Bioactive Terpenoids from Octocorallia. 4. Three New Cembrane-Type Diterpenoids from the Soft Coral *Lobophytum schoedei*

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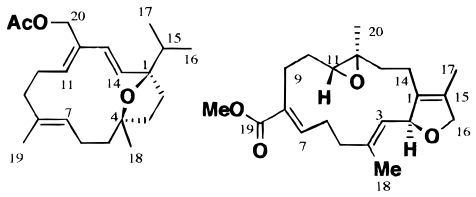
Three new cembrane-type diterpenoids, lobophynins A (**1**), B (**2**), and C (**3**), were isolated from the soft coral *Lobophytum schoedei*. Their structures and relative stereochemistries were determined by extensive 2D NMR studies. Lobophynin C (**3**) has ichthyotoxicity against the mosquito fish, *Oryzias latipes*, and lethal activity toward brine shrimps.

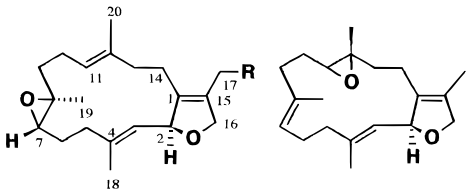
The soft corals have been shown to contain a variety of secondary metabolites, mainly diterpenoids, sesquiterpenoids, and steroids.[1] Many of these constituents are structurally unique and exhibit interesting biological activities. Recently, a number of diterpenoids were isolated from the Japanese soft corals.[2] As part of our search for biologically active constituents, we also conducted the isolation and structure elucidation of the biologically active diterpenoids from some Japanese soft corals and reported the structures of two hemolytic diterpenoids, litophytols A and B, isolated from the soft coral *Litophyton* sp.,[3] an ichthyotoxic diterpenoid, deoxyxeniolide B, isolated from the soft coral *Xenia elongata*,[4] and a diterpenoid possessing toxicity to brine shrimp, cladiellaperoxide, isolated from the soft coral *Cladiella sphaeroides*.[5] In the continuing search for biologically active constituents of other animals, we found that extracts of a soft coral *Lobophytum schoedei* (Alcyoniidae) collected from the Nichinan Coast in the Miyazaki Prefecture of Japan showed ichthyotoxicity to the mosquito fish, *Oryzias latipes*, and lethality to brine shrimps, *Artemia salina*, L. Bioassay-directed fractionation of the extracts has led to the isolation of three new compounds, designated lobophynins A (**1**), B (**2**), and C (**3**). We report herein the isolation and structure elucidation of these compounds on the basis of their spectral properties.

The *n*-hexane- and EtOAc-soluble parts of the MeOH/$CHCl_3$ extract obtained from the *L. schoedei* (9.2 kg) were subjected to Sephadex LH-20 to give five fractions. The fourth fraction was chromatographed on a silica gel column to furnish fractions containing diterpenoids that showed ichthyotoxicity and lethality to brine shrimps with an LC_{100} value of 30 ppm concentration, respectively. These fractions were purified by reversed-phase column chromatography by HPLC (ODS column) to give lobophynins A (**1**), B (**2**), and C (**3**) as a part of the active constituents.

Lobophynin A (**1**) was isolated as a colorless oil. The molecular formula of $C_{22}H_{34}O_3$ was determined by HR positive-ion FAB mass spectrometry. The IR spectrum of **1** showed signals due to carbon−carbon double bonds (3010 and 910 cm^{-1}) and ester (1730 and 1240 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** contained signals for two secondary methyls, two tertiary methyls, one acetoxymethyl, and four olefinic protons (Table 1). The ^{13}C-NMR spectrum of **1** suggested the presence of six methylene carbons, one methine carbon, one oxygen-bearing methylene carbon, two oxygen-bearing quaternary carbons, six olefinic carbons, and one ester carbon (Table 2). These data were confirmed by ^{1}H−^{13}C COSY (HETCOR) correlations between each proton and carbon as shown in Table 3. The ^{1}H−^{1}H COSY correlations of **1** (Table 3) clearly indicated the presence of four partial structures, $-CH_2CH_2-$, $-CH(CH_3)_2$, $-CH{=}CH-$, and $-CH_2CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C<$ in **1**. In addition, the presence of a $>C(CH_3)O-$ unit was also revealed by the above-mentioned facts and the value of the chemical shift in the ^{1}H-NMR spectrum. Connectivities of these partial structures were determined by detailed analysis of the ^{1}H−^{13}C long-range COSY (COLOC) spectrum of **1**. COLOC correlations of δ_C 78.6 (C-1) to δ_H 0.85 (H-16) and 5.72 (H-13) suggested the connectivities between C-1 and C-14 and between C-1 and C-15. COLOC correlations of δ_C 61.2 (C-20) to δ_H 5.72 (H-13) and NOESY correlations between δ_H 5.59 (H-11) and δ_H 5.91 (H-14) in the NOESY spectrum suggested the connectivities between C-12 and C-13 and between C-12 and C-20. By considering the previously mentioned data, the molecular formula, and the degrees of unsaturation, the planar structure of **1** was determined to be a cembrane-type diterpenoid. The location of the acetoxyl group at the C-20 position was indicated by the values of chemical shifts in the ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) and the NOESY correlations between the δ_H 2.07 (acetoxymethyl) and δ_H 4.57, 4.66 (H-20). The geometry between C-13 and C-14 was



