Cytotoxic Cembrenolide Diterpenes from the Formosan Soft Coral *Lobophytum crassum*

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Received December 15, 1999

A new cytotoxic cembrenolide diterpene, lobocrassolide (**1**), and a known cytotoxic cembrenolide, lobohedleolide (**2**), were isolated from the Formosan soft coral *Lobophytum crassum*. The structure of compound **1** was determined by 1D and 2D spectral analysis.

In search for bioactive substances from marine organisms, the soft coral *Lobophytum crassum* Von Marenzeller (family Alcyoniidae) was studied, because the hexane extracts showed significant cytotoxicity in A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[1,2] Bioassay-guided fractionations resulted in the isolation of a new cytotoxic cembrane diterpene, lobocrassolide (**1**), and a known cytotoxic cembrenolide, lobohedleolide (**2**).[3]

The hexane-soluble fraction of *L. crassum* was chromatographed over Si gel to give colorless prisms, $[\alpha]^{25}_D$ +81.6° (*c* 1.15, $CHCl_3$). HRFABMS and a DEPT spectrum of **1** established the molecular formula to be $C_{22}H_{30}O_4$. Thus, 8 degrees of unsaturation were determined for **1**. The UV absorption maximum at 231 nm exhibited the presence of an α,*β*-unsaturated carbonyl. ^{1}H and ^{13}C NMR spectral data (Table 1) showed that **1** contained two methyl-bearing trisubstituted double bonds (δ_C 15.0, q, C-18; 141.7, s, C-4; 120.5, d, C-3; 15.8, q, C-20; 134.2, s, C-12; 123.5, d, C-11; δ_H 1.67, 3H, br s, H-18; 1.56, 3H, br s, H-20; 5.01, 1H, d, J = 9.9 Hz, H-3; 4.92, 1H, t, J = 7.2 Hz, H-11), an α-methylene-*γ*-lactone (δ_C 170.7, s, C-16; 120.6, t, C-17; 138.7, s, C-15; 43.2, d, C-1; 78.0, d, C-2; δ_H 5.39, 1H, dd, J = 9.9, 7.8 Hz, H-2; 3.36, 1H, m, H-1; 6.26, 1H, d, J = 3.0 Hz, H-17; 5.52, 1H, d, J = 3.0 Hz, H-17), an acetoxymethyl-bearing trisubstituted double bond [δ_C 61.7, d, C-19; 132.1, s, C-7; 132.1, d, C-8; 171.1, s, C-21; 20.9, q, C-22; δ_H 4.55, 1H, br s, H-19; 5.06, 1H, dd, J = 6.0, 4.0 Hz, H-7; 2.05, 3H, s, H-22], and six methylene carbons [δ_C 39.5, 24.1, 36.0, 24.1, 36.3, 27.0]. Comparison of the ^{1}H and ^{13}C NMR spectra of **1** with those of lobohedleolide revealed that the carboxyl group in lobohedleolide was replaced by an acetoxymethyl in **1**. From the HMBC experiment (Table 1) of **1**, the positioning of the α,*β*-methylene-*γ*-lactone at C-15 (α), C-1 (*β*), C-2 (*γ*), and C-16 (carbonyl carbon) was confirmed from long-range correlations between H-17 to C-1, C-15, and C-16; H-2 to C-4, C-15, and C-16; and H-1 to C-3, C-15, and C-17. The vinyl methyl group attached at C-4 was confirmed by HMBC correlations between H-3 to C-5 and C-18; H-18 to C-3, C-4, and C-5; and H-2 to C-4, C-15, and C-16. The other vinyl methyl group attached at C-12 was revealed by the HMBC correlations between H-20 to C-11, C-12, and C-13 and H-11 to C-13 and C-20. The acetoxymethyl attached at C-8 was revealed by the HMBC correlations between H-19 and C-7, C-8, C-9, and C-21 and between H-7 and C-5, C-9, and C-19. The trisubstituted olefins and the *γ*-lactone were further connected by HMBC and COSY correlations as shown in Table 1. The cis stereochemistry at C-1 and C-2 was established by comparison of the coupling constant (J = 8 Hz) between H-1 and H-2 with those of lobohedleolide (**2**). The relative stereochemistry of **1** was further confirmed by NOESY correlations (Table 1). The identity of compound **2** as lobohedleolide was established by directed comparison of $[\alpha]_D$, IR, EIMS, and ^{1}H and ^{13}C NMR data with literature data.[3] Compound **1** exhibited cytotoxicity against A549, HT-29, KB, and P-388 with ED_{50} values of 2.99, 2.70, 2.91, and 0.012 μg mL^{-1}, respectively. Compound **2** showed cytotoxicity against P-388 with an ED_{50} value of 2.44 μg mL^{-1}, but was not cytotoxic against A549, HT-29, and KB cell lines.

