Journal of Natural Products
Vol. 51, No. 6, pp. 1257-1260, Nov-Dec 1988

PUERTITOLS: NOVEL SESQUITERPENES FROM *LAURENCIA OBTUSA*. STRUCTURE ELUCIDATION AND ABSOLUTE CONFIGURATION AND CONFORMATION BASED ON CIRCULAR DICHROISM

JESUS T. VAZQUEZ, MAYLAND CHANG, KOJI NAKANISHI,

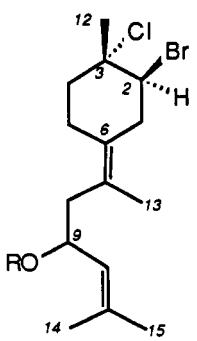
Department of Chemistry, Columbia University, New York, New York 10027

JULIO D. MARTIN,* VICTOR S. MARTIN, and RICARDO PEREZ

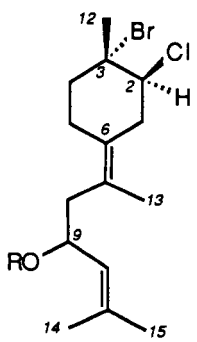
Centro de Productos Naturales Orgánicos Antonio González, Universidad de La Laguna, CSIC, Carretera de La Esperanza 2, 38206 La Laguna, Tenerife, Spain

Red algae of the genus *Laurencia* (Rhodomelaceae) are ubiquitous in the Canary Islands. A variety of compounds have been isolated from algae of this genus (1,2). One characteristic common to some of these marine metabolites is the presence of a C_{15} skeleton and one or more halogen atoms. We wish to describe the isolation and structure elucidation of two new regular sesquiterpenes, puertitols A [**1**] and B [**2**], from *Laurencia obtusa* (Huds.) Lamoroux, and the determination of the absolute configuration and conformation of the latter by application of the circular dichroic (cd) allylic benzoate method.

Chromatographic separation of the Et_2O extract of the alga afforded a mixture of compounds **1** and **2**. Because of the difficulty associated with the purification, these compounds were isolated as the corresponding acetates **3** and **4** in approximately a 2:1 ratio. Subsequent hydrolysis with K_2CO_3/MeOH gave alcohols **1** and **2** as oils.



1 R=H
3 R=Ac



2 R=H
4 R=Ac
5 R=*p*-BrBz

Eims of compounds **1** and **2** showed a molecular ion at *m/z* 334 ($C_{15}H_{24}BrClO$) with the characteristic isotope pattern consistent with one bromine and one chlorine atom. High resolution eims of the *p*-bromobenzoate derivative **5** confirmed this assignment with an ion at *m/z* 521.9923 (calcd 521.9996). Allylic cleavage resulted in ions at *m/z* 250, 252, and 254 corresponding to a dimethylmethylenecyclohexane ring containing methyl, bromo, and chloro groups. Cleavage of the C-C bond α to the oxygen atom gave a prominent peak at *m/z* 85 ($C_5H_9O^+$, base peak). The presence of a hydroxyl group in both compounds was evident from the broad ir band at 3400 cm^{-1}. There was an additional double bond (1670 cm^{-1}) which was trisubstituted as indicated by the presence of a single vinylic proton (δ 5.2, br d, H-10). ^1H nmr established the alcohol at the allylic position (δ 4.50, m, H-9). Table 1 shows the detailed ^1H-nmr assignments.

The relative configuration of the side chain for compounds **1** and **2** was determined by nOe difference experiments, which established the Me at C-7 in close spatial arrangement to H-1_{eq} (δ 2.8). These data, together with the COSY map which showed cross peaks correlating H-1 to H-2 (δ 4.4), fixed the position of the halogen at C-2 rather than at C-4. The triplet multiplicity and coupling constant $J_{2\text{-}1} = 3.5$ Hz of the resonance at δ 4.4 were consistent with H-2 in the equatorial position in acetates **3** and **4** and established the bromine as axial. The steric bulk of the chloro moi-

TABLE 1. ^{1}H-nmr Data for Compounds **1–5**.

