Cytotoxic Cembranoid Diterpenes from a Soft Coral *Sinularia gibberosa*

Guoqiang Li,[†,‡] Yanling Zhang,[†,‡] Zhiwei Deng,[§] Leen van Ofwegen,[⊥] Peter Proksch,[▽] and Wenhan Lin*[,†]

State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100083, People's Republic of China, Chemistry and Molecular Engineering College, Qingdao University of Science and Technology, Qingdao 266042, People's Republic of China, Chemical and Test Center, Beijing Normal University, Beijing 100073, People's Republic of China, National Museum of Natural History Naturalis, 2300 RA Leiden, The Netherlands, and Institute of Pharmaceutical Biology, Heinrich-Heine University, D-40225 Duesseldorf, Germany

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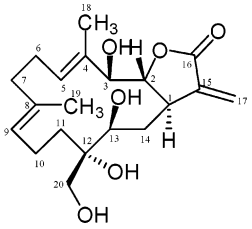
Five new α-methylene-γ-lactone-bearing cembranoid diterpenes, sinularolides A−E (**1**−**5**), along with 10 known cembranoids, were isolated from the soft coral *Sinularia gibberosa*. Their structures were determined by extensive spectroscopic (IR, MS, 2D NMR) data analysis. Compounds **2**−**5** showed moderate activity against selected tumor cell lines.

Cembranoids and their cyclized derivatives are the most abundant metabolites of soft corals and gorgonians.[1−6] They are produced as a defense against predators such as other corals and fishes and against settlement of microorganisms such as fungi or bacteria.[7,8] Previous bioassay results have shown that cembrane analogues possess significant biological activities, including ichthyotoxic,[22] cytotoxic, anti-inflammatory, and antiarthritic,[9,10] Ca-antagonistic,[11] and antimicrobial properties. Overall, the antitumor effects have been the most important activity of cembranoids reported so far. The soft coral genus *Sinularia* is a rich source of cembranoid diterpenes that has been extensively studied[12−22] and from which 13-, 14-, and 15-membered ring cembranoids were isolated.

Results and Discussion

A sample of species *S. gibberosa* (Tixier-Durivault) from the coral reef near Taiwan was investigated, and a number of monocyclic ring cembranes and oxygenated steroids were obtained.[23−28] During our study on the bioactive metabolites of marine organisms, the same soft coral, collected from the Bay of Sanya, Hainan Island of China, was selected for further chemical investigation. The EtOAc extract of this soft coral exhibited cytotoxicity against HL-60, Hela, BGC-823, MDA-MB-435, Bel-7402, and PC-3MIE8 tumor cell lines. Repeated column chromatography of this extract resulted in the isolation of 15 α-methylene-γ-lactone-containing cembranoid diterpenes, of which five (sinularolides A−E, **1**−**5**) were determined as new 14-membered cembranoids. The known cembranoids included sinulariol D (**6**),[19] mayolide A (**7**),[20] denticulatolide (**8**),[22] cembranolide B (**9**), cembranolide A (**10**),[22] two simple γ-lactonic cembranolides (**11** and **12**),[28,29] (3*E*,7*E*,11*E*)-18-hydroxy-3,7,11,15(17)-cembratetraen-16,14-olide (**13**),[30] lobophytol (**14**), and lobophytol acetate (**15**).[31] Compounds **6**−**10** were isolated from *S. mayi*, while **11**−**15** were obtained from the genus *Lobophytum*.

The molecular formula of sinularolide A (**1**) was found to be $C_{20}H_{30}O_6$ by HRFABMS (*m/z* 389.1935, calcd for $C_{20}H_{30}O_6Na$, 389.1934), implying six degrees of unsaturation. IR absorptions at 3421, 1756, and 1660 cm^{-1} suggested the presence of hydroxyl substituents and a α-methylene-γ-lactone group.[32] This assumption was supported by the NMR signals resonating at δ 5.86 (1H, d, *J* = 3.0 Hz, H-17a), 6.32 (1H, d, *J* = 3.0 Hz, H-17b), 169.4 (s, C-16), 138.2 (s, C-15), and 123.5 (t, C-17). The ^{1}H NMR spectrum of **1** further exhibited the signals for two olefinic methyl groups at δ 1.77 (3H, s, H-18) and 1.70 (3H, s, H-19), two olefinic protons at δ 5.45 (1H, dd, *J* = 5.5, 8.0 Hz, H-5) and 5.03 (1H, dd, *J* = 5.0, 7.5 Hz, H-9), oxymethylene protons at δ 3.50 (1H, d, *J* = 11.5 Hz, H-20a) and 3.54 (1H, d, *J* = 11.5 Hz, H-20b), and two oxymethine protons at δ 4.04 (1H, d, *J* = 8.0 Hz, H-3) and 3.91 (1H, brd, *J* = 10.0 Hz, H-13). These spectral features, in association with DQFCOSY correlations from H-13 to H-3, H-5 to H-7, and H-9 to H-11, as well as long-range COSY correlations



