HIV-Inhibitory Cembrane Derivatives from a Philippines Collection of the Soft Coral *Lobophytum* Species[1]

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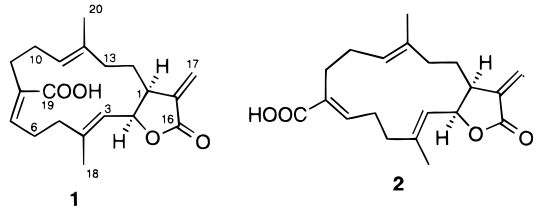
Bioassay-guided fractionation of an aqueous extract of a Philippine Islands collection of the soft coral *Lobophytum* sp. concentrated its HIV-inhibitory activity into fractions rich in cembranoid diterpenes. Lobohedleolide (**1**), (7*Z*)-lobohedleolide (**2**), and a new compound, 17-dimethylaminolobohedleolide (**3**), were purified from these fractions by HPLC. The structures of compounds **1**−**3** were elucidated by spectroscopic analyses and by comparison of their spectral data with previously reported values. The relative stereochemistry of the γ-lactone ring substituents of **3** was determined by 1D NOESY experiments. While several other cembranoids that contain a dimethylamino functional group have been reported from the soft coral *Sinularia* sp., compound **3** represents the first cembrane diterpene with this functional group isolated from a *Lobophytum* species. Diterpenoids **1**−**3** exhibited moderate HIV-inhibitory activity (EC_{50} approximately 3−5 μg/mL) in a cell-based in vitro anti-HIV assay.

Marine invertebrates in the family Alcyoniidae are a rich source of structurally diverse sesquitepenes and diterpenes.[2] In particular, soft corals of the genus *Lobophytum* have provided a number of cembranoid diterpenes,[3−13] including lobohedleolide (**1**) and (7*Z*)-lobohedleolide (**2**).[14] Numerous cembranoids have been reported to exhibit cytotoxic properties,[4,5,7,10,15−18] and recently a cembrane diterpene from *Lobophytum cristagally* was reported to inhibit Ras farnesyl transferase.[7] Our studies were initiated when an aqueous extract of *Lobophytum* sp., which had been collected in the Philippines, was found active in the U.S. National Cancer Institute (NCI)'s primary anti-HIV screen.[19,20] Bioassay-guided fractionation of the extract provided a new diterpene **3**, in addition to the known compounds **1** and **2**.



1

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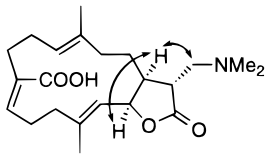

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Arrows indicate key NOE interactions

The freeze-dried aqueous extract of *Lobophytum* sp. was rehydrated in H_2O and then fractionated by reversed-phase vacuum flash chromatography on C_4 media, eluted with increasing concentrations of MeOH in H_2O. Fractions exhibiting HIV-inhibitory activity were further fractionated by gel permeation on Sephadex LH-20 using MeOH−H_2O (7:3). Final purification was achieved by C_{18} HPLC to afford three diterpenes, **1**−**3**. Two of these were readily identified as lobohedleolide (**1**) and (7*Z*)-lobohedleolide (**2**) by comparison of their physical and spectral data with previously reported values.[14] High-resolution FABMS of compound **3** provided a $(MH)^+$ ion at *m*/*z* 376.2496, which established its molecular formula as $C_{22}H_{33}NO_4$. The IR spectrum of **3** supported the presence of an α,β-unsaturated carboxyl group (ν 3400−3100 and 1674 cm^{-1}) and a γ-butyrolactone moiety (ν 1760 cm^{-1}). The ^1H and ^{13}C NMR spectra of **3** (Table 1) revealed close correspondence with spectra recorded for lobohedleolide (**1**). However, resonances appropriate for the *exo*-methylene group in **1** were absent from the spectra of **3** and were instead replaced with resonances indicative of a dimethylaminomethyl (−CH_2-NMe_2) residue. The two *N*-methyl groups were evident from a six-proton singlet at δ_H 2.97 that showed HSQC correlations with carbon resonances at δ_C 42.6 and 45.7. The CH_2 group of the dimethylaminomethyl moiety provided a carbon resonance at δ_C 57.9 and two proton signals at δ_H 3.36 (1H, dd, J = 13.5, 4.0 Hz) and 3.45 (1H, dd, J = 13.5, 10.5 Hz) that demonstrated both geminal and vicinal couplings. An α-methine proton (δ_H 3.18) was coupled to the aminomethylene protons and the C-1 bridgehead proton. This suggested that compound **3** was a dimethylamino adduct of lobohedleolide (**1**).

