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Cembranoids from the Soft Coral *Sinularia rigida* with Antifouling Activities

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Supporting Information

ABSTRACT: Chemical examination of the soft coral *Sinularia rigida* resulted in the isolation of 12 new cembranoids, namely, sinulariols T−Z_5 (**1**−**12**), together with a known analogue, **13**. Their structures were determined on the basis of 1D and 2D NMR (COSY, HSQC, HMBC, and NOESY) spectroscopic analyses in association with MS and IR data. Compounds **7** and **13** showed potent antifouling activity for the inhibition against the barnacle *Balanus amphitrite* and moderate inhibition against *Bugula neritina*. The primary structure−activity relationship is discussed.

KEYWORDS: *soft coral, Sinularia rigida, sinulariols T−Z_5, structural elucidation, antifouling activity*

INTRODUCTION

Soft corals of the genus *Sinularia* have been reported to contain a rich diversity of cembranoid-type diterpenoids, with a characteristic 14-membered carbocyclic skeleton.[1−3] Some of the typical diterpenoids were reported to have a wide range of pharmacological activities, such as cytotoxic, anti-inflammatory, and antibacterial activities.[1] Ecologically, the chemical constituents derived from *Sinularia* soft corals play a very important role in the survival of the soft corals in their natural environment, including feeding deterrence and competition (antifouling and allelopathy).[4,5] Soft corals that use a chemical defense strategy to keep their body surfaces free of fouling are regarded to produce antifouling secondary metabolites.[6] For example, some cembranoids from the Caribbean sea whip *Eunicea knighti* were reported to have potent antiquorum sensing activity and antibacterial activities against marine bacteria associated with heavily fouled surfaces.[7] Our preliminary investigation of a specimen of *Sinularia rigida* collected in the South China Sea revealed that some cembranoids showed potent inhibitory activities against the larval settlement of the barnacles *Balanus amphitrite* and *Bugula neritina*.[8] Other events reported in the literature also suggested soft corals to be promising sources of environmentally friendly antifouling agents. Although numerous cembranoids were isolated, new structural patterns of secondary metabolites are continually uncovered from various *Sinularia* species that inhabit distinct geographic locations. These facts imply that the number of cembranoid families derived from *Sinularia* and other marine organisms is unlimited. Previously, we reported a series of oxygenated cembranoids, sinulariols A−S, isolated from *S. rigida*.[8] Further examination of the minor diterpenoids from the same species led to the isolation of 12 new cembranoids (**1**−**12**) (Figure 1). In this paper we report the structural elucidation of these new compounds in addition to the antifouling evaluation.

MATERIALS AND METHODS

General Method. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were measured on Bruker Avance 400, 500, and 600 NMR spectrometers (400, 500, and 600 MHz for ^{1}H and 100, 125, and 150 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) referring to the solvent peaks δ_H 7.26 and δ_C 77.0 for $CDCl_3$ and δ_H 2.50 and δ_C 40.0 for DMSO-d_6 and coupling constants in hertz. Electrospray ionization mass spectrometry (ESIMS) and high-resolution electrospray ionization mass spectrometry (HRESIMS) spectra were obtained with a Thermo Scientific LTQ Orbitrap XL instrument. Si gel (160−200 and 200−300 mesh, Qingdao Marine Chemistry Co. Ltd.) and ODS (50 μm, YMC) were used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analyses. HPLC chromatography was performed on an Alltech instrument (426-HPLC pump) equipped with an Alltech uvis-200 detector at 210 nm and semipreparative reversed-phased columns (YMC-packed C8, 5 μm, 250 mm × 10 mm i.d.).

Animal Material. Soft coral *S. rigida* was collected from the inner coral reef at a depth of 10 m in Sanya Bay, Hainan Island of China, in May 2004. The fresh samples were frozen immediately. The specimen was identified by Dr. Leen van Ofwegen (National Museum of Natural History Naturalis). The coral (HSF-37) was deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, People's Republic of China, and also at the National Museum of Natural History Naturalis, The Netherlands.