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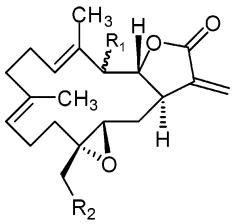
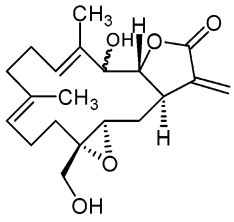


2. R_1 = β–OH, R_2 = OH
3. R_1 = α–OH, R_2 = OH
4. R_1 = H, R_2 = OH
5. R_1 = β– OH, R_2 = H



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* To whom correspondence should be addressed. Tel: +86-10-62062210. Fax: +86-10-82802724. E-mail: whlin@bjmu.edu.cn.
[†] Peking University.
[‡] Qingdao University of Science and Technology.
[§] Beijing Normal University
[⊥] National Museum of Natural History Naturalis.
[▽] Heinrich-Heine University.

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Table 1. ^{1}H NMR Data for Sinularolides A–E (**1**–**5**)

H	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
1	3.00 m	2.77 m	3.27 m	2.86 m	2.32 m
2	4.34 dd 6.5, 8.0	4.13 dd 7.0, 8.5	4.29 dd 1.5, 6.5	4.21 ddd 3.0, 6.5, 9.5	3.66 dd 7.5, 8.5
3	4.04 d 8.0	4.07 d 8.5	4.45 br	2.30 dd 9.5, 13.0; 2.61 brd 13.0	3.71 d 8.5
5	5.45 dd 5.5, 8.0	5.44 dd 5.0, 9.0	5.60 dd 4.0, 8.5	5.17 dd 5.5, 8.0	5.08 dd 4.5, 8.0
6	2.32 m	2.16 m	2.15 m	2.15 m	2.30 m
	2.48 m	2.48 m	2.54 m	2.40 m	2.32 m
7	2.18 m	2.08 m	2.18 m	2.12 m	1.87 m
	2.32 m	2.34 m	2.34	2.41 m	2.31 m
9	5.03 dd 5.0, 7.5	5.04 dd 6.5, 7.0	5.05 dd 7.0, 7.0	5.05 dd 7.0, 6.5	4.86 dd 4.5, 8.5
10	2.11 m	2.11 m	2.12 m	2.16 m	2.03 m
	2.49 m	2.29 m	2.21 m	2.23 m	2.07 m
11	1.58 m	1.21 ddd 3.5, 13.5, 14.0	1.28 m	1.35 ddd 3.0, 11.5, 14.0; 2.37 ddd 2.0, 6.5, 14.0	1.18 m
	1.68 m	2.41 ddd 3.0, 6.0, 14.0	2.38 m		1.94 m
13	3.91 brd 10.0	2.88 dd 3.5, 6.5	2.93 dd 3.5, 7.0	2.97 dd 4.0, 6.5	2.41 dd 2.0, 8.0
14	1.60 m	1.65 m	1.61 m	1.66 ddd 6.5, 8.5, 14.0	1.01 m
	1.80 m	1.84 ddd 3.0, 3.5, 14.5	1.78 ddd 3.5, 3.5, 14.5	1.88 ddd 4.0, 4.0, 14.0	1.54 ddd 2.0, 2.5, 14.5
17	5.86 d 3.0	6.01 d 3.0	5.97 d 3.0	5.94 d 3.0	6.17 d 3.0
	6.32 d 3.0	6.28 d 3.0	6.27 d 3.0	6.29 d 3.0	6.48 d 3.0
18	1.77 s	1.69 s	1.72 s	1.73 s	1.63 s
19	1.70 s	1.61 s	1.61 s	1.62 s	1.43 s
20	3.50 d 11.5	3.58 d 12.5	3.58 d 12.0	3.61 d 12.0	1.09 s
	3.54 d 11.5	3.83 d 12.5	3.82 d 12.0	3.85 d 12.0	

[a] Measured in $CDCl_3$, [b] Measured in C_6D_6.

