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Lobocrasol, a New Diterpenoid from the Soft Coral *Lobophytum crassum*

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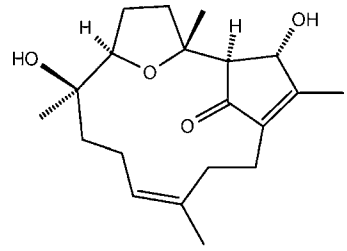
ABSTRACT



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Lobocrasol (1), possessing an unprecedented diterpenoid skeleton, was isolated from the soft coral *Lobophytum crassum*. The structure of lobocrasol was established by extensive analysis of spectroscopic data.

Soft corals of the genus *Lobophytum* (Alcyoniidae) have proven to be a rich source of macrocyclic diterpenoids.[1−3] Previous bioassay results of some macrocyclic diterpenoids have been shown to exhibit significant cytotoxic properties.[4−8] The continuing search for bioactive constituents prompted us to investigate the secondary metabolites of the soft coral *Lobophytum crassum* (Von Marenzeller, 1886). Our chemical examination of this soft coral has led to the isolation of lobocrasol (**1**), possessing an unprecedented diterpenoid skeleton.

The soft coral *Lobophytum crassum* was collected at Dongsha Island, Taiwan, in April 2007, at a depth of 6 m. The specimen was authenticated by Prof. C.-F. Dai, and a voucher specimen (TS-11) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University. The bodies of the soft coral were freeze-dried (1.20 kg), which was extracted with acetone (2.0 L × 4). After removal of solvent in vacuo, the residue (36 g) was chromatographed over silica gel 60 using *n*-hexane and *n*-hexane-EtOAc mixtures of increasing polarity. Elution by *n*-hexane-EtOAc (1:9) afforded a fraction containing compound **1**. Compound **1** (2 mg) was further purified by HPLC

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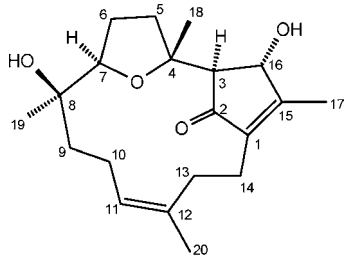
(LiChrosorb RP-18, 7 μ, 25 × 250 mm), eluting with MeOH-H_2O (65:35).



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Compound **1** was isolated as a colorless oil, $[\alpha]^{25}_D$ −186 (*c* 0.1, $CHCl_3$). The IR spectrum of **1** exhibited absorptions due to hydroxyl (3425 cm^{-1}) and conjugated enone (1698 cm^{-1}) functionalities. The presence of the conjugated enone was also confirmed by the UV spectrum [λ_{max} (log ϵ) 230 nm (3.58)]. HRESIMS exhibited a pseudo molecular ion peak at *m*/*z* 357.2044 $[M + Na]^+$, consistent with the molecular formula of $C_{20}H_{30}O_4$.

The structure of **1** was solved by a combination of 1D and 2D NMR methods. The resonances at δ_C 205.3 (qC), 141.3 (qC), and 166.7 (qC), in the ^{13}C NMR and DEPT spectra suggested the presence of a tetrasubstituted conjugated enone (Table 1). Furthermore, the presence of four oxygenated carbons was inferred from the carbon signals at δ_C 73.1 (qC), 82.3 (qC), 82.1 (CH), and 75.5 (CH), a trisubstituted olefin at δ_C 131.7 (qC) and 128.2 (CH). Six methylene groups were deduced from six triplet signals at δ_C 38.3, 37.1, 33.9, 25.7, 22.1, and 20.3, a methine signal at δ_C 61.1, and, finally, four methyl signals at δ_C 13.3, 28.5, 23.5, and 14.8.

Table 1. ^1H and ^{13}C NMR data of **1** (400 and 100 MHz, respectively, in $CDCl_3$) (δ in ppm relative to TMS)

pos.	$\delta_H{}^{a,b}$	$\delta_C{}^a$
1		141.3 (qC)
2		205.3 (qC)
3	2.05 m	61.1 (CH)
4		83.3 (qC)
5	1.60 m, 3.16 dt (12.4, 8.8)	33.9 (CH_2)
6	1.74 m, 1.76 m	25.7 (CH_2)
7	3.57 dd (10.0, 6.4)	82.3 (CH)
8		73.1 (qC)
9	1.54 m	38.3 (CH_2)
10	2.04 m, 1.92 m	22.1 (CH_2)
11	4.84 t (7.2)	128.2 (CH)
12		131.7 (qC)
13	2.48 m, 2.00 m	37.1 (CH_2)
14	2.48 m, 2.24 m	20.3 (CH_2)
15		166.7 (qC)
16	4.80 d (6.4)	75.5 (CH)
17	2.08 s	13.3 (CH_3)
18	1.37 s	28.5 (CH_3)
19	1.09 s	23.5 (CH_3)
20	1.59 s	14.8 (CH_3)

[a] Assigned by DEPT, COSY, HSQC, and HMBC experiments. [b] Coupling constant in Hz in parentheses.