Extraction and Isolation. The frozen sample (wet mass 2.5 kg) was homogenized and then extracted with 90% EtOH. The concentrated extract was desalted by being dissolved in MeOH to yield a residue (70.0 g). This residue was successively partitioned between H_2O and petroleum ether (PE; 60−90 °C), EtOAc, and 1-

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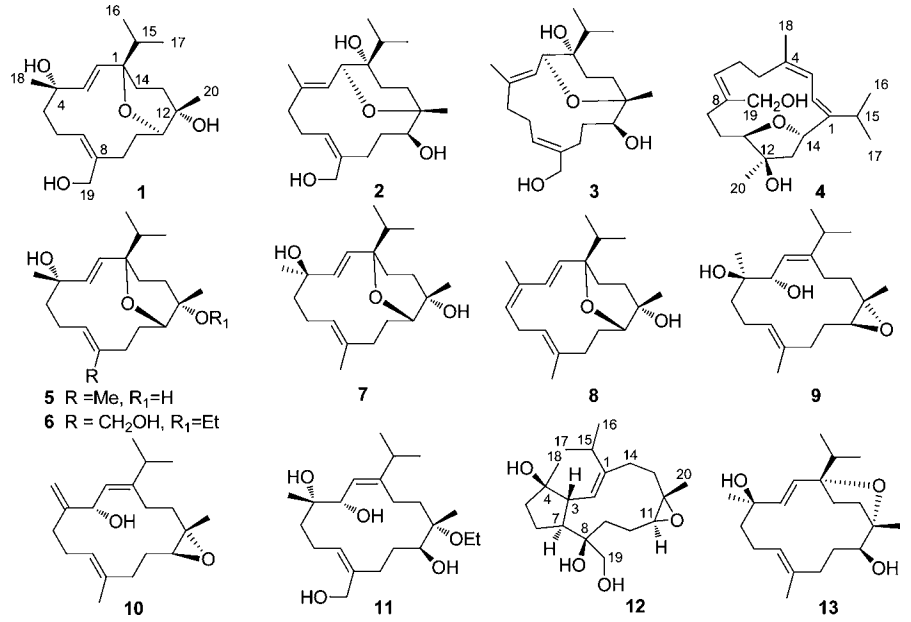

Figure 1. Structures of cembranoids isolated from *S. rigida*.

butanol to yield the corresponding extracts. The EtOAc fraction (5.5 g) was subjected to Si gel vacuum liquid chromatography (VLC), eluting with a gradient of PE (60−90 °C)−EtOAc (10:1, 5:1, 3:1, 1:1, 0:1) to obtain five subfractions (SF1 to SF5). Analyses of the ^{1}H NMR spectra of the subfractions indicated SF2 to SF5 were enriched with cembranoids. SF-2 (56.7 mg) was chromatographed on an ODS column eluting with MeOH−H_2O (65%) to yield **9** (2.4 mg), while for SF3 (37.8 mg) the same protocol as that for SF1 was followed to obtain **5** (0.7 mg) and **8** (5.0 mg). Using the same protocol as that for SF1, **9** (1.8 mg), **10** (3.7 mg), and **6** (1.5 mg) were separated from SF4 (60.5 mg). SF5 (70.1 mg) was separated upon semipreparative HPLC (C_{18}, 5 μm) with a mobile phase of 45% ACN−H_2O to yield **4** (1.9 mg), **13** (17.3 mg), **7** (4.0 mg), **2** (1.3 mg), **3** (1.9 mg), **11** (2.3 mg), **12** (1.6 mg), and **1** (2.8 mg).

*Data for sinulariol T (**1**):* colorless oil; $[\alpha]_D^{22}$ −41.4 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} 200 nm; IR (KBr) ν_{max} 3382, 2962, 2928, 2873, 1626, 1450, 1374, 1104, 1053, 1021 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 361.2336 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*Data for sinulariol U (**2**):* colorless oil; $[\alpha]_D^{21}$ +23.4 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} 200 nm; IR (KBr) ν_{max} 3331, 2959, 2875, 1659, 1549, 1452, 1003 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 361.2341 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*Data for sinulariol V (**3**):* colorless oil; $[\alpha]_D^{21}$ −46.1 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3402, 2929, 2872, 1660, 1450, 1381, 1114, 1075, 1029 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 361.2340 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*Data for sinulariol W (**4**):* colorless oil; $[\alpha]_D^{23}$ +25.6 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 202, 239 nm; IR (KBr) ν_{max} 3345, 2960, 2925, 2856, 1673, 1450, 1376, 1294, 1242, 1111, 1008 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 343.2240 [M + Na]$^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2244).