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1_{eq}	2.80 (dd, J = 3.7, 14.8)	2.82 (dd, J = 3.8, 14.8)	2.85 (dd, J = 3.5, 14.8)	2.78 (dd, J = 3.4, 14.7)	2.76 (dd, J = 3.8, 14.7)
H-1_{ax}	3.00 (br d, J = 14.8)	3.05 (br d, J = 14.8)	2.98 (br d, J = 14.8)	2.90 (br d, J = 14.7)	2.93 (br d, J = 14.7)
H-2	4.40 (m)	4.45 (m)	4.41 (t, J = 3.5)	4.45 (t, J = 3.4)	4.38 (t, J = 3.8)
H-9	4.50 (m)	4.50 (m)	5.62 (dt, J = 6.8, 9.5)	5.60 (dt, J = 6.5, 9.5)	5.82 (dt, J = 7.2, 9.4)
H-10	5.22 (br d, J = 9)	5.20 (br d, J = 9)	5.12 (d, J = 9.5)	5.12 (d, J = 9.5)	5.19 (d, J = 9.4)
H-12	1.71 (s)	1.91 (s)	1.70 (s)	1.90 (s)	1.87 (s)
H-13	1.73 (s)	1.74 (s)	1.70 (s)	1.71 (s)	1.72 (s)
H-14	1.73 (s)	1.67 (s)	1.70 (s)	1.71 (s)	1.74 (s)
H-15	1.73 (s)	1.72 (s)	1.70 (s)	1.71 (s)	1.74 (s)
CH_3-COO			2.00 (s)	2.00 (s)	
3,5-BrBz					7.54 (d, J = 8.5)
2,6-BrBz					7.85 (d, J = 8.5)

ety at C-3 indicated a *trans*-diaxial halide conformation for both compounds. This finds precedent in numerous bromochlorinated marine sesquiterpenes whose structures have been firmly established by X-ray crystallography (2–5). Finally, natural abundance ^{13}C-nmr spectroscopy (6,7) showed C-3 of compound **1** to be 28% more intense than other quaternary carbons, thereby establishing the location of the chlorine atom at C-3. This follows from the negligible contribution of the C-Cl scalar interactions to the spin-lattice relaxation times of chlorinated carbons which lead to enhanced values of nOe (6). On the other hand, brominated carbons are influenced by C-Br scalar interactions which shorten the ^{13}C-spin lattice relaxation times with concomitant reduction of nOe values (6). A similar experiment with compound **2** established the bromine group at C-3. ^{13}C-nmr assignments are given in Table 2. It is worth noting that compounds **1** and **2** are isomers differing only in the position of the halogens. With few exceptions (2,4), in the majority of *Laurencia* metabolites chlorine is at the tertiary position whereas bromine is located at a methine.

Once the relative configurations were established, the cd allylic benzoate method (8–11), an extension of the exciton chirality method, was applied to compound **2** to determine its absolute configuration. Benzoylation of puertitol B with 4-bromobenzoyl chloride in pyridine and subsequent hplc purification afforded the *p*-bromobenzoate derivative **5**. This compound showed a positive Cotton effect at 238 nm ($\Delta\epsilon = +16.1$) and established an *S* configuration at C-9 (9). This absolute value for $\Delta\epsilon$ is rather large compared to a simple acyclic allylic benzoate (9) and can be attributed to the combined additive effect (12,13) of the homoallylic 6-ene/9-benzoate and the allylic 10-ene/9-benzoate systems (14). In addition, cd spectroscopy established the conformation of

TABLE 2. ^{13}C-nmr Data for Compounds **1**, **2**, and **4**.

