Leptolide, a New Furanocembranolide Diterpene from *Leptogorgia alba*

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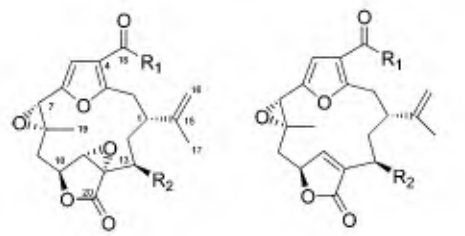
Six furanocembranolides (**1**−**6**) and one pseudopterolide (**7**) have been isolated from the octocorals *Leptogorgia alba* and *Leptogorgia rigida*, collected on the Pacific coast of Panama. Compound **1**, named leptolide, has a new structure closely related to the neurotoxin lophotoxin (**3**). The X-ray structures of **1**−**3** were determined, and the absolute configurations of **2**−**7** are discussed. Compounds **1**−**7** were evaluated in vitro against drug-resistant *Plasmodium falciparum*.

Gorgonian octocorals are marine invertebrates that are a well-known source of novel natural products,[1,2] including highly functionalized diterpenes such as the furanocembranolides.[2] Thought to be used as chemical defenses against fish predation,[3,4] the furanocembranolides display a range of biological activities.[5−7]

In the course of a joint program between the University of Santiago de Compostela and the Panama International Cooperative Biodiversity Groups program (Panama ICBG), several specimens of octocorals were collected by scuba in the Coiba National Park, a marine protected area located in the Gulf of Chiriquí, in the southwest Pacific of the Republic of Panama.[8] Among the octocorals collected were two species in the genus *Leptogorgia* (Gorgoniidae), *L. alba* and *L. rigida*, whose crude extracts showed antiplasmodial activity in a novel in vitro fluorescence-based bioassay developed by the Panama ICBG.[9] The crude extracts were subjected to bioassay-guided fractionation, yielding diterpenes **1**−**7**. Compound **1** is a novel natural product, and its structure was deduced by 1D and 2D NMR spectrometry and HREIMS and confirmed by single-crystal X-ray crystallography. Diterpenes **1**−**7** were evaluated for activity against a chloroquine-resistant strain of *Plasmodium falciparum*.

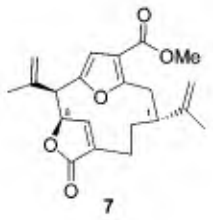
The MeOH extracts of *L. alba* and *L. rigida* showed antiplasmodial activity and were subjected to activity-guided fractionation. *L. alba* was subjected to liquid−liquid partition, and the active CH_2Cl_2-soluble material was fractionated using silica gel vacuum liquid chromatography (VLC) followed by silica gel flash chromatography, affording the new compound **1** and two known compounds identified as pukalide aldehyde (**2**)[6] and lophotoxin (**3**).[5] The MeOH extract of *L. rigida* was fractionated following the same protocol described for *L. alba*, resulting in the isolation of four known compounds identified as pukalide (**4**),[10] lopholide (**5**),[11] 13α-acetoxypukalide (**6**),[11] and deoxypseudopterolide (**7**).[12]

Compound **1** showed a molecular ion at *m/z* 358 in the EIMS. The ^{13}C NMR spectrum showed resonances for 20 carbon atoms, while DEPT and HSQC experiments revealed seven quaternary carbons, six methines, five methylenes, and two methyl groups. These data and the HREIMS indicated the molecular formula, $C_{20}H_{22}O_6$, requiring 10 degrees of unsaturation. Detailed examination of ^{13}C, ^1H, and DEPT NMR spectra and comparison with literature data indicated that compound **1** is a diterpene with a structure similar to 11β,12β-epoxypukalide,[13] with the primary difference being an aldehyde substituent bonded to C-4 of the furan ring. Evidence of the aldehyde function at C-4 was provided by correlations observed in 2D NMR experiments (Figure 1). The structure and relative stereochemistry of **1** were confirmed by single-crystal X-ray crystallography.