Table 1. NMR Data of **1**[a]

position	δ_H, mult., J[b]	δ_C, mult.[c]	HMBC	COSY	NOESY
1	3.36, m	43.2, d	3, 15, 17	2, 14, 16	2, 18
2	5.39, dd, 9.9, 7.8	78.0, d	4, 15, 16	3	1, 18
3	5.01, d, 9.9	120.5, d	5, 18	2	
4		141.7, s			
5		39.5, t	4, 6, 7		
6		24.1, t	7, 8		
7	5.06, dd, 6.0, 4.0	132.1, d	5, 9, 19	6	11, 18
8		132.1, s			
9		36.0, t	8, 10, 11		
10		24.1, t	8, 11, 12		
11	4.92, t, 7.2	123.5, d	13, 20	10, 20	7, 18
12		134.2, s			
13		36.3, t	14, 20		
14		27.0, t	1, 13		
15		138.7, s			
16		170.7, s		1	
17	5.52, d, 3.0 6.26, d, 3.0	120.6, t	1, 15, 16		
18	1.67, br s	15.0, q	3, 4, 5	3	
19	4.55, br s	61.7, t	7, 8, 9, 21		
20	1.56, br s	15.8, q	11, 12, 13	11	
21	2.05, s	20.9, q			
22		171.1, s	22		

[a] Spectra recorded in $CDCl_3$. [b] J values in Hz. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

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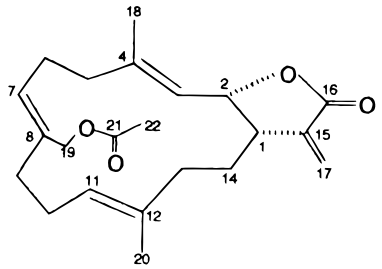
10.1021/np990620h CCC: $19.00
Published on Web 05/11/2000



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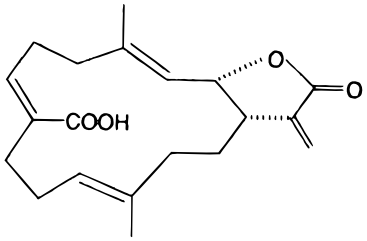

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Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are reported uncorrected. Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26-30 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX 400 NMR spectrometer at 400 and 100.6 MHz, respectively, in $CDCl_3$ using TMS as internal standard. EIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Materials. The soft coral *L. crassum* was collected at Green Island off Taiwan in September 1997 at a depth of 12 m and was stored for 1 day in a freezer until extraction. The voucher specimen, NSUGN-1025, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *L. crassum* were freeze-dried to give 910 g of a solid, which was sequentially extracted with CH_2Cl_2 (2 L × 3), CH_2Cl_2−MeOH (2 L × 3), and acetone−MeOH (2 L × 3). After removal of solvent in vacuo, the residue (69 g) was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction was further partitioned between *n*-hexane and 10% aqueous MeOH. The 10% aqueous MeOH-soluble fraction was then partitioned between CCl_4 and 20% aqueous MeOH. The 20% aqueous MeOH-soluble fraction was further partitioned between $CHCl_3$ and 35% aqueous MeOH. The *n*-hexane-soluble fraction was chromatographed over Si gel 60 using *n*-hexane and *n*-hexanes−EtOAc mixtures of increasing polarity as eluting solvent. Elution by *n*-hexanes−EtOAc (5:1) afforded fractions containing **1**. Compound **1** was finally purified by chromatography over Si gel 60 using *n*-hexanes−EtOAc (3:1) as eluting solvent. The $CHCl_3$-soluble fraction was chromatographed over Si gel 60 using *n*-hexanes−EtOAc mixtures of increasing polarity. Elution by *n*-hexanes−EtOAc (1:1) afforded **2**.

Lobocrassolide (1): colorless prisms (55 mg); mp 98−100 °C; $[\alpha]^{25}_D$ +81.6° (*c* 1.2, $CHCl_3$); IR (KBr) ν_{max} 1735, 1648, 1234 cm^{-1}; UV(MeOH) λ_{max} (log ϵ) 231 nm (3.29); ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 358 $[M]^+$ (0.1), 298 (2), 255 (1), 227 (2), 197 (3), 171 (8), 157 (9), 155 (1), 43 (100); HREIMS *m*/*z* 358.2134 (calcd for $C_{22}H_{30}O_4$, 358.2136).

Cytotoxicity Testing. KB and P-388 cells were supplied by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxicity asssays were carried out according to the procedure described previously.[4]

Acknowledgment. We thank Prof. J. M. Pezzuto, Program for Collaborative Research in Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, for the provision of P-388 and KB cell lines. This work was supported by a grant from the National Science Council of Taiwan.

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NP990620H