1 **3**



2 R = OAc
4 R = H

5

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Table 1. ^{1}H-NMR Data of Lobophynins A (**1**), B (**2**), and C (**3**) in $CDCl_3$[a]

position	**1**	**2**	**3**
1			
2a	2.03 (1H, m)	5.59 (1H, dt, *J* = 4.8, 10.0 Hz)	5.53 (1H, m)
2b	2.28 (1H, m)		
3a	1.29 (1H, m)	5.26 (1H, dd, *J* = 1.3, 10.0 Hz)	5.28 (1H, dd, *J* = 1.0, 9.9 Hz)
3b	1.87 (1H, m)		
4			
5a	1.63 (1H, m)	2.34 (2H, dd, *J* = 3.8, 10.1 Hz)	1.73 (1H, m)
5b	2.00 (1H, m)		2.37 (1H, dt, *J* = 5.1, 11.8 Hz)
6a	2.04 (1H, m)	1.68 (2H, m)	1.85 (1H, m)
6b	2.27 (1H, m)		2.18 (1H, m)
7	5.24 (1H, dd, *J* = 3.4, 8.9 Hz)	2.70 (1H, dd, *J* = 10.1, 12.8 Hz)	6.73 (1H, dd, *J* = 6.7, 10.1 Hz)
8			
9a	1.65 (1H, m)	0.99 (1H, dt, *J* = 3.4, 16.9 Hz)	1.73 (1H, m)
9b	1.86 (1H, td, *J* = 3.4, 14.2 Hz)	2.12 (1H, ddd, *J* = 3.4, 5.1, 16.9 Hz)	2.28 (1H, m)
10a	2.20 (1H, m)	2.23 (1H, ddd, *J* = 3.4, 6.1, 15.2 Hz)	1.61 (1H, m)
10b	2.48 (1H, dq, *J* = 7.5, 14.2 Hz)	2.30 (1H, m)	1.90 (1H, m)
11	5.59 (1H, t, *J* = 7.5 Hz)	5.11 (1H, ddd, *J* = 1.0, 6.1, 10.1 Hz)	2.59 (1H, dd, *J* = 1.7, 8.6 Hz)
12			
13a	5.72 (1H, d, *J* = 15.5 Hz)	1.90 (1H, dd, *J* = 4.8, 10.4 Hz)	0.89 (1H, dt, *J* = 3.4, 7.8 Hz)
13b		1.94 (1H, dd, *J* = 5.4, 10.4 Hz)	2.19 (1H, m)
14a	5.91 (1H, d, *J* = 15.5 Hz)	1.75 (1H, m)	1.73 (1H, m)
14b		2.65 (1H, dd, *J* = 7.8, 11.8 Hz)	2.14 (1H, m)
15	1.60 (1H, q, *J* = 6.6 Hz)		
16a	0.85 (3H, d, *J* = 6.6 Hz)	4.62 (1H, dd, *J* = 2.4, 5.6)	4.47 (1H, dd, *J* = 3.3, 11.8 Hz)
16b		4.64 (1H, dd, *J* = 2.4, 5.6 Hz)	4.57 (1H, ddd, *J* = 1.0, 5.9, 11.8 Hz)
17	0.85 (3H, d, *J* = 6.6 Hz)	4.68 (2H, d, *J* = 1.0 Hz)	1.70 (3H, s)
18	1.33 (3H, s)	1.83 (3H, d, *J* = 1.3 Hz)	1.79 (3H, s)
19	1.67 (3H, s)	1.27 (3H, s)	
20a	4.57 (1H, d, *J* = 12.3 Hz)	1.60 (3H, d, *J* = 1.0 Hz))	1.28 (3H, s)
20b	4.66 (1H, d, *J* = 12.3 Hz)		
OAc	2.07 (3H, s)	2.07 (3H, s)	
OMe			3.76 (3H, s)

[a] Spectra were recorded at 23 °C. Chemical shifts were given in ppm.

