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TWO NEW CHAMIGRENE SESQUITERPENOIDS FROM THE TROPICAL RED ALGA *LAURENCIA OBTUSA*

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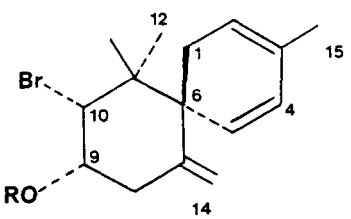
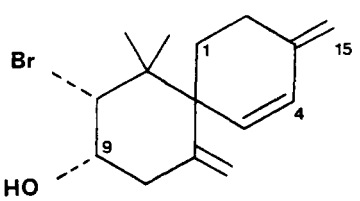
ABSTRACT.—Two new brominated sesquiterpenes of the chamigrene type, obtusadiene [**1**] and isoobtusadiene [**2**], have been isolated from the red alga *Laurencia obtusa*. Relative stereochemistry of **1** was deduced from a combination of coupling constant analyses and nOeds, while absolute stereochemistry was determined by the exciton chirality method on the benzoate derivative **3**.

Marine algae of the genus *Laurencia* occur worldwide in diverse marine habitats and are an abundant source of novel natural products (1-3). In the course of our survey work on the biomedicinal potential of Caribbean seaweeds,[1] we initiated a study on the chemistry of the red alga *Laurencia obtusa* (Hudson) Lamouroux. The crude lipid extracts of our Caribbean collections of *L. obtusa* have yielded two new brominated sesquiterpenes of the chamigrene type in addition to several known halogenated sesquiterpenes. We report here the structures of the two new algal metabolites **1** and **2**, which were established from analyses of their respective ^{1}H- and ^{13}C-nmr spectra and by comparison to known natural products. Relative stereochemistry of **1** was deduced from a combination of coupling constant analyses and nuclear Overhauser enhancement difference spectroscopy (nOeds) experiments, while absolute stereochemistry of **1** was determined by the exciton chirality method on the benzoate derivative **3** (4). We propose the trivial names obtusadiene and isoobtusadiene for compounds **1** and **2**, respectively.





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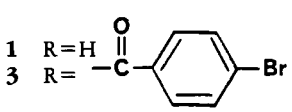
1 R=H
3 R= 

A collection of *L. obtusa* from northeastern Puerto Rico was preserved in iPrOH and later extracted for its lipids according to standard methodology. This extract was repeatedly chromatographed over Si gel to yield obtusadiene [**1**], isoobtusadiene [**2**], and the known compounds (+)-elatol (5) and obtusol (6).

The major new compound **1** was a colorless and optically active oil with a molecular formula by hreims of $C_{15}H_{21}OBr$ and, thus, contained 5 degrees of unsaturation. The ir spectrum of **1** showed absorptions for a hydroxyl functionality and olefinic bonds, two of which were in conjugation within a ring (uv λ max=262 nm). By ^{13}C-nmr spectros-

[1]W.H. Gerwick, work in progress.

copy obtusadiene had a total of six olefinic carbons and was, thus, bicarbocyclic (Table 1).

There were two isolated spin systems in the ^{1}H-nmr spectrum of obtusadiene [**1**]. One was nearly identical, including coupling constants, to the array found in the A ring of elatol and several other well-characterized β-chamigrenes of marine origin (1,7) and

TABLE 1. ^{13}C-nmr Data of the New Brominated Sesquiterpenes Obtusadiene [**1**] and Isoobtusadiene [**2**]

Carbon No.	Compound	
	1[a,b]	**2**[b,c]
C-1	30.2 t	26.5
C-2	119.7 d	26.8[h]
C-3	129.7 s	143.7[i]
C-4	129.1 d[e]	131.5[j]
C-5	129.6 d[e]	132.7[j]
C-6	48.8 s	51.5
C-7	146.4 s	142.3[i]
C-8	38.2 t	37.9
C-9	71.9 d[f]	72.1
C-10	71.5 d[f]	70.6
C-11	43.5 s	42.8
C-12	21.3 q[g]	21.5
C-13	26.8 q	26.5[h]
C-14	113.3 t	117.6[k]
C-15	20.7 q[g]	111.3[k]

[a]Obtained in C_6D_6 at 125 MHz. Multiplicity assignments obtained from a ^{13}C off-resonance decoupling experiment.

