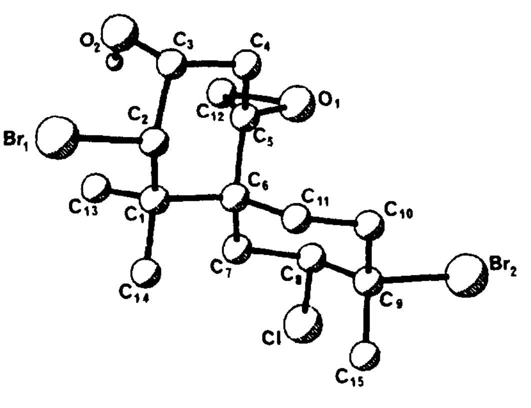

Fig. 1. PLUTO drawing of obtusol epoxide (**2**) showing the enantiomer identified in *Laurencia obtusa*.

Compound (**4**) was isolated as an oil $[\alpha]_D^{25} = +93°$ ($CHCl_3$; *c* 0.36). Its spectroscopic data (see Experimental) were closely related to those of **3** suggesting the presence of a second acetoxy group in the molecule. This was confirmed by treatment of **3** with acetic anhydride–pyridine, yielding a compound whose physical and spectroscopical properties were identical to those found for 4 establishing the structure as 2-bromo-3,11-diacetoxy β-chamigrene.

EXPERIMENTAL

Mps: uncorr. Optical rotations were obtained in $CHCl_3$ using a 10 cm cell, IR spectra in $CHCl_3$. LR and HRMS were recorded at 70 eV. ^1H and ^{13}C NMR were measured at 200 MHz in $CDCl_3$; chemical shifts are in ppm relative to TMS. The ^1H–^1H homonuclear (COSY) correlation expts were performed with a modified Bruker microprogram.

Analytical and prep. TLC were done on commerical silica gel and compounds were detected either by UV or by spraying with H_2SO_4–HOAc–H_2O soln followed by heating at 120°. Dry CC was performed on silica gel (60 G or 60, 70–230 mesh), gel filtration CC on Sephadex LH-20. Wet CC was carried out on silica gel using a peristaltic pump. All solvents were purified by standard techniques. Na_2SO_4 was used for drying solns.

Extraction and chromatographic separation. Shade-dried and ground plant material was extracted in a Soxhlet apparatus with Me_2CO. Solvent was removed *in vacuo* to give a dark-green viscous mass (38 and 47.6 g; 2.5 and 2.3% of dry wt, respectively) which was chromatographed on silica gel (6 × 100 cm). The column was eluted with increasing concns of EtOAc in hexane. Frs of 250 ml were collected and those exhibiting similar TLC profiles were combined to give different frs in order of increasing polarity.

Isolation of obtusol epoxide (**2**). Fraction D of *L. obtusa* collected at Gran Canaria (5.6 g, 0.37% dry wt of alga) was subjected to chromatography on Sephadex LH-20 with

Table 1. ^{13}C and ^{1}H NMR for compound 3*†

C	^{13}C	^{1}H	Multiplicity	Coupling constant (Hz)
1	43.46 *s*	—		
2	71.02 *d*	4.60	*d*	3
3	72.47 *d*	4.14	*ddd*	3, 6, 2.5
4	38.21 *t*	2.88	*dd*	6, 14.5
		2.55	*dd*	2.5, 14.5
5	141.10 *s*	—		
6	48.75 *s*	—		
7	30.36 *t*	2.21		
		2.15		
8	124.33 *d*	5.48	*br s*	
9	132.00 *s*	—		
10	32.26 *t*	2.15	*dd*	10, 13
		1.58	*dd*	13, 3
11	72.47 *d*	4.91	*dd*	3, 10
12	115.95 *t*	4.74	*s*	
		5.07	*s*	
13	24.45 *q*	1.00	*s*	
14	20.42 *q*	1.04	*s*	
15	21.39 *q*	1.58	*d*	1
16	171.15 *s*	—		
17	18.52 *q*	2.04	*s*	

*The spectra were recorded at 200 MHz in $CDCl_3$ solution.

Chemical shifts are reported in ppm relative to TMS.

†COSY 2D NMR data support the proton assignments.

$CHCl_3$–MeOH (1:1) to give pure **2** (11 mg, 0.0007% dry wt of alga); colourless needles, mp 103–105°; $[\alpha]_D^{25} = +5.9°$ ($CHCl_3$, *c* 1.69); IR ν_{max} cm^{-1}: 3450, 3060, 2970, 1450, 1370, 1230, 1090, 845; ^{1}H NMR (200 MHz, $CDCl_3$): δ1.09 (3H, *s*, H_3-13), 1.20 (3H, *s*, H_3-14), 1.82 (3H, *s*, H_3-15), 2.74 (1H, *d*, $J = 4$ Hz, H-12), 3.10 (1H, *dd*, $J = 2.4$ Hz, H-12), 4.23 (1H, *d*, $J = 3$ Hz, H-3), 4.42 (1H, *d*, $J = 3$ Hz, H-2) and 5.0 (1H, *dd*, $J = 5$, 12 Hz, H-8); ^{13}C NMR (90 MHz, $CDCl_3$): δ45.0 (*s*, C-1), 69.4 (*d*, C-2), 71.9 (*d*, C-3), 36.4 (*t*, C-4), 58.4 (*s*, C-5), 46.2 (*s*, C-6), 40.0 (*t*, C-7), 67.7 (*d*, C-8), 67.2 (*s*, C-9), 37.3 (*t*, C-10), 25.2 (*t*, C-11), 50.0 (*t*, C-12), 24.12 (*q*, C-13), 20.51 (*q*, C-14) and 24.97 (*q*, C-15); LREIMS 70 eV, *m/z* (rel. int.): 335 (1), 333 (3), 331 (2.7) $[M-Br-H_2O]^+$, 317 (2), 315 (7), 313 (5) $[M-Br-2H_2O]^+$, 269 (7.5), 267 (22.5), 265 (16), 253 (16), 251 (43), 233 (20), 223 (18), 221 (22), 215 (24), 203 (14), 197 (25), 195 (17), 193 (21), 169 (43), 107 (22), 55 (72), 53 (48), 48 (37), 41 (100), 39 (55); HREIMS *m/z*: 350.0603 (Calc. for $C_{15}H_{23}O_2{}^{79}Br^{35}Cl$ 350.0560) $[M-Br]^+$, 333.0527 (Calc. for $C_{15}H_{23}O^{79}Br^{35}Cl$ 333.0621) $[M-O-Br]^+$, 251.1123 (Calc. for $C_{15}H_{20}O^{35}Cl$ 251.1203) $[M-H_2O-Br-BrH]^+$.