It was possible to trace all of the proton−proton spin systems in **3** with data from a COSY-45 experiment. Heteronuclear correlation experiments (HMBC, HSQC, and HSQC-TOCSY; Table 1) allowed unambiguous assignment of all ^1H and ^{13}C NMR resonances in **3**. HMBC correlations from the *N*-methyl protons at δ 2.97 to the methylene at δ_C 57.9 confirmed placement of the dimethylamino moiety at C-17. The relative stereochemistry of the γ-butyrolactone ring of **3** was determined by selective 1D NOESY experiments. Irradiation at the resonance frequency of H-2 produced significant NOESY correlations with H-1, H-3,

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Table 1. NMR Spectral Data for 17-Dimethylaminolobohedleolide (**3**) in CD_3OD

position	^{13}C	^{1}H	mult *J* (Hz)	COSY	HSQC-TOCSY	HMBC
1	44.0	2.47	m	1.52, 1.88, 3.18, 5.54	C-2, C-3, C-13, C-14, C-15, C-17	C-2, C-14, C-15, C-17
2	80.6	5.54	dd 11.0, 7.5	2.47, 5.40	C-1, C-3, C-13, C-14, C-15, C-17	C-3, C-4, C-15, C-16
3	119.9	5.40	br d 11.0	1.75, 5.54	C-1, C-2, C-13, C-14, C-15, C-17	C-2, C-5, C-18
4	145.0					
5	40.7	2.30	m	2.36, 2.38, 3.17		
		2.38	m	2.30, 2.36, 3.17		
6	27.3	2.36	m	2.30, 2.38, 3.17, 5.62		
		3.17	m	2.30, 2.36, 2.38, 5.62	C-5, C-7	C-7
7	145.1	5.62	dd 9.0, 3.5	2.36, 3.17	C-5, C-6	C-5, C-9, C-19
8	131.6					
9	36.5	1.84	m	2.13, 2.69		
		2.69	m	1.84, 2.13	C-10, C-11	C-7, C-8, C-10, C-11, C-19
10	25.9	2.13 (2H)	m	1.84, 2.69, 4.95		C-8, C-9, C-11, C-12
11	124.0	4.95	br t 7.7	1.50, 2.13	C-9, C-10, C-20	C-9, C-20
12	136.0					
13	37.2	1.71	m	1.88, 2.01		C-1, C-11, C-12, C-14, C-20
		2.01	m	1.52, 1.71, 1.88		C-11, C-12, C-14
14	27.9	1.52	m	1.88, 2.01, 2.47		
		1.88	m	1.52, 1.71, 2.01		
15	40.1	3.18	m	2.47, 3.36, 3.45	C-1, C-2, C-3, C-13, C-17	C-1, C-14, C-16, C-17
16	178.9					
17	57.9	3.36	dd 13.5, 4.0	3.18, 3.45	C-1, C-2, C-3, C-13, C-14, C-15	C-1, C-15, C-16, N-Me_2
		3.45	dd 13.5, 10.5	3.18, 3.36	C-1, C-2, C-3, C-13, C-14, C-15	C-1, C-15, C-16, N-Me_2
18	15.1	1.75 (3H)	s	5.40		C-3, C-4, C-5
19	171.0					
20	16.2	1.50 (3H)	s	4.95		C-11, C-12, C-13
NMe_2	42.6	2.97 (3H)	s			C-17, N-Me
	45.7	2.97 (3H)	s			C-17, N-Me

and the C-4 olefinic methyl group, while H-1 showed strong NOESY correlations to H-2 and both of the C-17 methylene proton resonances. This established a cis relationship between H-1, H-2, and the C-15 dimethylaminomethyl substituent in **3**, which is the same relative stereochemistry found in the sinulamines.[21] The new diterpene was thus identified as 17-dimethylaminolobohedleolide (**3**). While nitrogen-containing cembranoid diterpenes are rare, cembranolides containing a dimethylamino group have previously been reported from Okinawan collections of the soft coral *Sinularia* sp.[21,22]

Compounds **1**−**3** inhibited the cytopathic effect of in vitro HIV-1 infection in a cell-based assay described elsewhere.[23] However, they were cytoprotective only over a modest concentration range (**1**: $EC_{50} = 3.6$ μg/mL, $IC_{50} = 9.0$ μg/mL; **2**: $EC_{50} = 4.6$ μg/mL, $IC_{50} = 7.6$ μg/mL; **3**: $EC_{50} = 3.3$ μg/mL, $IC_{50} = 10.2$ μg/mL) with maximum cellular protection of approximately 55−70%.