Carbon	Compound		
	1	**2**	**4**
1	35.6	35.5	35.5
2	62.0	67.8	67.7
3	72.3	70.1	70.3
4	37.5	38.5	38.4
5	25.8	27.1	27.1
6	129.4	127.9	127.8
7	126.9	126.9	126.1
8	42.5	42.4	39.6
9	66.4	67.5	70.5
10	127.3	127.7	123.5
11	135.1	135.0	137.2
12	31.8	31.6	31.7
13	18.3	19.0	19.1
14	18.3	18.2	18.4
15	25.8	25.9	25.9
CH_3COO			21.4
CH_3COO			170.2

compound **5** as depicted in Figure 1. It should be noted that the ^{1}H-nmr spectrum of compound **5** was recorded in $CDCl_3$, while its cd spectrum was taken in MeCN. Although it is conceivable that different conformations may be preferred in the two solvents, the nmr spectrum obtained in CD_3CN showed no appreciable differences from that in $CDCl_3$. The conformation shown in Figure 1 was further confirmed by the coupling constant $J_{9\text{-}10} = 9.4$ Hz and the triplet multiplicity observed for H-9 when H-10 was irradiated.

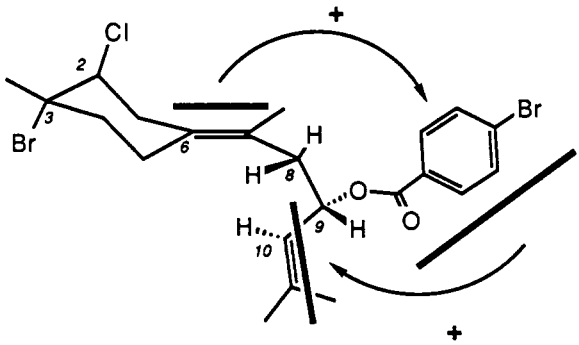


FIGURE 1. Conformation of compound **5**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All reagents were of the best grade commercially available. Solvents were either spectrophotometric grade or glass-distilled prior to use. Cc was performed on Si gel G (230–400 mesh) obtained from Merck. Thin layer plates (Si gel G) were obtained from Analtech and were visualized by spraying with HOAc-H_2O-H_2SO_4 (80:16:4) followed by heating. Preparative hplc was performed on a semi-preparative Si gel column (5 μm) attached to a Dupont pump and monitored at 254 nm with a Schoeffel SF 770 detector. Ir spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer. The uv spectrum was obtained on a Perkin-Elmer Model 320 spectrophotometer. The cd spectrum was recorded on a Jasco J-500A spectropolarimeter interfaced with a Jasco DP500N data processor and an IBM-PC. The concentration of the cd sample was ascertained from the uv spectrum using the standard ε value of 21,300 for the mono-*p*-bromobenzoate chromophore. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter. ^{1}H-nmr spectra were recorded on Bruker Models WP 200SY and WM 250 spectrometers at 200 and 250 MHz, respectively. The nOe experiments used a selective 180° irradiation of a given resonance followed after a delay by a 90° observed pulse. The spectrum was acquired simultaneously with one in which the selective pulse was further downfield. The two spectra were computer-subtracted to observe the enhancements. COSY experiments utilized the $(\pi/2\text{-}\Delta\text{-}t_1\text{-}\pi/2\text{-}\Delta\text{-}t_2)_n$ pulse sequence (15). ^{13}C-nmr spectra were obtained at 50 MHz on a Varian CFT-20 spectrometer interfaced with a Varian 620/F-100 data processor. Samples for nOe and natural abundance ^{13}C-nmr experiments were degassed by bubbling He through the solutions. Mass spectra were determined on a Ribermag R10-10 (dci-NH_3) or a VG Micromass Model ZAB-2F (ei, 70 eV, 200°) spectrometer.