Table 2. ^{13}C-NMR Data of Lobophynins A (**1**), B (**2**), C (**3**), Sarcophytoxide (**4**), and Isosarcophytoxide (**5**) in $CDCl_3$

position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[c]
1	78.6 (s)	127.3 (s)	132.4 (s)	133.5	132.7
2	24.1 (t)	83.8 (d)	83.3 (d)	83.8	84.6
3	23.3 (t)	125.2 (d)	127.4 (d)	126.4	125.5
4	73.9 (s)	140.3 (s)	139.5 (s)	139.2	140.8
5	45.0 (t)	37.7 (t)	38.0 (t)	37.6	39.7
6	35.4 (t)	25.3 (t)	25.6 (t)[d]	25.4	24.6
7	126.3 (d)	61.9 (d)	141.6 (d)	61.9	125.5
8	136.8 (s)	59.8 (s)	131.8 (s)	59.8	133.1
9	33.2 (t)	39.8 (t)	25.5 (t)[d]	39.7	37.0
10	36.2 (t)	23.6 (t)	22.9 (t)	23.5	24.2
11	134.3 (d)	124.0 (d)	62.9 (d)	123.7	61.2
12	131.6 (s)	136.4 (s)	61.4 (s)	136.7	60.7
13	129.9 (d)	36.7 (t)	38.6 (t)	36.7	35.4
14	137.4 (d)	26.4 (t)	25.9 (t)[d]	26.0	20.4
15	38.9 (d)	139.0 (s)	129.4 (s)	127.5	128.4
16	16.6 (q)	75.8 (t)	78.1 (t)	78.4	78.3
17	17.7 (q)	58.1 (t)	9.9 (q)	10.1	10.2
18	29.6 (q)	15.7 (q)	14.8 (q)	15.6	15.1
19	14.9 (q)	16.9 (q)	167.9 (s)	17.0	15.1
20	61.2 (t)	15.2 (q)	16.7 (q)	15.2	7.7
OAc	21.0 (q)	20.8 (q)			
	170.1 (s)	170.9 (s)			
OMe			51.8 (q)		

[a] Spectra were aquired at 23 °C at 67.8 MHz. Chemical shifts were given in ppm. Multiplicity was given in DEPT. [b] These δ_C values were cited from ref 6. [c] These δ_C values were cited from ref 7. [d] Signals may be interchanged.

Table 3. ^{1}H-NMR Data, ^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY, and COLOC Correlations for Lobophynin A (**1**)

		COSY correlations		
	^{1}H		^{1}H−^{13}C	
position	(δ_H)	^{1}H−^{1}H	(δ_C)	COLOC
1				
2a	2.03	H-2b	24.1	
2b	2.28	H-2a, H-3a, H-3b	24.1	
3a	1.29	H-2b, H-3b	23.3	
3b	1.87	H-2b, H-3a	23.3	
4				
5a	1.63	H-5b, H-6a	45.0	C-20
5b	2.00	H-5a	45.0	
6a	2.04	H-5a, H-6b	35.4	
6b	2.27	H-6a, H-7	35.4	
7	5.24	H-6b	126.3	
8				
9a	1.65	H-9b, H-10a	33.2	
9b	1.86	H-9a, H-10b	33.2	
10a	2.20	H-9a, H-10b, H-11	36.2	
10b	2.48	H-9b, H-10a, H-11	36.2	
11	5.59	H-10a, H-10b	134.3	
12				
13	5.72	H-14	129.9	C-1, C-20
14	5.91	H-13	137.4	
15	1.60	H-16, H-17	38.9	
16	0.85	H-15	16.6	C-1, C-15, C-17
17	0.85	H-15	17.7	C-1, C-15, C-16
18	1.33		29.6	C-4, C-5
19	1.67		14.9	C-7, C-8, C-9
20a	4.57	H-20b	61.2	
20b	4.66	H-20a	61.2	
OAc	2.07		21.0	C-1′ (OAc)