*Data for sinulariol X (**5**):* colorless oil; $[\alpha]_D^{24}$ −18.3 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} 202 nm; IR (KBr) ν_{max} 3380, 2962, 2931, 2863, 1623, 1465, 1370, 1253, 1163 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 345.2401 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2400).

*Data for sinulariol Y (**6**):* colorless oil; $[\alpha]_D^{20}$ −35.6 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} 202 nm; IR (KBr) ν_{max} 3343, 2962, 2932, 2872, 1712, 1566, 1454, 1377, 1136, 1111, 1078, 1018 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 389.2643 [M + Na]$^+$ (calcd for $C_{22}H_{38}O_4Na$, 389.2662).

*Data for sinulariol Z (**7**):* colorless oil; $[\alpha]_D^{23}$ −61.6 (*c* 0.22, $CHCl_3$); UV (MeOH) λ_{max} 204 nm; IR (KBr) ν_{max} 3340, 2956, 1703, 1451, 1380, 1134 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 345.2397 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2400).

*Data for sinulariol Z_1 (**8**):* colorless oil; $[\alpha]_D^{24}$ −33.0 (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} 241 nm; IR (KBr) ν_{max} 3431, 2964, 2935, 2873, 1627, 1456, 1379, 1135 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 305.2462 [M + H]$^+$ (calcd for $C_{20}H_{33}O_2$, 305.2475).

*Data for sinulariol Z_2 (**9**):* colorless oil; $[\alpha]_D^{23}$ −22.4 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} 203 nm; IR (KBr) ν_{max} 3388, 2961, 2928, 2858, 1715, 1460, 1381, 1287, 1077 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 345.2398 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2400).

*Data for sinulariol Z_3 (**10**):* colorless oil; $[\alpha]_D^{20}$ +16.3 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} 207 nm; IR (KBr) ν_{max} 3338, 2959, 2932, 2872, 1723, 1443, 1386, 1091, 1008 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 327.2297 [M + Na]$^+$ (calcd for $C_{20}H_{32}O_2Na$, 327.2295).

*Data for sinulariol Z_4 (**11**):* colorless oil; $[\alpha]_D^{23}$ −32.3 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3367, 2965, 2928, 2875, 1608, 1460, 1376, 1137, 1110, 1080, 1016 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 407.2773 [M + Na]$^+$ (calcd for $C_{22}H_{40}O_5Na$, 407.2768).

*Data for sinulariol Z_5 (**12**):* colorless oil; $[\alpha]_D^{20}$ +47.8 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3418, 2958, 2931, 2868, 1460, 1381, 1122, 1065 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m*/*z* 361.2342 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

Antifouling Bioassay. Adults of the barnacle *Ba. amphitrite* Darwin which were exposed to air for more than 6 h were collected from the intertidal zone in Hong Kong (22°19′ N, 114°16′ E) and then were placed in a container filled with fresh 0.22 μm filtered seawater (FSW) to release nauplii. The collected nauplii were reared to cyprid stage according to the method described by Thiyagarajan et al.[14] When kept at 26−28 °C and fed with *Chaetoceros gracilis*, larvae developed to cyprids within four days. Fresh cyprids were used in the tests. Adults of *Bu. neritina* were collected from submerged rafts at the fish farms in Yung Shue O, Hong Kong (114°21′ E, 22°24′ N), and larvae were obtained according to the method described by Dobretsov et al.[9]

The anti-larval-attachment activity was determined using cyprid larvae of *Ba. amphitrite* and *Bu. neritina*. Larval settlement assays were