1 $R_1 = R_2 = H$
3 $R_1 = H$; $R_2 = OAc$
5 $R_1 = OMe$; $R_2 = OAc$

2 $R_1 = R_2 = H$
4 $R_1 = OMe$; $R_2 = H$
6 $R_1 = OMe$; $R_2 = OAc$



7

Compounds **2**, **4**, **6**, and **7** have an α,β-unsaturated γ-lactone moiety as common feature, and although they have been previously described in the literature,[6,10−12] the absolute stereochemistry of these compounds had not been established. Previous studies with the α,β-unsaturated γ-lactone chromophore showed that the sign of the Cotton effect due to the n−π* and π−π* transitions may be correlated directly with the absolute configuration of the stereogenic center at the γ-carbon of the butenolide moiety.[14,15] Interpretation of the CD data for compounds **2**, **4**, **6**, and **7** (Table 1) following the butenolide configurational rule[15] and the exciton chirality method[16] revealed that the configuration at C-10 in compounds **2**, **4**, and **6** is *S*, while

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Figure 1. Selected 2D NMR correlations of compound **1**.

Table 1. CD Data of Compounds **2**, **4**, **6**, and **7**[a]

compound	CD, $\Delta\epsilon$ (nm)	
	n−π*	π−π*
2	−0.8 (276)	7.4 (218)
4	−0.7 (262)	9.3 (226)
6	−0.6 (263)	8.3 (226)
7	0.6 (274)	8.7 (224)

[a] Measured in MeCN at $c = 1 \times 10^{-4}$ M.

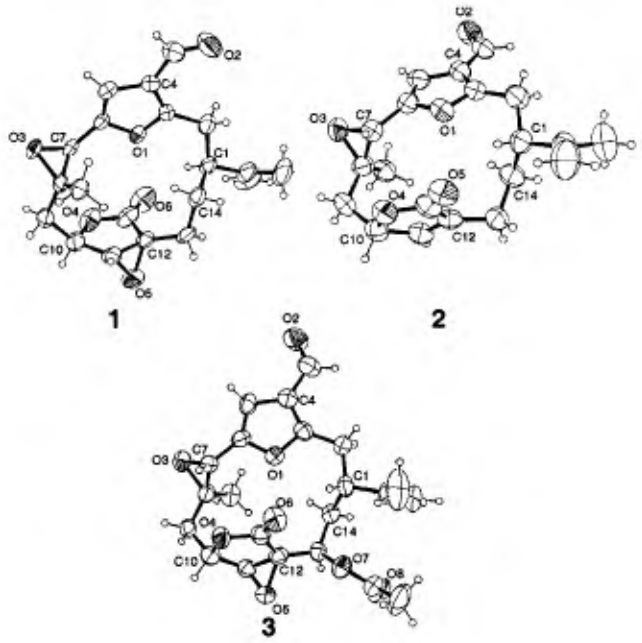

Figure 2. ORTEP diagrams for compounds **1**−**3** with 30% thermal probability ellipsoids. Absolute configuration is implied only in the case of compound **2**.

the configuration at C-8 in compound **7** is *R*. Monocrystals suitable for X-ray analysis of compounds **1**−**3** were obtained and examined (Figure 2). Compound **2** crystallized in association with a molecule of chloroform ($C_{20}H_{22}O_5 \cdot CHCl_3$), and the anomalous dispersion of the chlorine atoms allowed us to assign its absolute configuration as 1*R*, 7*S*, 8*S*, 10*S* (Figure 2), a result in agreement with the data obtained from the CD experiments.

While Fenical and colleagues carried out a series of chemical interconversions to show that compounds **3**, **4**, **5**, and **6** belong to the same stereochemical series, their absolute configurations remained unknown.[5] The determination of the absolute configuration of **4** and **6** in the present study allowed us to use the interconversion data to assign the absolute configuration of **3** and **5** as depicted in the structure block.