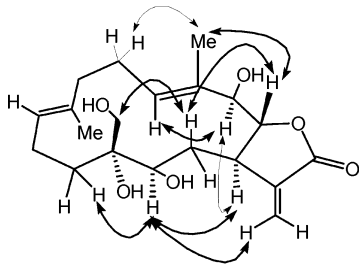
Table 2. ^{13}C NMR Data for Sinularolides A–E (**1**–**5**)

C	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
1	38.1	41.4	36.9	44.7	42.0
2	83.2	82.4	81.8	79.8	81.6
3	79.7	80.2	73.4	45.2	80.9
4	132.0	131.7	131.1	129.4	132.4
5	131.2	131.6	127.3	130.3	130.7
6	23.6	24.5	24.3	24.8	24.5
7	37.5	38.5	38.2	38.8	38.7
8	137.3	134.8	134.6	135.0	134.0
9	124.3	124.2	124.0	124.4	124.6
10	21.6	23.6	23.1	23.3	24.6
11	32.5	32.8	32.5	32.7	38.2
12	75.4	62.9	62.6	62.8	59.1
13	71.9	63.2	63.0	63.0	62.9
14	34.9	31.4	32.7	31.7	31.5
15	138.2	138.8	139.7	139.8	139.8
16	169.4	169.4	170.0	169.8	168.3
17	123.5	123.9	122.8	122.9	122.8
18	13.1	12.5	15.2	17.1	11.9
19	17.3	15.6	15.6	15.6	14.8
20	67.5	61.8	61.7	62.1	16.3

[a] Measured in $CDCl_3$. [b] Measured in C_6D_6.

between H-1/H_2-17, H_3-18/H-5, and H_3-19/H-9, suggested **1** to have a 14-membered cembrane-type diterpenoid skeleton. The DQFCOSY and HMQC spectra connected all protons and the protonated carbons in the molecule (Tables 1 and 2). The ^{13}C NMR and DEPT analysis showed two methyls, six sp^3 methylenes, one sp^2 methylene, four sp^3 methines, two sp^2 methines, one sp^3 quaternary carbon, and four sp^2 quaternary carbons involving five oxygen-bearing carbons at δ 83.2 (d, C-2), 79.7 (d, C-3), 75.4 (s, C-12), 71.9 (d, C-13), and 67.5 (t, C-20). With exception of the position at C-2 assigned to the γ-carbon of γ-lactone, the remaining four oxygenated carbons were assumed to be substituted with hydroxyl groups due to the degrees of molecular unsaturation, which were fully accounted for by a bicyclic system, three vinyl groups, and a carbonyl group. Analysis of COSY and HMBC correlations, such as the HMBC correlations of H_2-20 to C-11, C-12, and C-13; H-3 to C-18 (δ 13.1, q), C-5, and C-1; and H-13 (δ 3.91, brd) to C-20 and C-1, led to the assignment of the hydroxyl groups at C-3, C-12, C-13, and C-14. The location of the two double bonds at C-4/C-5 and C-8/C-9 and the fusion of the α-methylene-γ-lactone unit at C-1/C-2 were clarified by analysis of the HMBC spectrum. The geometry of the trisubstituted olefins was determined as being *E*-configurated due to the ^{13}C chemical shift values of the olefinic methyl signals for C-18 and C-19 (less than 20 ppm)[32] and due to the NOESY correlations between H-5 and H-3 and between H-9 and H-7. The *J* value between H-1 and H-2 (6.5 Hz) was comparable to that of lobolide and its derivatives,[30] implying a *trans*-fused lactone ring. The NOESY correlations between H-1/H-3, H-1/H-13, H-3/H-5, H-3/H_3-18, H-3/H-14b (δ 1.80), H-20/H-14b, H-13/H-17 (δ 5.86), and H-13/H-11 (δ 1.68) confirmed the geometry of the fused lactone ring and indicated that H-1, H-3, and H-13 are located on the same side of the ring system, whereas H-2 and H_2-20 are oriented toward the opposite side.

