

Cembrane Diterpenes from the Gorgonian *Leptogorgia laxa*

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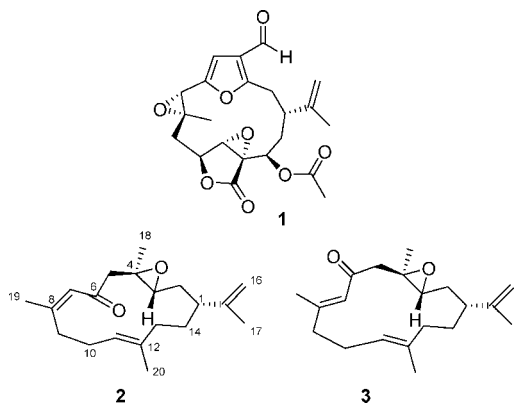
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The new cembrane diterpenes leptodienone A (**2**) and leptodienone B (**3**) and the known compounds lopholide, lophodiol B, lophodione, and lophotoxin (**1**) have been isolated from the gorgonian *Leptogorgia laxa* collected in the Gulf of California. The structures of the new metabolites have been established by spectroscopic techniques. The *in vitro* cytotoxicity of the new compounds has been tested against three human tumor cell lines.

Cembrane-type diterpenoids are a large and structurally varied group of natural products isolated from both terrestrial and marine organisms. In the marine environment, coelenterates of the orders Alcyonacea and Gorgonacea are recognized as the most prominent source of cembranoids, which usually exhibit cyclic ether, lactone, or furane moieties around the cembrane framework.¹ Moreover, diterpenoids of the cembrane family have been shown to play an ecological role in the chemical defense of corals.² From a biomedical perspective, cytotoxicity is the most remarkable property of this class of diterpenoids.^{3,4}

In our continuing research on bioactive metabolites from marine organisms we have studied specimens of the gorgonian *Leptogorgia laxa* (Hickson, 1928) collected in the Gulf of California (Mexico). Previous chemical research on gorgonians of the genus *Leptogorgia* has resulted in the isolation of different classes of secondary metabolites. Caribbean and eastern Pacific *Leptogorgia* species have yielded highly functionalized cembrane diterpenoids such as lophotoxin (**1**) and related cembranolides,⁵ whereas the Mediterranean *L. sarmentosa* contains polyoxygenated steroids⁶ and the South African *L. gilchristi* has recently been shown to contain prenylated alkaloids.⁷ The natural product content herein reported for Pacific specimens of *L. laxa* comprises four furanocembranolid-related compounds together with the new metabolites leptodienones A (**2**) and B (**3**), which represent the first account of simple cembranes in gorgonians of the genus *Leptogorgia*.



Freeze-dried specimens of *L. laxa* were extracted with acetone/MeOH (1:1), and the resulting residue was partitioned between H₂O and Et₂O. The organic extract was subjected to column chroma-

tography eluting with hexanes/Et₂O mixtures of increasing polarity, then CHCl₃/MeOH mixtures, and finally MeOH. Repeated HPLC separations of selected fractions afforded the new diterpenes leptodienone A (**2**) and leptodienone B (**3**), along with the known compounds lopholide,^{5b,8} lophodiol B,^{4c} lophodione,⁹ and lophotoxin (**1**).^{5b,10}

Leptodienone A (**2**) had the molecular formula C₂₀H₃₀O₂, as derived from HRCIMS data. The ¹H NMR spectrum (Table 1) exhibited four methyl groups at 1.89 (d, *J* = 1.2 Hz, Me-19), 1.65 (br s, Me-17), 1.57 (br s, Me-20), and 1.44 (s, Me-18) that suggested a diterpenoid structure. The presence of a β -substituted- α,β -unsaturated ketone was evident from the NMR resonances at δ_C 197.7 (C), δ_C 126.2 (CH)/ δ_H 6.12 (s), and δ_C 160.7 (C). The NMR spectra also included the signals of a trisubstituted double bond [δ_C 135.6 (C) and δ_C 121.7 (CH)/ δ_H 5.01] and those of a *gem*-disubstituted double bond [δ_C 148.1 (C) and δ_C 110.9 (CH₂)/ δ_H 4.73 and 4.68]. In addition, two resonances in the ¹³C NMR spectrum at δ_C 58.8 (C) and 60.6 (CH), the latter one coupled in the HSQC spectrum with the proton at δ_H 2.92, were assigned to a trisubstituted oxirane ring. All of these functional groups accounted for five degrees of unsaturation, and therefore, compound **2** had to contain an additional ring. COSY and HMBC correlations (Figure 1) indicated that **2** possessed a 14-membered cembrane nucleus and defined the positions of the functional groups mentioned above. Thus, the presence of the isopropenyl group attached to C-1 of the 14-membered carbocycle was supported by the HMBC correlations of the olefinic methylene carbon [δ_C 110.9 (C-16)] with the methyl group at δ_H 1.65 (Me-17) and the methine proton at δ_H 2.11 (H-1). This methine proton showed COSY correlations with two methylene protons at δ_H 1.80 (H-2) and 1.39 (H-2), which correlated in turn with the epoxide proton (δ_H 2.92, H-3), thereby establishing the location of the oxirane ring at C-3,C-4. The methine carbon of the epoxide function [δ_C 60.6 (CH, C-3)] exhibited HMBC correlations with an isolated methylene at δ_H 2.86 (d, *J* = 13.0 Hz, H-5) and 2.32 (d, *J* = 13.0 Hz, H-5), which showed additional correlations with the ketone carbonyl [197.7 (C, C-6)] and the olefinic methine at δ_C 126.2 (CH, C-7). These data indicated the location of the ketone group at C-6 and the conjugated double bond at C-7. The position of the remaining double bond at C-11 was supported by the HMBC correlations of the olefinic carbon C-8 (δ_C 160.7) with the methylene protons at δ_H 2.32 (H-10) and 2.24 (H-10), which were correlated in the COSY with the olefinic proton at δ_H 5.01 (H-11). Finally the HMBC correlations of the methyl groups at δ_H 1.44 (Me-18), 1.89 (Me-19), and 1.57 (Me-20) with the resonances assigned to carbons C-3, C-7, and C-11, respectively, confirmed the location of the methyl substituents attached to C-4, C-8, and C-12 of the carbocycle. The *Z* configuration at C-7 and the *E* configuration at C-11 were readily inferred from NOE interactions of the olefinic proton H-7 with Me-19 and