Compounds **1**−**7** were tested in vitro against a chloroquine-resistant strain of *P. falciparum*.[9] Compounds **2**, **5**, and **6** exhibited moderate activity (IC_{50} = 134, 105, 109 μM, respectively), whereas compounds **3** and **7** showed more significant activity against the parasite (IC_{50} = 74 and 50 μM, respectively). Compounds **1** and **4** showed no measurable activity against the parasite. This is the first report of activity against *P. falciparum* for any member of the furanocembranolide class of compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 polarimeter using a 1 dm path length cell. UV spectra were measured in ethanol in a Hewlett-Packard 8452A diode array spectrophotometer. CD spectra were obtained in MeCN in a JASCO J-720 spectropolarimeter. IR spectra were recorded in a Bruker IFS-66v (film, KBr). NMR spectra were recorded in *d*-chloroform at 250, 400, or 500 MHz using a Bruker DPX-250, Varian Inova-400, and Bruker AMX-500, respectively. HREIMS were measured on a Micromass Autospec mass spectrometer. X-ray crystallographic data collection was carried out with a Bruker Nonius FR591-Kappa-CCD-2000 diffractometer or with a Bruker Smart-CCD-1000 diffractometer. Column chromatography was performed on silica gel 60 (70−230 mesh for VLC and 230−400 mesh for flash columns). Semipreparative HPLC purification was carried out on a Waters apparatus equipped with a refractive index detector and a Spherisorb 10 μm silica semiprep column (10 × 250 mm).

Animal Material. Specimens of *L. alba* and *L. rigida* (vouchers C7 and S3, respectively) were collected by scuba in the Coiba National Park at 3−10 m depth in August 2002. The samples were frozen immediately after collection and stored at −80 °C until extraction. Voucher specimens are maintained for reference at the Smithsonian Tropical Research Institute. Specimen C7 was consistent with the original description for *L. alba* (Duchassaing and Michelotti 1864).[17] Colonies are white or light pink in color. Colonies may grow up to 40 cm in height and 40 cm in width, flabelliform in nature, with long, slender pinnate branches, short stems, and thin holdfasts. The specimen S3 is consistent with the description for *L. rigida* (Verrill, 1868).[18] Colonies are upright, measuring up to 40 cm in height. Stems may reach 30 mm in width. Branches are mostly cylindrical, especially at the ends. The color is deep purple, but in some cases, yellowish rings of sclerites surrounding the polyp apertures are observed.

Extraction and Isolation. The octocoral *L. alba* (121.9 g, fresh weight) was cut into small pieces and extracted with MeOH and CH_2Cl_2. The extracts were pooled and concentrated under reduced pressure in a rotary evaporator to yield 3.4 g of crude extract that was subjected to solvent partition, affording four fractions (*n*-hexane, CH_2Cl_2, *n*-butanol, and water). The CH_2Cl_2 fraction (350 mg) was active against *P. falciparum* and was subjected to VLC on silica gel eluting with CH_2Cl_2−MeOH mixtures (from 5% to 100% MeOH), affording five fractions. Fraction 2 (174 mg) showed the most significant antiplasmodial activity and was subjected to silica gel flash chromatography eluting with *n*-hexane−EtOAc mixtures (33%, 50%, and 100% EtOAc), yielding 10 fractions. Fractions 4 and 6 yielded compounds **1** (42 mg) and **2** (11 mg), respectively, in pure form. Fraction 7 (37 mg) was subjected to silica gel flash chromatography eluting with $CHCl_3$−MeOH (0.30% of MeOH), yielding 25 mg of compound **3**.

The octocoral *L. rigida* was extracted and partitioned in the same way as described for *L. alba*, of which the CH_2Cl_2 fraction (744 mg) showed activity in the antiplasmodial bioassay. The CH_2Cl_2 fraction was subjected to VLC on silica gel, eluting with CH_2Cl_2−MeOH mixtures (from 3% to 100% MeOH), affording six fractions. Significant antiplasmodial activity was located in fraction 2 (382 mg), which was fractionated on a silica gel flash column eluting with mixtures of CH_2Cl_2−MeOH (from 0.5% to 3.0% MeOH), yielding compounds **4** (105 mg) and **5** (15 mg) from fractions 3 and 5, respectively. Fraction 6 (94 mg) was chromatographed using silica gel flash column eluting with 0.5% MeOH in CH_2Cl_2, affording compound **6** (23 mg).