COLLECTION, EXTRACTION, AND ISOLATION.—*L. obtusa* was collected by hand in the intertidal zone at Puertito de Güimar, Tenerife, Canary Islands. A voucher specimen is deposited in the Departamento de Biología Vegetal, Universidad de La Laguna. The dried alga (3 kg) was extracted in a Soxhlet apparatus with Et_2O. The extract (50 g) was subsequently chromatographed on a Si gel column eluted with EtOAc/*n*-hexane mixtures of increasing polarity. The EtOAc-*n*-hexane (10:90) fraction were combined, evaporated to dryness (6 g), and acetylated in dry pyridine and excess Ac_2O. The solution was stirred at room temperature overnight and then poured into excess H_2O. The products were extracted with Et_2O, dried over anhydrous Na_2SO_4, and purified by Si gel chromatography with EtOAc-*n*-hexane (3:97). The previously known isoobtusol acetate (16) and elatol acetate (17) were isolated as major components, together with a mixture (640 mg) of minor compounds. Successive chromatography of this mixture afforded pure compounds **3** (54 mg) and **4** (24 mg).

HYDROLYSES OF COMPOUNDS **3** AND **4**.—These compounds were separately hydrolyzed with excess K_2CO_3 in MeOH. Typically, the so-

lution was allowed to stand at room temperature for 15 min before quenching with H_2O. The alcohols were extracted with Et_2O and dried over anhydrous Na_2SO_4, and solvent was evaporated in vacuo. The residue was chromatographed [EtOAc-*n*-hexane (5:95)] to give puertitols A (45 mg) and B (18 mg), corresponding to 0.0015 and 0.0006% of the dry weight of the alga, respectively.

Puertitol A [**1**].—Oil, $[\alpha]_D$ −37° (*c* = 1.62, $CHCl_3$); ir (film) 3400, 2940, 1670, 1450, 1380, 1240, 1110, 810 cm^{-1}; eims *m/z* 334, 336, $[M]^+$ 338 (<0.1%), 250, 252, $[M-C_5H_9O]^+$ 254 (4%, allylic cleavage), 171, $[M-C_5H_9O-Br]^+$ 173 (3%), $[M-C_5H_9O-Br-Cl]^+$ 136 (12%), $[C_5H_9O]^+$ 85 (100%); ^1H nmr ($CDCl_3$) see Table 1; ^{13}C nmr ($CDCl_3$) see Table 2.

Puertitol B [**2**].—Oil, $[\alpha]_D$ +66° (*c* = 0.25, $CHCl_3$); ir (film) 3400, 2940, 1670, 1455, 1390, 1240, 1200, 1140, 840 cm^{-1}; eims *m/z* 334, 336, $[M]^+$ 338 (<1%), 250, 252, $[M-C_5H_9O]^+$ 254 (10%, allylic cleavage), 204, 206 (2%), 171, $[M-C_5H_9O-Br]^+$ 173 (2%), $[M-C_5H_9O-Br-Cl]^+$ 136 (28%), $[C_5H_9O]^+$ 85 (100%); ^1H nmr ($CDCl_3$) see Table 1; ^{13}C nmr ($CDCl_3$) see Table 2.

Puertitol A acetate [**3**].—Oil; eims *m/z* 376, 378, $[M]^+$ 380, 316, 318, $[M-HOAc]^+$ 320, 281, $[M-HOAc-Cl]^+$ 283, 237, $[M-HOAc-Br]^+$ 239; ^1H nmr ($CDCl_3$) see Table 1.

Puertitol B acetate [**4**].—Oil; eims *m/z* 376, 378, $[M]^+$ 380, 316, 318, $[M-HOAc]^+$ 320, 281, $[M-HOAc-Cl]^+$ 283, 237, $[M-HOAc-Br]^+$ 239; ^1H nmr ($CDCl_3$) see Table 1; ^{13}C nmr ($CDCl_3$) see Table 2.