determined by the value of the coupling constant $J_{13,14}$ = 15.5 Hz. The chemical shift of the C-19 methyl carbon (δ_C 14.9) suggested a 7*E*-geometry. In addition, the NOESY cross peaks between δ_H 2.03 (H-2a) and δ_H 5.91 (H-14), between δ_H 2.28 (H-2b) and δ_H 1.60 (H-15), between δ_H 1.29 (H-3a) and δ_H 2.27 (H-6b), between δ_H 2.27 (H-6b) and δ_H 1.67 (19-CH_3), between δ_H 5.72 (H-13) and δ_H 0.85 (16-CH_3), and between δ_H 0.85 (17-CH_3) and δ_H 1.33 (18-CH_3) were observed, respectively. The cisoid geometry (from C-11 to C-14) was determined by NOESY correlations between δ_H 5.59 (H-11) and δ_H 5.91 (H-14) and dreiding stereomodel based on the above-mentioned NOESY correlations. The relative stereochemistry of lobophynin A (**1**) was thus determined as shown in Figure 1.

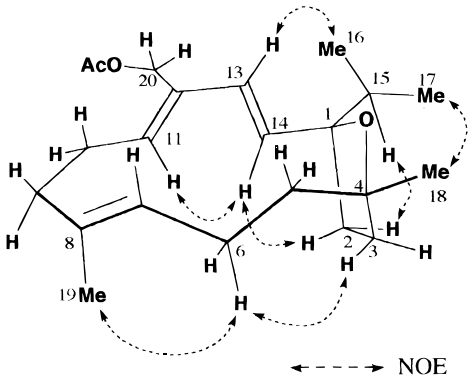

Figure 1. NOESY correlations for **1**.

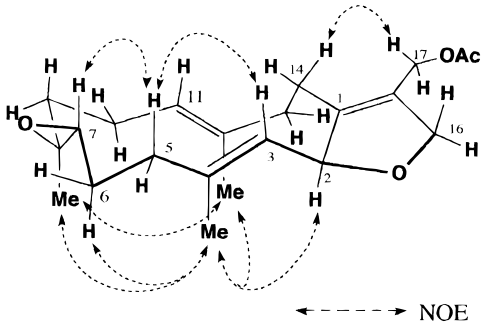

Figure 2. NOESY correlations for **2**.

Lobophynin B (**2**) was isolated as a colorless oil. HR positive-ion FAB mass spectrometry of **2** indicated a molecular formula of $C_{22}H_{32}O_4$. Compound **2** showed signals due to carbon−carbon double bonds (3040 and 910 cm^{-1}) and ester (1740 and 1240 cm^{-1}) groups in the IR spectrum. The ^{1}H- and ^{13}C-NMR spectra of **2** resembled those of sarcophytoxide (**4**) obtained from another soft coral *Sarcophyton* sp.,[6] except for the signals ascribable to an additional acetoxyl group [δ_H 2.07 (3H, s), δ_C 20.8, 170.9] and oxygen-bearing methylene [δ_H 4.68 (2H, d, J = 1.0 Hz), δ_C 58.1] (Tables 1 and 2). The position of the acetoxyl group at the C-17 was revealed by the COLOC correlation between the ^{1}H-signal at δ_H 4.68 (17-CH_2) and the ^{13}C-signal at δ_C 139.0 (C-15). The chemical shifts of the C-18 and C-20 methyl carbons (δ_C 15.7 and 15.2, respectively) suggested 3*E*- and 11*E*-geometries. The relative stereochemistry of **2** was confirmed on the basis of a NOESY experiment as shown in Figure 2.

Lobophynin C (**3**) was isolated as a colorless oil. The molecular formula of $C_{21}H_{30}O_4$ was determined by HR positive-ion FAB mass spectrometry. The IR spectrum of **3** displayed absorption bands for carbon−carbon double bonds (3030 and 930 cm^{-1}) and ester (1710 and 1240 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra of **3** resembled those of isosarcophytoxide (**5**) obtained from another soft coral *Sarcophyton* sp.[7] except for resonances due to the ester carbonyl carbon [δ_C 167.9] and methoxy group [δ_H 3.76 (3H, s), δ_C 51.8] (Tables 1 and 2). These data indicated that **3** was the carboxylic acid methyl ester derivative of **5**. The location of the methyl ester group at the C-8 position was indicated by the COLOC correlations between the ^{1}H-signal at δ_H 2.28 (H-9) and the ^{13}C-signal at δ_C 131.8 and 167.9 (C-8, 19, respectively). The location was also suggested by the fact that the ^{1}H-signal at δ_H 6.73 of H-7 in **3** shifted to a lower field than that of **5**. The chemical shift of the C-18 methyl carbon (δ_C 14.8) suggested a 3*E*-geometry,