[b]δ in ppm from TMS.

[c]Obtained in $CDCl_3$ at 22.5 MHz.

[d]Assignments made on the basis of comparison to model compounds (10).

[e-k]Assignments may be reversed.

accounted for two degrees of unsaturation. The remaining spin system contained a homoannular diene and showed extensive long-range coupling (Table 2), a detailed analysis of which allowed formulation of the B ring.

The relative stereochemistry at the spiro juncture, C-6, was investigated by nOeds in which enhancements of the exocyclic methylene proton band (H_2-14) and the lower field methyl protons (H_3-12) were observed upon irradiation of the olefinic protons at δ 5.85 (H-4 and H-5, Figure 1). The absolute configuration was determined by the chiral exciton coupling method using the *p*-bromobenzoate derivative [**3**] (4). The positive benzoate Cotton effect at 244 nm indicated a right-handed chirality between the C-7–14 olefin and benzoate chromophores, thus predicting the 6*R*, 9*S*, 10*R* enantiomer. Furthermore, prediction of a left-hand skewness in the *cisoid* diene by the empirical diene helicity rules (8) was confirmed by a negative Cotton effect at λ max 265 nm in the cd spectrum of obtusadiene [**1**]. The absolute configurations at C-9 and C-10 for obtusadiene [**1**] are identical to those found in (+)-elatol, which suggests that these chamigrenes may arise via analogous biosynthetic steps (7,9).

Isoobtusadiene [**2**] was also a colorless and optically active oil that possessed an identical molecular formula and similar spectral features to obtusadiene [**1**]. Metabolite **2** displayed uv absorptions consistent with a heteroannular diene, a conclusion further supported by analysis of the ^{1}H-nmr spectrum (Table 2). An additional exocyclic methylene replaced the vinyl methyl group (C-15) and the olefin protons at H-4 and H-

TABLE 2. ^{1}H-nmr Data of the Brominated Sesquiterpenes Obtusadiene [**1**] and Isoobtusadiene [**2**][a]

Hydrogen Atom	Compound		
	1 ($CDCl_3$)[b,c]	**1** (C_6D_6)[c,d,e]	**2** ($CDCl_3$)[c,f]
H-1a	2.05-2.62, m	1.87, ddq	1.98-2.64, m
H-1b	2.05-2.62, m	2.13, m	1.98-2.64, m
H-2	5.32, ddddq	5.13, m	1.98-2.64, m
H-4	5.87, d	5.73, bs	5.84, bd
H-5	5.87, d	5.73, bs	6.27, d
H-8a	2.05-2.62, m	2.37, dd	1.98-2.64, m
H-8b	2.05-2.62, m	2.13, m	1.98-2.64, m
H-9	4.08, m	3.75, bddd	4.15, m
H-10	4.62, d	4.17, d	4.65, d
H-12	1.15, s	1.19, s	1.23, s
H-13	1.05, s	0.98, s	1.02, s
H-14a	4.93, m	4.93, bd	4.80, s[g]
H-14b	4.93, m	4.87, bd	4.80, s[g]
H-15	1.70, ddd	1.56, ddd	5.12, bs[g] 4.86, bs[g]

[a]δ in ppm from TMS.

[b]Obtained at 80 MHz.

[c]*J*(Hz)-**1** ($CDCl_3$): 2,1a=3; 2,1b=3; 2,15=1.5; 2,4=1.5; 2,5=1.5; 9,10=3.1; 15,1a or 1b=1.5; 15,4 or 5=1.5; **1** (C_6D_6): 1a,1b=17.8; 1a,2=4.5; 1a,15=1.8; 15,1b=1.8; 15,2=1.8; 15,4 or 5=1.8; 8a,8b=14.6; 9,8b=3.4; 10,9=3.0; 14a,14b=1.5; **2**: 4,5=10.2; 9,10=3.1.

[d]Obtained at 500 MHz.

[e]Assignments were made by aid of spin decoupling experiments.

[f]Obtained at 90 MHz.

[g]Assignments may be reversed.