The HRFABMS and NMR spectral data of sinularolide B (**2**) provided the molecular formula as $C_{20}H_{28}O_5$, which is 18 mass units smaller than that of **1**. Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of **1** and of (7*E*,11*E*)-13,18-dihydroxy-3,4-epoxy-7,11,15(17)-cembratrien-16,14-olide (**16**)[30] showed that **2** shared most spectral features of **1** and **16**. A detailed 2D NMR (COSY, HMQC, and HMBC) analysis of **2** revealed that its planar structure was the same as that of **16**, but the chemical shifts of the epoxide group at C-12 (δ 62.9, s) and C-13 (δ 63.2, d) in **2** assigned



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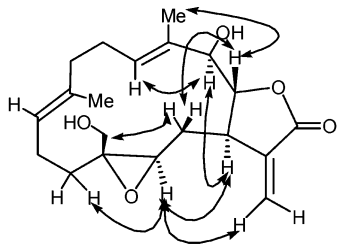

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Figure 1. Key NOE correlations of **1** and **2**.

by DEPT differed when compared to those of **16** [δ 63.0 (d), 61.9 (s)].[30] This evidence suggested that the two compounds may possess a different geometry with regard to the stereochemistry of the epoxide group. Analysis of the NOESY spectrum of **2** showed the presence of NOE correlations between H-13 (δ 2.88, dd)/H-17a (δ 6.01, d), H-13/H-1 (δ 2.77, m), H-13/H-11 (δ 1.21, ddd), H-1/H-3 (δ 4.07, d), H-2 (δ 4.13, dd)/H_3-18 (δ 1.69, s), H-2/H-14a(δ 1.84, ddd), and H-20a (δ 3.58, d)/H-14b (δ 1.65, m). NOESY also indicated that **2** possesses the same geometry of the fused lactone ring and the same configuration of the hydroxyl group at C-3, while the geometry of the epoxide group was determined as the *trans*-form. The absence of a NOE correlation between H-13 and H_2-20 supported the supposed configuration of the epoxide group, since the *cis*-form of the epoxide group as present in stolonidiol and in a dollabelane diterpenoid[32] is characterized by showing the respective NOE correlation. The ^{1}H and ^{13}C chemical shifts from C-1 to C-5 in **2** were found to be almost identical compared to those of **16**, indicating the same relative configuration at C-3 for both compounds, which had previously not been assigned for **16** in the literature.[30] Accordingly, the structure of **2** was determined to be an epimer of **16** at C-13.

The molecular formula of sinularolide C (**3**) was identical to that of **2** as indicated by HRFABMS. The IR and ^{1}H and ^{13}C NMR spectra of **3** were also comparable to those of **2**, with the exception of the ^{13}C chemical shift of C-3 in **3**, which resonated at δ 73.4 (d) in contrast with δ 80.2 (d, C-3) in **2**, and the signal of H-3 (δ 4.45, brs), which appeared as a broad singlet instead of a doublet as observed for **2**. The remaining NMR data of **3** were almost identical to those of **2**, indicating the same relative configurations at C-1, C-2, C-12, and C-13 for both compounds. The NOE correlation between H-2 (δ 4.29, dd) and H-3 and between H-3 and H_3-18 (δ 1.72, s) suggested that H-3 is in a β-orientation. The structure of **3** was thus determined as an epimer of **2** at C-3.

The HRFABMS of sinularolide D (**4**) indicated the molecular formula $C_{20}H_{28}O_4$, which is 16 mass units smaller than that of **3**. Comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) of both compounds showed that **4** exhibited the same framework of an α-methylene-γ-lactone-containing cembrane-type diterpenoid as **3**, with the exception of signals assigned to C-3, where the hydroxyl methine in **3** was replaced by a methylene [δ_H 2.30 (dd, J = 9.5, 13.0 Hz) and 2.61 (brd, J = 13.0 Hz), δ_C 45.2 (t)] in **4**. The observed COSY correlation between H-3 and H-2 (δ_H 4.21, ddd, J = 3.0, 6.5, 9.5 Hz) and the HMBC correlations from H-3 to C-18 (δ_C 17.1, q), C-5 (δ_C 130.3, d), and C-1 (δ_C 44.7, d) further confirmed the location of the new methylene group. The relative stereochemistry of **4** was in agreement with that of **3** due to the similar NOE correlations [H-13 (δ 2.97, dd)/H-17a (δ 5.94, d), H-13/H-1 (δ 2.86, m), H-13/H-11 (δ 1.35, ddd), H-1/H-3α (δ 2.30, dd), H-2/H_3-18 (δ 1.73, s), H-2/H-14β (δ 1.88, ddd), and H_2-20 (δ 3.61, 3.85)/H-14β] and the comparable NMR data of both compounds.