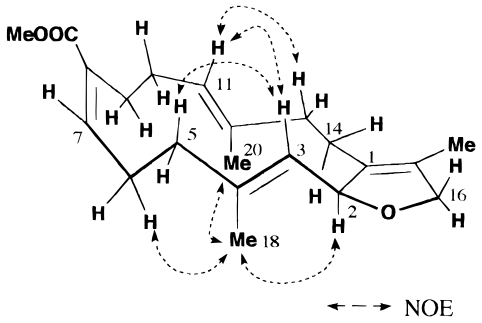

Figure 3. NOESY correlations for **3**.

whereas 7*E*-geometry was assigned by the chemical shift of H-7.[8] Furthermore, the NOESY correlations between δ_H 5.53 (H-2) and δ_H 1.79 (18-CH_3), between δ_H 5.28 (H-3) and δ_H 2.37 (H-5b), between δ_H 5.28 (H-3) and δ_H 2.59 (H-11), between δ_H 1.85 (H-6a) and δ_H 1.79 (18-CH_3), between δ_H 2.59 (H-11) and δ_H 2.19 (H-13b), and between δ_H 1.79 (18-CH_3) and δ_H 1.28 (20-CH_3) and suggested the relative stereochemistry of **3** as shown in Figure 3.

Lobophynin C (**3**) displayed ichthyotoxicity with an LC_{100} value of 30 ppm and exhibited toxicity with an LC_{50} value of 22.5 ppm in the brine shrimp lethality bioassay. Lobophynins A (**1**) and B (**2**) showed lethality to brine shrimp at a concentration of 30 ppm with 20% and 25% lethal rates, respectively. The ichthyotoxicities of compounds **1** and **2** could not be examined, but both fractions containing these compounds similarly showed ichthyotoxicity at a concentration of 30 ppm with 100% lethal rate.

A large number of cembrane-type diterpenoids have been isolated from the soft corals.[1] Many of them exhibit biological activities, e.g., cytotoxicity, inhibition of cholinesterase,[9] Ca-antagonistic action,[10] and so on. In particular, the ichthyotoxic diterpenoids sarcophine[11] and lobolide[12] that have been isolated from the soft corals *Sarcophytum glaucum* and *Lobophytum* sp., respectively, are believed to play a role in the protective mechanism of the corals against predators. Consequently, it is thought that lobophynins A (**1**), B (**2**), and C (**3**) may also play the same role.

Lobophynin A (**1**) is unique in that it has ether bonds between C-1 and C-4 and an acetoxyl group at C-20, while lobophynin B (**2**) is an unusual dihydrofuran-type cembranoid with an acetoxyl group at C-17.

Experimental Section

General Experimental Procedures. Spectra were recorded using the following instruments: specific optical rotations, JASCO DIP-370 digital polarimeter; IR, JASCO IR-700 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured at 270 and 67.8 MHz, respectively, with a JEOL GX-270 spectrometer. Chemical shifts were given on a δ (ppm) scale with TMS as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). FABMS/HR positive FAB mass spectra were recorded using a JEOL DX-300 (6 kV, xenon atom beam) with JMA-3500 data system, and the spectra were measured in $CHCl_3$/*m*-NBA (*m*-nitrobenzyl alcohol) solution. The EIMS spectrum was recorded using a JEOL JMS-DX-300 with JMA-3500 data system, an accelerating potential of 3 kV, ionizing potential of 30 eV, and sample temperature of 150−200 °C. Column chromatography was performed with Sepha-

dex LH-20 (Pharmacia), Kieselgel 60 (No. 7734, Merck), and Cosmosil 5C18 (Nacalai Tesque) columns. HPLC was conducted with a JASCO BIP-I model and a RID-300 RI detector with a Wakosil 5C18 (ODS) column (Wako). TLC was performed with a Kieselgel 60 F_{254} (No. 5715, Merck).

Animal Material. The soft coral *L. schoedei* was collected on a coral reef off Nangou-cho (Miyazaki Prefecture, Japan) in May 1992 at a depth of 2−3 m and identified as *L. schoedei* Moser, 1919 by Mr. Yukimitsu Imahara. A voucher specimen (No. WMNH-94-INV-12) is on deposit at the Wakayama Prefectural Museum of Natural History (Wakayama, Japan).