Fraction 1 (10 mg) was subjected to purification by HPLC eluting with 50% EtOAc–isooctane, yielding compound **7** (2 mg).

Leptolide (1): red glassy solid; $[\alpha]^{25}_{D}$ +10.4° (*c* 1.9, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 234 (3.34), 262 (3.38), 292 (3.36) nm; IR (KBr) ν_{max} 3489, 2932, 1778, 1680, 1084 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.82 (1H, s, H-18), 6.50 (1H, s, H-5), 5.06 (1H, s, H-16a), 4.96 (1H, s, H-16b), 4.73 (1H, dd, J = 3.0, 4.0 Hz, H-10), 4.04 (1H, s, H-7), 4.05 (1H, s, H-11), 3.49 (1H, dddd, J = 3.0, 8.5, 11.0, 11.0 Hz, H-1), 3.05 (1H, dd, J = 3.0, 18.0 Hz, H-2a), 2.97 (1H, dd, J = 11.0, 18.0 Hz, H-2b), 2.55 (1H, ddd, J = 1.5, 11.0, 15.0 Hz, H-14a), 2.50 (1H, dd, J = 3.0, 15.5 Hz, H-9a), 2.03 (1H, dd, J = 4.0, 15.5 Hz, H-9b), 1.75 (3H, s, H-17), 1.66 (1H, m, H-13a), 1.48 (1H, ddd, J = 1.5, 8.5, 15.0 Hz, H-14b), 1.37 (1H, m, H-13b), 1.14 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 184.4 (CH, C-18), 171.2 (C, C-20), 162.2 (C, C-3), 149.8 (C, C-6), 144.8 (C, C-15), 123.1 (C, C-4), 113.8 (CH_2, C-16), 105.1 (CH, C-5), 76.8 (CH, C-10), 63.2 (CH, C-11), 62.4 (C, C-12), 56.3 (C, C-8), 55.3 (CH, C-7), 40.6 (CH, C-1), 39.1 (CH_2, C-9), 30.7 (CH_2, C-2), 26.5 (CH_2, C-13), 23.6 (CH_2, C-14), 20.2 (CH_3, C-19), 18.9 (CH_3, C-17); EIMS *m/z* 358 $[M]^+$ (9), 329 (5), 301 (3), 231 (5), 203 (10), 189 (15), 175 (19), 149 (53), 137 (81), 121 (24), 107 (37), 91 (54), 79 (52), 77 (47), 55 (78), 43 (94), 39 (53), 28 (36), 18 (100); HREIMS *m/z* 358.1424 (calcd for $C_{20}H_{22}O_6$, 358.1416).

X-ray Crystal Structure of Leptolide (1).[19] A red prism was obtained by slow evaporation from methanol. Crystal data. Empirical formula = $C_{20}H_{22}O_6$, M_r = 358.38, T = 120(2) K, crystal system = orthorhombic, space group = $P2_12_12_1$, unit cell dimensions: a = 10.091(5) Å, b = 11.064(5) Å, c = 16.970(5) Å, V = 1894.6(14) Å^3, Z = 4, D_{calc}= 1.256 Mg/m^3, λ = 0.7107 Å, μ(Mo Kα) = 0.093 mm^{-1}, F_{000} = 760, crystal size = 1.00 × 0.58 × 0.53 mm, theta range for data collection: θ = 2.19–24.04°, index ranges = $-12 \le h \le 12$, $0 \le k \le 13$, $0 \le l \le 21$, reflections collected = 9896, independent reflections = 3734 (R_{int} = 0.0635), completeness to theta = 26.37°, 97.3%, absorption correction = semiempirical from equivalents, maximum and minimum transmission = 0.9525 and 0.9131, respectively. Refinement method: full-matrix least-squares on F^2, data/restraints/parameters = 3734/0/250, S = 1.003, final R indices [$I > 2\delta(I)$]: R1 = 0.0571, wR2 = 0.1430, R indices (all data): R1 = 0.1079, wR2 = 0.1646, Flack parameter = −0.5(18), extinction coefficient = 0.007(2), largest difference peak and hole = 0.212 and −0.180 e Å^{-3}.