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Table 1. NMR Spectroscopic Data (600 MHz, CDCl₃) for Compounds **2** and **3**^a

position	2		3	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	42.6	2.11 dddd (10.1, 9.5, 7.8, 3.0)	41.6	2.11 dddd (9.6, 9.3, 5.2, 2.3)
2	32.8	1.80 ddd (14.0, 9.5, 4.1) 1.39 ddd (14.0, 9.3, 3.0)	33.6	1.80 ddd (13.9, 9.6, 3.5) 1.31 ddd (13.9, 10.2, 2.3)
3	60.6	2.92 dd (9.3, 4.1)	62.4	3.00 dd (10.2, 3.5)
4	58.8		58.6	
5	53.7	2.86 d (13.0) α 2.32 d (13.0) β	54.6	2.81 d (11.9) α 2.35 d (11.9) β
6	197.7		197.1	
7	126.2	6.12 s	123.7	6.04 s
8	160.7		160.8	
9	31.4	3.01 m, 2.89 m	40.9	2.29 m
10	25.0	2.32 m, 2.24 m	24.3	2.29 m
11	121.7	5.01 br t (6.5)	123.3	5.04 br t (6.4)
12	135.6		135.8	
13	34.9	1.98 ddd (15.0, 6.7, 6.7) 1.82 ddd (15.0, 7.9, 7.9)	34.9	2.02 ddd (15.5, 5.4, 5.4) 1.87 m
14	30.0	1.55 m	30.4	1.61 m
15	148.1		147.5	
16	110.9	4.73 dq (1.5, 1.5) 4.68 br s	111.5	4.71 dq (1.4, 1.4) 4.59 br s
17	19.1	1.65 br s	18.3	1.61 br s
18	19.1	1.44 s	17.2	1.27 s
19	24.3	1.89 d (1.2)	19.4	2.16 d (1.2)
20	17.5	1.57 br s	17.5	1.61 br s

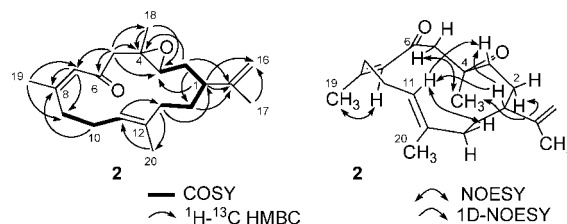
^a Assignments aided by COSY, HSQC, HMBC, and NOESY experiments.

of the olefinic proton H-11 with the methylene protons H-13, respectively. The relative configurations at C-1, C-3, and C-4 as depicted in formula **2** were proposed from NOESY and 1D-NOESY experiments (Figure 1, energy minimized using MM2). Thus, the NOESY correlations of Me-18 with H-5 α and the methylene protons H-2 were consistent with a *trans* relationship between Me-18 and H-3. Following this, the *cis* relationship between H-3 and H-1 was deduced from the NOE enhancements of H-3 and H-11 upon irradiation of H-1 and the NOE enhancements of H-3 and H-1 upon irradiation of H-11.

The molecular formula C₂₀H₃₀O₂ determined by HRCIMS analysis of leptodienone B (**3**), together with the close similarities of its NMR spectra (Table 1) with those of compound **2**, suggested that **3** was a stereoisomer of **2**. In fact, the analysis of the COSY and HMBC correlations confirmed that **3** was a cembrane diterpene containing the same functional groups as **2** at identical positions. Diagnostic differences between the NMR spectra of **3** and **2** were observed for the resonances of Me-19 and the methylene at C-9. In particular, the upfield shift of the carbon resonance of Me-19 in **3** (δ_C 19.4) with respect to **2** (δ 24.3) and the downfield shift of C-9 in **3** (δ_C 40.9) with respect to **2** (δ 31.4) indicated an *E* geometry for the double bond at C-7 in compound **3**. The remaining stereochemical features of the molecule, deduced from 1D-NOESY experiments, were shown to be identical to those of compound **2**.