Experimental Section

General Experimental Procedures. HPLC was performed on a Varian-Rainin system employing a Dynamax C_{18} column (1 × 25 cm), using a flow rate of 3 mL/min and UV detection at 220 nm. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on a Beckman DU-640 and Perkin-Elmer 1600 FTIR spectrometer, respectively. The ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Varian INOVA 500 spectrometer, and the chemical shifts are reported in ppm relative to the residual nondeuterated solvents. The number of attached protons for ^{13}C signals was determined using the DEPT pulse sequence. Inverse detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{CH} = 140$ Hz) and HMBC (optimized for $^nJ_{CH} = 8.5$ and 3.5 Hz) pulse sequences. High-resolution mass spectra were acquired on a JEOL SX102 mass spectrometer.

Animal Material. Samples of the soft coral *Lobophytum* sp. were collected in 1990 by E. Menez and subsequently identified by G. Williams (California Academy of Science). A voucher specimen (Voucher ID # 0ALQ0068) for this collection is maintained at the Smithsonian Institution, Washington, D.C.

Extraction and Isolation. The frozen samples were ground into a coarse powder (582 g) and extracted with H_2O. The H_2O-soluble fraction was freeze-dried to provide 30.4 g of aqueous extract. A 5.7 g aliquot of the crude extract was subjected to C_4 vacuum flash chromatography eluting with increasing concentrations of MeOH in H_2O. Anti-HIV activity was concentrated in fractions that eluted with MeOH−H_2O (1:2) and MeOH−H_2O (2:1). Sephadex LH-20 chromatography of the MeOH−H_2O (1:2) fraction (189 mg) on a 2.5 × 100 cm column eluted with MeOH−H_2O (7:3) afforded two diterpene-rich fractions. Further purification of these fractions by C_{18} HPLC using a linear gradient from 55% to 65% MeCN in H_2O (0.1% TFA) over 20 min yielded, in order of elution, compound **3** (7 mg; $t_R = 5.0$ min), compound **2** (3 mg; $t_R = 14.7$ min), and compound **1** (14 mg; $t_R = 16.7$ min). Similar purification of the MeOH−H_2O (2:1) fraction from the C_4 column also provided compounds **1**−**3**, as evident by identical retention times and ^1H NMR spectra.

Lobohedleolide (1): white amorphous powder; $[\alpha]_D$ +97.3° ($c = 0.38$, $CHCl_3$) (lit.[14] +104.2°); UV, IR, MS, ^1H NMR, and ^{13}C NMR data were in agreement with previously reported values.[14]

(7*Z*)-Lobohedleolide (2): white gum; $[\alpha]_D$ +35.0° ($c = 0.07$, $CHCl_3$) (lit.[14] +61.4°); UV, IR, MS, ^1H NMR, and ^{13}C NMR data were in agreement with published values.[14]

17-Dimethylaminolobohedleolide (3): white gum; $[\alpha]_D$ +13.1° ($c = 0.25$, $CHCl_3$); UV (EtOH) λ_{max} 222 (log $\epsilon = 3.56$), 218 (log $\epsilon = 3.60$) nm; IR (film) ν_{max} 3400−3100, 1760, 1674, 1200, 1176, 961 cm^{-1};^1H NMR and ^{13}C NMR, see Table 1; HR-FABMS *m/z* $[MH]^+$ 376.2494 (calcd 376.2488 for $C_{22}H_{34}NO_4$).

Anti-HIV Assay. The crude extract, chromatographic fractions, and purified compounds were dissolved in DMSO−H_2O (1:1) or 100% DMSO, diluted to the desired concentration, and tested in an XTT-based in vitro anti-HIV assay, the experimental details of which have been reported previously.[23]

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