Bioassays. The ichthyotoxicity assay was conducted using the mosquito fish, *O. latipes*. The extracts and crude fractions were assayed at 30 ppm by dissolution in 0.5 mL of EtOH and then diluted to 50 mL with distilled H_2O. Control tests were carried out with each test run. The toxicity was evaluated for producing lethality. The brine shrimp, *A. salina*, L, lethality assay was performed according to standard protocols.[13,14] The crude extracts and fractions were assayed at 30 ppm by dissolution in 200 μL of DMSO and then diluted to 2 mL with filtered sea water. LC_{50} values, in ppm, were determined only for lobophynin C (**3**).

Extraction and Isolation. Wet specimens (9.2 kg) were homogenized with MeOH (8.7 L) and left at room temperature for a few hours. After filtration, the residue was extracted with MeOH/$CHCl_3$ (1:1). These MeOH and MeOH/$CHCl_3$ extracts were combined and concentrated *in vacuo* to an aqueous suspension (2650 mL). The obtained suspension was extracted in succession with *n*-hexane (800 mL × 3) and EtOAc (400 mL × 3) . The organic layers were subsequently dried separately over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residues from both fractions (114.6 g and 9.9 g, respectively) showed ichthyotoxicity and lethality to brine shrimps and were combined and loaded onto a Sephadex LH-20 (750 g dry weight with 115 cm × 6.5 cm column) column and eluted with MeOH/$CHCl_3$ (1:1) to yield five fractions: fraction 1 (7.0 g), 2 (25.6 g), 3 (35.8 g), 4 (30.8 g), and 5 (4.0 g). The fourth fraction was chromatographed on a silica gel column. Elution was performed with *n*-hexane/acetone (98:2 → 0:100) to yield 16 fractions (fractions 6−21). Fraction 12 (850 mg) was chromatographed on Cosmosil 5C18 using MeOH−H_2O (80% → 100%) as the eluent to give 12 fractions (fractions 22−33). Fraction 24 was chromatographed on silica gel with *n*-hexane/acetone (90:10 → 0:100) to give 8 fractions (fractions 34−41). Fraction 36 was subjected to reversed-phase HPLC (70% MeOH−H_2O) to give lobophynin B (**2**) (3.3 mg, 0.00003%) and C (**3**) (7.1 mg, 0.00007%). Fraction 37 was purified by reversed-phase HPLC (70% MeOH−H_2O) to obtain lobophynin A (**1**) (2.2 mg, 0.00002%).

Lobophynin A (1): colorless oil; $[\alpha]^{22}_D$ −22.9° (*c* 0.12 $CHCl_3$); EIMS *m/z* $[M]^+$ 346 (8), 328 (30), 321 (22), 303 (13), 286 (21), 268 (35), 243 (39), 225 (40), 185 (36), 159 (41), 145 (43), 133 (69), 121 (52), 81 (67), 69 (41), 55 (42); HR positive-ion FAB MS *m/z* 347.2585, calcd for $C_{22}H_{35}O_3$ $[M + H]^+$ 347.2586; IR ($CHCl_3$) ν_{max} 3010, 1730, 1240, 910 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Tables 1 and 2.

Lobophynin B (2): colorless oil; $[\alpha]^{25}_D$ +122.6° (*c* 0.13 $CHCl_3$); EIMS *m/z* $[M]^+$ 360 (14), 342 (13), 318 (8), 300 (58), 282 (28), 175 (43), 161 (82), 148 (82), 133 (66), 119 (51), 93 (37), 81 (49), 69 (54), 55 (39); HR positive-ion FAB MS *m/z* 359.2223, calcd for $C_{22}H_{31}O_4$ $[M - H]^+$ 359.2222;[15] IR ($CHCl_3$) ν_{max} 3030, 1740, 1240, 910 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Tables 1 and 2.

Lobophynin C (3): colorless oil; $[\alpha]^{25}_D$ +109.3° (*c* 0.31 $CHCl_3$); EIMS *m/z* $[M]^+$ 346 (21), 328 (25), 288 (11), 204 (42), 161 (35), 149 (82), 135 (89), 121 (67), 109 (61), 91 (100), 81 (35), 69 (35), 55 (29); HR positive-ion FAB MS *m/z* 345.2063, calcd for $C_{21}H_{29}O_4$ $[M - H]^+$ 345.2066;[16] IR ($CHCl_3$) ν_{max} 3030, 1710, 1240, 930 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Tables 1 and 2.

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