Sinularolide E (**5**) had the molecular formula $C_{20}H_{28}O_4$ as determined by HRFABMS (*m/z* 355.1878, calcd for $C_{20}H_{28}O_4Na$, 355.1879). The IR and ^{1}H and ^{13}C NMR spectra of **5** closely resembled those of **2**, and the ^{13}C NMR data for the basic skeleton of **5** were in agreement with those reported for one of the epimeric 3-acetoxy derivatives from the coral *Lobophytum crassum*,[29,33] indicating a α-methylene-γ-lactone-fused cembranoid. However, the ^{1}H NMR and DEPT spectra of **5** exhibited an additional tertiary methyl group [δ 1.09 (3H, s, H-20), 16.3 (q, C-20)], which replaced the methyleneoxy group (C-20) of **2**. The HMBC correlations of H_3-20 to C-11 (δ 38.2, t) and to the trisubstituted epoxide carbons at δ 59.1 (s, C-12) and 62.9 (d, C-13) suggested that **5** is a 20-dehydroxyl derivative of **2**. The relative stereochemistry of **5** was determined to be identical to that of **2** based on the $J_{H-1/H-2}$ value (7.5 Hz) and the observed NOE correlations between H-3/H-1, H-3/H-5, H-2/H_2-14, and H_2-14/H_3-20.

Table 3. Cytotoxicity Data of Sinularolides A−E (**1**−**5**)[a]

		IC_{50} (μg/mL)				
cell line	method	**1**	**2**	**3**	**4**	**5**
HL-60	MTT	NA	5.2	5.1	2.3	6.0
BGC-823	SRB	NA	6.3	5.2	6.1	8.6
MDA-MB-435	SRB	NA	8.0	7.7	NA	2.1

[a] Results are expressed as IC_{50} in μg/mL; NA: IC_{50} > 10 μg/mL.

Sinularolides A−E (**1**−**5**) were tested for in vitro cytotoxicity against cultured human tumor cell lines HL-60, Bel-7402, and Hela. The bioassay results showed that **2**−**4** possess moderate cytotoxicity toward the selected tumor cell lines (Table 3). The primary bioassay results suggested that the epoxide unit at C-12/C-13 is required for the observed activity against the tested cell lines.

The possible biogenetic relationship of the cembranoid diterpenes from this soft coral is included as Supporting Information. Geranylgeranyl pyrophosphate[30] is considered to be the precursor that, through a series of ring cyclizations, dehydrogenations, epoxidation, hydroxylations, double-bond rearrangements, oxidated ring cleavages, and cyclic peroxidization, would yield the cembranoids isolated from the soft coral.

Experimental Section

General Experimental Procedures. Optional rotations were measured with a Perkin-Elmer 243B polarimeter. IR spectra were determined on Thermo Nicolet Nexus 470 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on an Avance-500 FT 500 MHz NMR spectrometer using TMS as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants (J) are reported in hertz (Hz). EIMS was performed on a Bruker APEX II mass spectrometer, and ESIMS were recorded in the MDS-SCIEX-QSTAR (ABI, USA). HRFABMS were obtained from GCT-MS instruments. Column chromatography was carried out with Si gel (200−300 mesh), and GF_{254} Si gel for TLC was provided by Qingdao Marine Chemistry Co. Ltd.

Animal Material. The soft coral *Sinularia gibberosa* was collected from the inner coral reef at a depth of 8 m in Hainan Island of China in May 2003 and frozen immediately. The specimen was identified by one of the authors (L.v.O.). Voucher specimens are deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China (HSE-16), and at the National Museum of Natural History Naturalis, The Netherlands (RMNH Coel 32244).