5 formed an AX spin system. The methylenes at C-1 and C-2 were coupled and formed part of a more complex 6-proton multiplet in the δ 1.9-2.7 region. Neither the relative stereochemistry at the spiro center nor the absolute stereochemistry was determined for isoobtusadiene [**2**].

Natural products **1** and **2** were devoid of significant antimicrobial (Gram-positive, Gram-negative, yeast) or cytotoxic activities (KB).

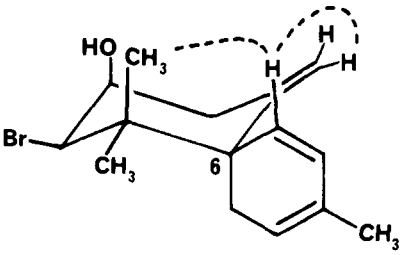


FIGURE 1. Perspective representation of obtusadiene [**1**] with key nOe interactions that defined the relative stereochemistry at C-6

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on a Perkin-Elmer 143 polarimeter, uv spectra on a Beckman DB-GT grating spectrophotometer, and ir spectra with a Perkin-Elmer 283 spectrophotometer. Nmr spectra were recorded on Varian FT-80A, JEOL FX90Q, and Bruker

HX500 spectrometers and nOeds was performed on a Bruker AM-400 spectrometer. Low resolution mass spectra were obtained on a Hewlett Packard 5995A mass spectrometer, and high resolution mass spectra were recorded on a Kratos MS 50 mass spectrometer. Cd spectra were recorded on a Jasco J-41A spectropolarimeter. All solvents were distilled from glass prior to use.

COLLECTION, EXTRACTION, AND ISOLATION.—*L. obtusa* was collected at a depth of 0.1-1 m at Isleta Marina on the east coast of Puerto Rico in May 1984. The alga was preserved by storage in iPrOH, and a taxonomic voucher is deposited in the herbarium of the Department of Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico.

The preserved alga (dry weight, 1.01 kg) was homogenized and extracted three times with $CHCl_3$-MeOH (2:1, v/v) to yield, after in vacuo removal of extraction solvents and H_2O, 28.5 g of a dark green tar (2.8% of dry weight). The crude extract was chromatographed on a gravity-driven Si gel column employing a gradient of isooctane/EtOAc/MeOH, and fractions eluting with 5% EtOAc/isooctane contained metabolites **1** and **2**. Fractions eluting with 5-20% EtOAc/isooctane contained the known metabolites (+)-elatol which showed $[\alpha]^{25}D+53.1°$ ($c=1.19$, $CHCl_3$) and obtusol. Metabolites **1** and **2** were further purified using semipreparative normal phase hplc (Alltech RSIL Silica, 50 cm×10 mm, 10% EtOAc/isooctane), followed by analytical normal phase hplc (μPorasil, 2×25 cm×3.9 mm, 1.0% EtOAc/isooctane), and yielded pure **1** (410 mg, 1.44% of extract) and **2** (32.6 mg, 0.11% of extract) as colorless oils. Other collections of *L. obtusa* from the east coast of the Island of Tobago in the Lesser Antilles in December 1982, and from La Parguera on the southwestern coast of Puerto Rico in February 1983, were also found to contain metabolites **1** and **2**.

OBTUSADIENE [**1**].—The colorless oil showed $[\alpha]^{25}D-52.0°$ ($c=2.8$, $CHCl_3$); uv λ max (MeOH) 262 nm (log $\epsilon=3.5$); cd (MeOH) $\epsilon=-13.5$ l mol^{-1} cm^{-1} (λ max 265 nm); ir (CCl_4) 3570, 3460, 3090, 3030, 2940, 1641, 1470, 1445, 1373, 1208, 1055, 902, 867, 610 cm^{-1}; lreims (70 eV) *m/z* 298 (1.0), 296 (1.0), 217 (1.0), 216 (1.0), 199 (10.4), 161 (6.6), 157 (12.5), 133 (46.7), 132 (48.9), 119 (22.7), 117 (32.6), 115 (25.8), 105 (82.3), 91 (46.4), 85 (100.0), 65 (19.7), 55 (37.0); hreims *m/z* 298.0753 (M^++2, $C_{15}H_{21}O^{81}Br$, −0.3 mamu dev., 7.1%), 296.0778 (M^+, $C_{15}H_{21}O^{79}Br$, 0.3 mamu dev., 7.5%), 217.1599 ($C_{15}H_{21}O$, 0.6 mamu dev., 5.6%), 199.1495 ($C_{15}H_{19}$, 0.8 mamu dev., 32.1%), 161.0970 ($C_{11}H_{13}O$, 0.3 mamu dev., 14.3%), 157.1025 ($C_{12}H_{13}$, 0.8 mamu dev., 20.6%), 133.1020 ($C_{10}H_{13}$, 0.3 mamu dev., 66.6%), 119.0855 (C_9H_{11}, −0.6 mamu dev., 30.6%), 105.0705 (C_8H_9, 0.0 mamu dev., 100.0%).

