# Cembrane Diterpenes from the Gorgonian *Leptogorgia laxa*

María J. Ortega,[†] Eva Zubía,*[,†] M. Carmen Sánchez,[†] and J. Luis Carballo[‡]

*Departamento de Química Orgánica, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Apartado 40, 11510-Puerto Real (Cádiz), Spain, and Instituto de Ciencias del Mar y Limnología, UNAM, Apartado 811, Mazatlán 82000, Sinaloa, Mexico*

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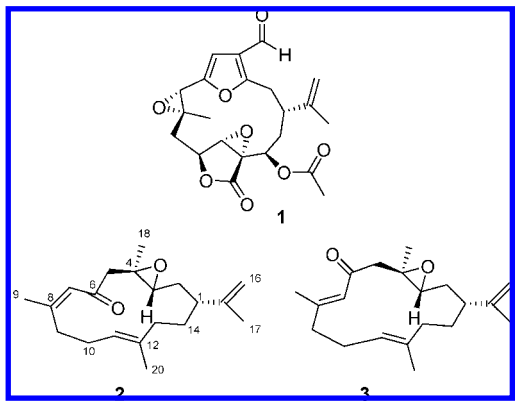
The new cembrane diterpenes leptodienone A (**2**) and leptodienone B (**3**) and the known compounds lopholide, lophodiol B, lophodione, and lophotoxin (**1**) have been isolated from the gorgonian *Leptogorgia laxa* collected in the Gulf of California. The structures of the new metabolites have been 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 steroids[6] 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 furanocembranolide-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 $H_2O$ and $Et_2O$. The organic extract was subjected to column chromatography eluting with hexanes/$Et_2O$ mixtures of increasing polarity, then $CHCl_3$/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 $C_{20}H_{30}O_2$, as derived from HRCIMS data. The $^1$H 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 $\beta$-substituted-$\alpha,\beta$-unsatutated ketone was evident from the NMR resonances at $\delta_C$ 197.7 (C), $\delta_C$ 126.2 (CH)/$\delta_H$ 6.12 (s), and $\delta_C$ 160.7 (C). The NMR spectra also included the signals of a trisubstituted double bond [$\delta_C$ 135.6 (C) and $\delta_C$ 121.7 (CH)/$\delta_H$ 5.01] and those of a *gem*-disubstituted double bond [$\delta_C$ 148.1 (C) and $\delta_C$ 110.9 ($CH_2$)/$\delta_H$ 4.73 and 4.68]. In addition, two resonances in the $^{13}$C NMR spectrum at $\delta_C$ 58.8 (C) and 60.6 (CH), the latter one coupled in the HSQC spectrum with the proton at $\delta_H$ 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 [$\delta_C$ 110.9 (C-16)] with the methyl group at $\delta_H$ 1.65 (Me-17) and the methine proton at $\delta_H$ 2.11 (H-1). This methine proton showed COSY correlations with two methylene protons at $\delta_H$ 1.80 (H-2) and 1.39 (H-2), which correlated in turn with the epoxide proton ($\delta_H$ 2.92, H-3), thereby establishing the location of the oxirane ring at C-3,C-4. The methine carbon of the epoxide function [$\delta_C$ 60.6 (CH, C-3)] exhibited HMBC correlations with an isolated methylene at $\delta_H$ 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 $\delta_C$ 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 ($\delta_C$ 160.7) with the methylene protons at $\delta_H$ 2.32 (H-10) and 2.24 (H-10), which were correlated in the COSY with the olefinic proton at $\delta_H$ 5.01 (H-11). Finally the HMBC correlations of the methyl groups at $\delta_H$ 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

* To whom correspondence should be addressed. Tel: +34 956016021. Fax: +34 956016193. E-mail: eva.zubia@uca.es.
[†] Universidad de Cádiz.
[‡] UNAM.

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**Table 1.** NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compounds **2** and **3**[a]

| | **2** | | **3** | |
|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ 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)<br>1.39 ddd (14.0, 9.3, 3.0) | 33.6 | 1.80 ddd (13.9, 9.6, 3.5)<br>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<br>2.32 d (13.0)*β* | 54.6 | 2.81 d (11.9)α<br>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)<br>1.82 ddd (15.0, 7.9, 7.9) | 34.9 | 2.02 ddd (15.5, 5.4, 5.4)<br>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)<br>4.68 br s | 111.5 | 4.71 dq (1.4, 1.4)<br>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-5α 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 $C_{20}H_{30}O_2$ 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** ($\delta_C$ 19.4) with respect to **2** ($\delta$ 24.3) and the downfield shift of C-9 in **3** ($\delta_C$ 40.9) with respect to **2** ($\delta$ 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 $GI_{50}$ values of 16.2 and 14.9 $\mu$M, respectively. Compound **3** was more potent and inhibited the growth of the three cell lines with $GI_{50}$ values of 6.3, 5.6, and 10.9 $\mu$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 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.

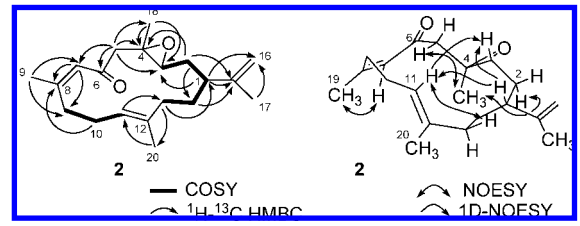


**Figure 1.** Key COSY, HMBC, and NOE correlations for leptodienone A (**2**).

## 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. $^1$H and $^{13}$C NMR spectra were recorded on a Varian INOVA 600 spectrometer using $CDCl_3$ as solvent. Proton chemical shifts were referenced to the residual $CHCl_3$ signal at $\delta$ 7.26, and $^{13}$C NMR spectra were referenced to the central peak of $CDCl_3$ at $\delta$ 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$^{top}$ spectrometer. High-resolution mass spectra (HRCIMS) were obtained on an Autospec-Q mass spectrometer using $CH_4$ 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 $H_2O$ and $Et_2O$. The organic layer was evaporated to dryness to give an extract (740 mg), which was chromatographed on a $SiO_2$ column using solvents of increasing polarities from hexanes to $Et_2O$, then $CHCl_3$/MeOH (8:2), and finally MeOH. The fraction eluted with hexanes/$Et_2O$ (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/$Et_2O$ (1:9) and $CHCl_3$/MeOH (8:2) were subjected to repeated normal-phase HPLC separations using hexane/EtOAc (7:3) and $CHCl_3$/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; $[\alpha]^{25}_D$ +32.7 (*c* 0.24, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 243 (3.95) nm; IR (film) $\nu_{max}$ 1682, 1614 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 600 MHz) see Table 1; $^{13}$C NMR ($CDCl_3$, 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 $C_{20}H_{30}O_2$, 302.2246).

**Leptodienone B (3):** amorphous solid; $[\alpha]^{25}_D$ +108.1 (*c* 0.77, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 242 (4.05) nm; IR (film) $\nu_{max}$ 1685, 1610 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 600 MHz) see Table 1; $^{13}$C NMR ($CDCl_3$, 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 $C_{20}H_{31}O_2$, 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:** $^{1}H$ and $^{13}C$ 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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