Extraction and Isolation. The frozen soft coral *S. gibberosa* (2.5 kg) was homogenized, then extracted with MeOH. The MeOH extract (55.0 g) was partitioned between H_2O and petroleum ether, EtOAc, and *n*-butanol. The EtOAc fraction (11.5 g) was subjected to Si gel column chromatography eluting with a gradient of acetone−petroleum ether to obtain five fractions (A−E). Fraction B (20:1, 1.4 g) was separated and purified on a Si gel column by eluting with petroleum ether−acetone (15:1) to obtain **6** (18.9 mg), **8** (6.5 mg), and **9** (4.2 mg). Fraction D (5:1, 1.1 g) was treated in the same way as fraction B by eluting with petroleum ether−EtOAc (4:1) to yield **10** (4.4 mg), **11** (65.5 mg), **12** (3.5 mg), **13** (80.8 mg), **14** (2.0 mg), **15** (14.6 mg), **7** (63.6 mg), and **5** (2.0 mg). Fraction E (2:1, 2.1 g) was chromatographed on a Si gel column and eluted with a gradient of petroleum ether−acetone of increasing polarity

from 20:1 to 5:1 to yield **4** (5.0 mg), **3** (48.1 mg), **2** (100.0 mg), and **1** (8.3 mg).

Sinularolide A (1): colorless oil; $[\alpha]_D^{25}$ +2.17° (*c* 0.05, $CHCl_3$); IR (KBr) ν_{max} 3421, 2927, 1756, 1660, 1437, 1271, 1058 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)ESIMS *m/z* 365 $[M - H]^-$ (100), 317 (44), 253 (19), 223 (27); HRFABMS *m/z* 389.1935 (calcd for $C_{20}H_{30}O_6Na$, 389.1934).

Sinularolide B (2): colorless crystal; mp 137−138 °C; $[\alpha]_D^{25}$ −134.3° (*c* 0.05, $CHCl_3$); IR (KBr) ν_{max} 3441, 2924, 1762, 1660, 1436, 1263, 1140, 1045 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)ESIMS *m/z* 347 $[M - H]^-$ (100), 329 (13), 317 (10), 311 (9), 299 (8), 273 (5), 123 (7), 111 (18); HRFABMS *m/z* 371.1826 (calcd for $C_{20}H_{28}O_5Na$, 371.1829).

Sinularolide C (3): colorless oil; $[\alpha]_D^{25}$ −56.3° (*c* 0.07, $CHCl_3$); IR (KBr) ν_{max} 3425, 2926, 2856, 1762, 1663, 1441, 1269, 1144, 1043 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)ESIMS *m/z* 347 $[M - H]^-$ (57), 329 (6), 317 (100), 299 (12), 273 (5); HRFABMS *m/z* 371.1833 (calcd for $C_{20}H_{28}O_5Na$, 371.1829).

Sinularolide D (4): colorless oil; $[\alpha]_D^{25}$ −31.6° (*c* 0.04, $CHCl_3$); IR (KBr) ν_{max} 3420, 2927, 2850, 1764, 1663, 1446, 1268, 1147, 1040 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 355 $[M + Na]^+$ (50), 350 $[M + NH_4]^+$ (66), 333 $[M + H]^+$ (100); HRFABMS *m/z* 355.1879 (calcd for $C_{20}H_{28}O_4Na$, 355.1880).

Sinularolide E (5): colorless oil; $[\alpha]_D^{25}$ −29.7° (*c* 0.08, $CHCl_3$); IR (KBr) ν_{max} 3487, 2918, 2850, 1763, 1661, 1462, 1383, 1261, 1138, 1085 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)ESIMS *m/z* 331 $[M - H]^-$ (54), 315 (27), 297 (29), 271 (21); HRFABMS *m/z* 355.1878 (calcd for $C_{20}H_{28}O_4Na$, 355.1879).

Cytotoxicity Assays. The cytotoxic properties of the compounds were tested using human cancer cell lines, including HL-60 (human leukemic cancer cell), Hela (human cervical cancer cell), BGC-823 (human gastric cancer cell), MDA-MB-435 (human breast cancer cell), Bel-7402 (human hepatoma cancer cell), and PC-3MIE8 (human prostate cancer cell). The bioassay procedure was the same as described previously.[34]

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Supporting Information Available: ^1H and ^{13}C NMR, IR, and MS spectra of compounds **1**−**5**; scheme of a possible biogenetic relationship of the cembranoid diterpenes from this soft coral. This material is available free of charge via the Internet at http//pubs.acs.org.

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