BENZOYLATION OF PUERTITOL B [**2**].—To a solution of **2** (5.2 mg, 15.5 μmol) in dry pyridine (1 ml), 4-bromobenzoyl chloride (6.8 mg, 31.0 μmol) was added. After stirring at 60° overnight, the reaction mixture was quenched with a few drops of MeOH, and the solvent was removed in vacuo in the presence of toluene. The residue was chromatographed on a preparative tlc plate [R_f 0.7, EtOAc/*n*-hexane (10:90)] followed by hplc purification [silica, 1.0 cm i.d. × 25 cm, EtOAc-*n*-hexane (3:97) 1 ml/min, 254 nm) to afford 6.4 mg (12.3 μmol) of the desired compound **5**. Yield 80%; uv (MeCN) λ max 242 nm; cd (MeCN): λ ext 238 nm, $\Delta\epsilon = +16.1$; dcims (NH_3) *m/z* 534, 536, $[M+NH_4]^+$ 538 (2%), 517, 519, $[M+H]^+$ 521 (<1%), 334, 336, $[M+NH_4-BrBzOH]^+$ 338 (93%), 317, 319, $[M+H-BrBzOH]^+$ 321 (80%), $[M+H-BrBzOH-Br-Cl]^+$ 203 (100%); hreims *m/z* 521.9923 (calcd 521.9996 for $C_{22}H_{27}O_2{}^{81}Br_2{}^{37}Cl$); ^1H nmr ($CDCl_3$) see Table 1.

ACKNOWLEDGMENTS

This work was supported by the U.S.-Spanish Joint Committee for Scientific and Technological Cooperation Grant CCA85 10-079 and a National Institutes of Health Postdoctoral Fellowship GM1270-01 (MC). JTV thanks the Comunidad Autónoma de Canarias-Caja General de Ahorros de Canarias, Spain, for a postdoctoral fellowship.

LITERATURE CITED

1. A.G. González, J.M. Arteaga, J.J. Fernández, J.D. Martín, M. Norte, and J.Z. Ruano, *Tetrahedron*, **40**, 2751 (1984).
2. A.G. González, J. Darias, J.D. Martín, and C. Pérez, *Tetrahedron Lett.*, 1249 (1974).
3. K.H. Hollenbeak, F.J. Schmitz, M.B. Hossain, and D. Van der Helm, *Tetrahedron*, **35**, 541 (1979).
4. J.D. Martín and J. Darias, in: "Marine Natural Products, Chemical and Biological Perspectives." Ed. by P.J. Scheuer, Academic Press, New York, 1978, Vol. 1, pp. 125–173.
5. K. Erickson, in: "Marine Natural Products." Ed. by P.J. Scheuer, Academic Press, New York, 1983, Vol. 5, pp. 131–258.
6. R.S. Norton, *Tetrahedron*, **33**, 2577 (1977).
7. A.G. González, J.D. Martín, V.S. Martín, and M. Norte, *Tetrahedron Lett.*, 2719 (1979).
8. N. Harada, J. Iwabuchi, Y. Yokota, H. Uda, and K. Nakanishi, *J. Am. Chem. Soc.*, **103**, 5590 (1981).
9. N.C. Gonella, K. Nakanishi, V.S. Martín, and K.B. Sharpless, *J. Am. Chem. Soc.*, **104**, 3775 (1982).
10. N. Harada, Y. Yokota, J. Iwabuchi, H. Uda, and M. Ochi, *J. Chem. Soc., Chem. Commun.*, 1220 (1984).
11. N. Harada and K. Nakanishi, "Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry," University Science Books, Mill Valley, California, 1983.
12. W.T. Wiesler, J.T. Vázquez, and K. Nakanishi, *J. Am. Chem. Soc.*, **109**, 5586 (1987).
13. R. Takeda, A. Zask, K. Nakanishi, and M.H. Park, *J. Am. Chem. Soc.*, **109**, 914 (1987).
14. Y. Naya, K. Yoshihara, T. Iwashita, H. Komura, and K. Nakanishi, *J. Am. Chem. Soc.*, **103**, 7009 (1981).
15. A. Bax, "Two Dimensional NMR in Liquids," Delft University Press, Delft, Holland, 1982.
16. A.G. González, J.D. Martín, V.S. Martín, M. Martínez-Ripoll, and J. Fayos, *Tetrahedron Lett.*, 2717 (1979).
17. J.J. Sims, G.H.Y. Lin, and R.M. Wing, *Tetrahedron Lett.*, 3487 (1974).

Received 1 April 1988