p-BROMOBENZOATE DERIVATIVE OF **1** [**3**].—A solution of compound **1** (47 mg) in CH_2Cl_2 was treated with excess *p*-bromobenzoyl chloride in pyridine. The crude reaction mixture, after refluxing for 3 days, was worked up as usual and the *p*-bromobenzoate derivative of **1** isolated by preparative tlc (100% C_6H_6) and normal phase hplc (μPorasil, 2% EtOAc/isooctane) to yield 13.4 mg of **3** (26.2% yield) as a colorless oil: uv λ max (MeOH) 244 nm (log $\epsilon=4.5$); cd (MeOH) $\epsilon=+7.6$, 0.0, −16.5 l mol^{-1} cm^{-1} (λ max 244, 250, 267 nm); ^{1}H nmr ($CDCl_3$) δ 7.90 (2H, d, $J=8.8$ Hz), 7.58 (2H, d, $J=8.8$ Hz), 5.91 (2H, m), 5.47 (1H, ddd, $J=3.6$, 3.6, 3.6), 5.37 (1H, m), 4.96 (1H, bs), 4.86 (1H, bs), 4.66 (1H, d, $J=3.6$), 2.90-1.85 (4H, m), 1.71 (3H, ddd, $J=1.8$, 1.8, 1.8), 1.26 (3H, s), 1.13 (3H, s).

ISOOBTUSADIENE [**2**].—Pure **2** showed $[\alpha]^{25}D-11.7°$ ($c=0.7$, $CHCl_3$); uv λ max (MeOH) 234 nm (log $\epsilon=4.1$); ir (CCl_4) 3570, 3080, 3030, 2980, 1640, 1480, 1432, 1375, 1205, 1080, 1030, 910, 890, 605 cm^{-1}; lreims (70 eV) *m/z* 298 (7.1), 296 (5.9), 217 (7.7), 216 (5.1), 199 (12.8), 161 (16.0), 143 (20.9), 133 (25.8), 117 (53.0), 115 (45.5), 105 (37.3), 91 (100.0), 85 (24.9), 69 (51.6), 65 (31.3), 55 (52.9); hreims *m/z* 298.0754 (M^++2, $C_{15}H_{21}O^{81}Br$, −0.1 mamu dev., 6.9%), 296.0733 (M^+, $C_{15}H_{21}O^{79}Br$, −0.3 mamu dev., 9.1%), 217.1601 ($C_{15}H_{20}O$, 1.2 mamu dev., 14.9%), 199.1487 ($C_{15}H_{19}$, −0.1 mamu dev., 47.4%), 161.0953 ($C_{11}H_{13}O$, −1.3 mamu dev., 48.8%), 143.0859 ($C_{11}H_{11}$, −1.5 mamu dev., 34.2%), 133.1016 ($C_{10}H_{13}$, −0.2 mamu dev., 31.2%), 117.0703 (C_9H_9, −0.1 mamu dev., 66.4%), 115.0549 (C_9H_7, 0.1 mamu dev., 38.1%), 105.0693 (C_8H_9, −1.1 mamu dev., 53.1%), 91.0548 (C_7H_7, 0.0 mamu dev., 100.0%), 69.0575 (C_5H_9, 0.1 mamu dev., 65.1%), 65.0395 (C_5H_5, 0.3 mamu dev., 25.2%).

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