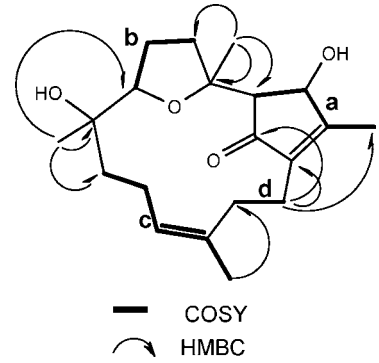


Figure 1. Key COSY and HMBC correlations of **1**.

The combined use of $^1H-^1H$ COSY and HMQC on **1** allowed us to distinguish four spin systems (see **a**−**d** in Figure 1). A HMBC experiment was used to assemble the skeletal fragments through quaternary carbons and heteroatoms. Thus, these substructures were connected through HMBC correlations between the protons H_2-14 (δ_H 2.48 and 2.24) and the carbons C-1 (δ_C 141.3), C-2 (δ_C 205.3), C-15 (δ_C 166.7), and C-12 (δ_C 131.7), between the methyl protons Me-20 (δ_H 1.59) and the carbons C-13 (δ_C 37.1), between the methine proton H-3 (δ_H 2.05) and carbon C-2, between the methyl protons Me-19 and the carbon C-7, C-8 and C-9, between the methyl protons Me-18 (δ_H 1.37) and carbons C-3 (δ_C 61.1), C-4 (δ_C 83.3), and C-5 (δ_C 33.9). These relationships are represented in Figure 1.

All of these data allowed us to identify compound **1** as a new diterpenoid with a novel skeleton. With the gross structure of **1** in hand, the relative stereochemistry of compound **1** was deduced from NOESY correlations and Chem3D Ultra 9.0 (Figure 2). The *Z* geometry of the Δ^{11} double bond was established by the NOESY correlation observed between H-11 and H_3-20. NOESY correlations of H-7 with H_3-19 indicated that these protons are on the same face of the ring system, whereas those of H_3-18 with H-16 were used to place them on the opposite face of the ring system, thereby establishing the relative configuration of **1**.

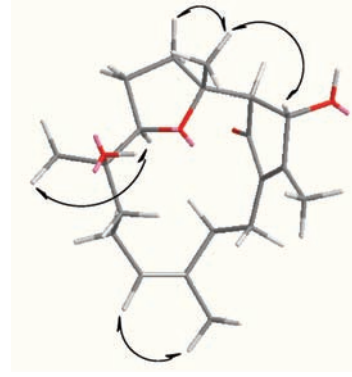


Figure 2. Key NOESY correlations of **1**.

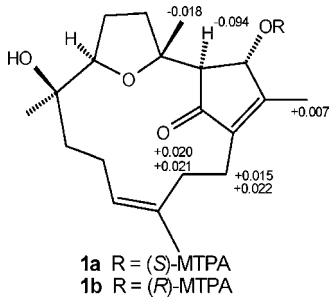
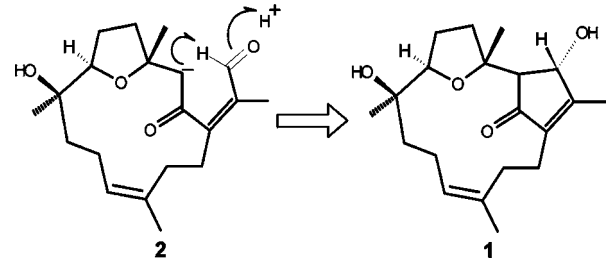


Figure 3. Absolute stereochemistry of **1**: δ values ($\delta_S - \delta_R$) in ppm for the two MTPA esters **1a** and **1b**.

The α proton at C-5 appearing at δ_H 3.16 (1.5 ppm downfield from its companion) was due to the deshielding effect of C-2 carbonyl, which was oriented to the α face of the molecule as shown in Figure 2.

The absolute configuration of **1** was determined by application of the modified Mosher method.[9] Treatment of **1** with (*S*)-MTPA chloride and (*R*)-MTPA chloride afforded the (*R*)-MTPA ester (**1a**) and (*S*)-MTPA ester (**1b**), respectively. The difference in chemical shift values ($\delta_S - \delta_R$) for the diastereomeric esters **1b** and **1a** was calculated in order to assign the absolute configuration at C-11. Calculations for all of the relevant signals suggested the 16*S* absolute configuration. Therefore, the 3*S*, 4*S*, 7*R*, and 8*R* absolute configuration was proposed for **1** on the basis of the $\Delta\delta$ results summarized in Figure 3.

Scheme 1. Plausible Biosynthetic Pathway for **1**



Compound **1** exhibited cytotoxicity against the P-388 cell with ED_{50} of 3.2 μg/mL. Biogenetically, lobocrasol (**1**) may be an aldol condensation product of an aldehyde analogue (**2**) (Scheme 1).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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