Isolation of compounds **3** and **4**. Fraction E from *L. obtusa* collected at Lanzarote was chromatographed on Sephadex LH-20 using *n*-hexane–$CHCl_3$–MeOH (2:1:1). The last frs were combined and subjected to PLC (silica gel-60, *n*-hexane–EtOAc, 3:2) to give a mixt. containing two main components (122 mg, 0.005% dry wt of alga). Prep. TLC using *n*-hexane–EtOAc (9:1, 4 developments) yielded compounds **3** (17.2 mg) and **4** (32.7 mg).

2-*Bromo*-3-*hydroxy*-11-*acetoxy β-chamigrene* (**3**). Oil, $[\alpha]_D^{25} = +49°$ ($CHCl_3$; *c* 0.13); IR ν_{max} cm^{-1}: 3520, 3000, 2985, 1720, 1650, 1445, 1430, 1365, 1245, 1200, 1075, 1030, 1020, 950, 900, 885, 805; LREIMS 70 eV, *m/z* (rel. int.): 316, 314 (7:7) $[M-CH_2CO]^+$, 298, 296 (2:2) $[M-HOAc]^+$, 238 (8) $[M-MeCO-Br]^+$, 216 (7) $[M-MeCO-Br-H_2O]^+$, 173 (7), 161 (6), 135 (16), 132 (11), 119 (14), 117 (13), 107 (11), 105 (24), 91 (22), 85 (11), 77 (10), 55 (16), 43 (45), 41 (23), 32 (24), 28 (100); HREIMS *m/z*: 316.0839 (Calc. for $C_{15}H_{23}O_2{}^{81}Br$ 316.0862) $[M-CH_2CO]^+$, 298.0749 (Calc. for $C_{15}H_{21}O^{81}Br$ 298.0756) $[M-HOAc]^+$, 234.1591 (Calc. for $C_{15}H_{22}O_2$ 234.1620) $[M-HOAc-Br]^+$, 216.1461 Calc. for $C_{15}H_{20}O$ 216.1514) $[M-HOAc-Br-H_2O]^+$; ^{1}H and ^{13}C NMR see Table 1.

2-*Bromo*-3,11-*diacetoxy β-chamigrene* (**4**). Oil, $[\alpha]_D^{25} = +93°$ ($CHCl_3$; *c* 0.36); IR ν_{max} cm^{-1}: 2990, 1710, 1360, 1240, 1025; LREIMS 70 eV, *m/z* (rel. int.): 358, 356 (13:13) $[M-CH_2CO]^+$, 340, 338 (0.3:0.3) $[M-CH_2CO-H_2O]^+$, 298, 296 (0.3:0.3) $[M-CH_2CO-HOAc]^+$, 277 (2) $[M-CH_2CO-Br]^+$, 217 (27), 199 (62), 183 (8), 171 (42), 157 (35), 145 (23), 135 (10), 119 (35), 105 (45), 93 (13), 91 (30), 85 (19), 77 (12), 69 (8), 55 (15), 43 (100), 41 (17); HREIMS *m/z*: 358.0971 (Calc. for $C_{17}H_{25}O_3{}^{81}Br$ 358.0966) $[M-CH_2CO]^+$, 259.1702 (Calc. for $C_{17}H_{23}O_2$ 259.1698) $[M-HOAc-Br]^+$, 199.1408 (Calc. for $C_{15}H_{19}$ 199.1487) $[M-2HOAc-Br]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ1.03 (6H, *s*, H-13 and H-14), 1.59 (3H, *s*, H-15), 1.95 (3H, *s*, H-19), 2.03 (3H, *s*, H-17), 4.47 (1H, *d*, $J = 3$ Hz, H-2), 4.73 (1H, *s*, H-12), 4.94 (1H, *s*, H-12), 5.16 (1H, *dd*, $J = 3$, 7 Hz, H-3), 5.26 (1H, *dd*, $J = 3$, 7 Hz, H-11), 5.64 (1H, *br s*, H-8); ^{13}C NMR (90 MHz, $CDCl_3$): δ42.56 (*s*, C-1), 67.86 (*d*, C-2), 73.85 (*d*, C-3), 36.47 (*t*, C-4), 145.62 (*s*, C-5), 44.87 (*s*, C-6), 30.29 (*t*, C-7), 123.78 (*d*, C-8), 132.36 (*s*, *C*-9), 32.93 (*t*, C-10), 72.03 (*d*, C-11), 115.14 (*t*, C-12), 25.37 (*q*, C-13), 24.13 (*q*, C-14), 24.13 (*q*, C-15), 170.90 (*s*, C-16), 21.39 (*q*, C-17), 171.44 (*s*, C-18), 18.52 (*q*, C-19).

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