The new compounds **2** and **3** isolated from *L. laxa* were tested in cytotoxicity assays against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Compound **2** showed growth inhibitory activity of MDA-MB-231 and HT-29 cells with GI₅₀ values of 16.2 and 14.9 μ M, respectively. Compound **3** was more potent and inhibited the growth of the three cell lines with GI₅₀ values of 6.3, 5.6, and 10.9 μ M against MDA-MB-231, A-549, and HT-29, respectively.

This chemical study of *L. laxa* extends the structural range of diterpenes isolated from gorgonians of the genus *Leptogorgia* to

**Figure 1.** Key COSY, HMBC, and NOE correlations for leptodienone A (**2**).

metabolites other than the furanocembranoids. From a biomedical point of view, leptodienones A (**2**) and B (**3**) represent the first record of cytotoxic diterpenes from *Leptogorgia* species.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a GBC Cintra-101 spectrometer. IR spectra were recorded on a Perkin-Elmer FT-IR System Spectrum BX spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Varian INOVA 600 spectrometer using CDCl₃ as solvent. Proton chemical shifts were referenced to the residual CHCl₃ signal at δ 7.26, and ¹³C NMR spectra were referenced to the central peak of CDCl₃ at δ 77.0. COSY, HSQC, HMBC, and NOESY experiments were performed using standard Varian pulse sequences. Low-resolution mass spectra were recorded on a Finnigan Voyager GC8000^{op} spectrometer. High-resolution mass spectra (HRCIMS) were obtained on an Autospec-Q mass spectrometer using CH₄ as ionizing gas. Column chromatography was carried out on Merck silica gel 60 (70–230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrospher Si-60 (Merck) columns in normal phase, using a RI-71 differential refractometer or a L-7400 UV detector. All solvents were spectroscopic grade or were distilled prior to use.

Collection and Identification. Specimens of *L. laxa* (family Gorgoniidae, order Gorgonacea, subclass Octocorallia, class Anthozoa) were collected by hand using scuba in the Bay of Ohuira (Gulf of California, Mexico) and immediately frozen. A voucher specimen is stored in the collection of the UNAM under the code M-568.

Extraction and Isolation. Freeze-dried specimens of the octocoral *L. laxa* (51.4 g) were extracted with 1.25 L of acetone/MeOH (1:1) at room temperature. After filtration, the solution was evaporated under reduced pressure to obtain a residue, which was partitioned between H₂O and Et₂O. The organic layer was evaporated to dryness to give an extract (740 mg), which was chromatographed on a SiO₂ column using solvents of increasing polarities from hexanes to Et₂O, then CHCl₃/MeOH (8:2), and finally MeOH. The fraction eluted with hexanes/Et₂O (8:2) was repeatedly purified by normal-phase HPLC using mixtures of hexanes/EtOAc (99:1 and 95:5) to yield leptodienone A (**2**) (3.6 mg, 0.007% dry wt) and leptodienone B (**3**) (9.6 mg, 0.019% dry wt). Fractions of the general chromatography eluted with hexanes/Et₂O (1:9) and CHCl₃/MeOH (8:2) were subjected to repeated normal-phase HPLC separations using hexane/EtOAc (7:3) and CHCl₃/MeOH (99:1) to obtain lopholide (7.4 mg, 0.014% dry wt), lophodiol B (6.9 mg, 0.013% dry wt), lophodione (2.5 mg, 0.005% dry wt), and lophotoxin (**1**) (32.0 mg, 0.062% dry wt).

Leptodienone A (2): colorless oil; [α]_D²⁵ +32.7 (*c* 0.24, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 243 (3.95) nm; IR (film) ν_{max} 1682, 1614 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) see Table 1; ¹³C NMR (CDCl₃, 150 MHz) see Table 1; CIMS *m/z* 302 (3) [M]⁺, 284 (98), 175 (65), 148 (85), 107 (100); HRCIMS(+) *m/z* 302.2226 (calcd for C₂₀H₃₀O₂, 302.2246).

Leptodienone B (3): amorphous solid; [α]_D²⁵ +108.1 (*c* 0.77, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 242 (4.05) nm; IR (film) ν_{max} 1685, 1610 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) see Table 1; ¹³C NMR (CDCl₃, 150 MHz) see Table 1; CIMS *m/z* 303 (5) [M + H]⁺, 284 (15), 257 (10), 175 (25), 135 (85), 107 (70), 82 (100); HRCIMS(+) *m/z* 303.2326 (calcd for C₂₀H₃₁O₂, 303.2324).

Cytotoxicity Assays. Compounds **2** and **3** were tested against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). A colorimetric assay using sulforhodamine B (SRB) reaction was adapted for

quantitative measurement of cell growth and viability as described in the literature.¹¹

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Supporting Information Available: ¹H and ¹³C NMR, HMBC, and 1D-NOESY spectra of compounds **2** and **3**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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