X-ray Crystal Structure of Pukalide Aldehyde (2).[19] A colorless needle was obtained by slow evaporation from chloroform. Crystal data. Empirical formula = $C_{20}H_{22}O_5 \cdot CHCl_3$, M_r = 461.74, T = 293(2) K, crystal system = hexagonal, space group = $P6_5$, unit cell dimensions: $a = b$ = 20.6319(12) Å, c = 9.8314(7) Å, V = 3624.3(4) Å, Z = 6, D_{calc} = 1.269 Mg/m^3, λ = 1.54184 Å, μ(Cu Kα) = 3.666 mm^{-1}, F_{000} = 1440, crystal size = 0.55 × 0.88 × 0.03 mm, theta range for data collection: θ = 4.29–50.71°, index ranges = $0 \le h \le 20$, $0 \le k \le 20$, $-9 \le l \le 9$, reflections collected = 10 161, independent reflections = 2491 (R_{int} = 0.0702), completeness to theta = 50.71° 99.3%, absorption correction = semiempirical from equivalents, maximum and minimum transmission = 0.775 and 0.376, respectively. Refinement method: full-matrix least-squares on F^2, data/restraints/parameters = 2491/39/278, S = 1.111, final R indices [$I > 2\delta(I)$]: R1 = 0.0992, wR2 = 0.2659, R indices (all data): R1 = 0.1225, wR2 = 0.2904, Flack parameter = 0.02(7), extinction coefficient = 0.0110(18), largest difference peak and hole = 0.425 and −0.322 e Å^{-3}.

X-ray Crystal Structure of Lophotoxin (3).[19] A colorless plate was obtained by slow evaporation of chloroform. Crystal data. Empirical formula = $C_{22}H_{24}O_8 \cdot H_2O$, M_r = 432.41, T = 293(2) K, λ = 1.54184 Å, crystal system = monoclinic, space group = $P2_1$, unit cell dimensions: a = 9.696(5) Å, b = 6.921(4) Å, c = 17.237(8) Å, β = 91.18(4)°, V = 1156.4(11) Å^3, Z = 2, D_{calc} = 1.242 Mg/m^3, μ(Cu Kα) = 0.818 mm^{-1}, F_{000} = 456, crystal size = 0.75 × 0.10 × 0.03 mm, theta range for data collection: θ = 5.19–50.48°, index ranges = $-9 \le h \le 9$, $-6 \le k \le 6$, $0 \le l \le 17$, reflections collected = 8270, independent reflections = 2366 (R_{int} = 0.0924), completeness to theta = 50.48°, 99.3%, absorption correction = semiempirical from equivalents, maximum and minimum transmission = 1.053 and 0.692, respectively. Refinement method: full-matrix least-squares on F^2, data/restraints/parameters: 2366/1/283, S = 1.105, final R indices [$I > 2\delta(I)$]: R1 = 0.0847, wR2 = 0.2368, R indices (all data): R1 = 0.1009, wR2 = 0.2666, Flack parameter = 0.0(7), largest difference peak and hole = 0.375 and −0.239 e Å^{-3}.

Biological Evaluation. Antiplasmodial activity was determined in a chloroquine-resistant *P. falciparum* strain (W2) utilizing a novel microfluorimetric assay to measure the inhibition of the parasite growth based on the detection of the parasitic DNA by intercalation with PicoGreen.[9] *P. falciparum* was cultured according to the methods described by Trager and Jensen.[20] The parasites were mantained at 2% haematocrit in flat-bottom flasks (75 mL) with RMPI 1640 medium (GibcoBRL) supplemented with 10% human serum.

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Supporting Information Available: CD spectra of compounds **2**, **4**, **6**, and **7**, crystallographic information of compounds **1**–**3**, and photos of the collected organisms are available free of charge via the Internet at http://pubs.acs.org.

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(19) Crystallographic data for structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition numbers CCDC 246192 for compound **1**, CCDC 246195 for compound **2**, and CCDC 246262 for compound **3**. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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