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Table 1. ^{1}H NMR Data for Sinulariols T−Z_1 (1−8)

position	1[a]	2[a]	3[a]	4[a]	5[a]	6[b]	7[a]	8[a]
2	5.49 d (16.0)	4.45 d (10.9)	4.36 d (8.9)	6.55 br d (11.2)	5.34 d (16.5)	5.44 d (16.4)	5.45 d (16.0)	5.24 d (16.1)
3	6.06 d (16.0)	5.48 d (10.9)	5.62 d (8.8)	6.09 br d (11.2)	6.03 d (16.5)	6.20 d (16.4)	5.94 d (16.0)	6.72 d (16.1)
5	1.81 ddd (13.2, 8.6, 1.5)	2.28 m	2.18 m	2.20 m	1.91 m	1.98 m	1.89 m	5.58 dd (7.9, 2.8)
	1.70 m	2.06 m	1.92 dd (11.5, 11.0)	2.30 m	1.55 m	1.63 m	1.62 m	
6	2.55 m, 2.13 m	2.42 m, 2.22 m	2.10 m, 1.97 m	2.36 m, 2.19 m	2.46 m, 2.06 m	2.58 m, 2.12 m	2.23 m, 2.10 m	3.09 ddd (13.1, 12.5, 2.0)
								2.55 ddd (13.1, 8.5, 2.5)
7	5.21 dd (9.0, 5.4)	5.20 dd (10.5, 5.7)	5.54 dd (8.3, 7.3)	5.74 dd (6.0, 4.0)	5.21 dd (7.3, 6.9)	5.39 dd (9.6, 5.6)	5.17 t (7.0)	5.25 dd (2.5, 2.0)
9	2.37 m	2.44 ddd (12.4, 5.6, 3.0)	2.18 m	2.42 m	2.21 m	2.32 m	2.21 m	2.28 ddd (12.9, 3.0, 3.0)
	2.15 m	2.02 ddd (12.4, 12.0, 2.0)	1.99 m	2.27 m	1.98 m	2.11 m	2.00 m	1.93 ddd (12.9, 12.9, 4.3)
10	1.85 m, 1.81 m	1.81 m	2.02 m	1.81 m	1.80 m	2.00 m	1.84 m, 1.37 m	1.85 m, 1.43 m
		1.36 dddd (14.4, 9.0, 6.0, 1.8)	1.24 m	1.73 m	1.38 m	1.45 m		
11	3.49 dd (8.6, 4.9)	3.84 d (8.9)	3.56 d (10.6)	3.45 br d (8.1)	3.16 dd (6.0, 2.0)	3.41 dd (7.0, 1.5)	3.16 dd (7.0, 2.5)	3.18 br d (5.4)
13	1.68 m, 1.70 m	1.93 m, 1.80 m	1.98 m, 1.60 m	2.32 m, 1.77 m	1.68 m, 1.55 m	1.85 m, 1.56 m	1.68 m, 1.59 m	1.50 m
								1.64 m
14	1.92 dt (13.8, 3.7)	1.64 m	1.79 m	4.62 br t (7.3)	1.58 m	2.05 m	1.60 m	1.48 m
	1.41 dt (13.8, 3.8)	1.58 m	1.64 m		1.64 m	1.52 m	1.70 m	1.64 m
15	2.40 dq (6.8)	1.81 dq (6.8)	1.66 dq (6.8)	2.69 dq (6.5)	1.77 dq (6.8)	1.82 dq (6.8)	1.76 dq (7.0)	1.67 dq (6.8)
16	0.94 d (6.8)	0.91 d (6.8)	0.85 d (6.8)	0.99 d (6.5)	0.79 d (6.8)	0.78 d (6.8)	0.78 d (7.0)	0.85 d (6.8)
17	0.80 d (6.8)	0.94 d (6.8)	0.91 d (6.8)	1.08 d (5.8)	0.87 d (6.8)	0.92 d (6.8)	0.86 d (7.0)	0.81 d (6.8)
18	1.31 s	1.64 s	1.85 s	1.84 s	1.37 s	1.38 s	1.31 s	1.80 s
19	4.29 d (11.1)	4.22 d (11.7)	4.15 d (12.5)	4.40 d (11.6)	1.65 s	4.35 d (12.0)	1.61 s	1.72 s
	4.09 d (11.1)	3.99 d (11.7)	4.03 d (12.5)	4.02 d (11.6)		4.05 d (12.0)		
20	1.23 s	1.02 s	1.18 s	1.25 s	1.16 s	1.12 s	1.15 s	1.16 s
−OH						3.43, 3.39 dq (8.5,7.0)		
						1.13 t (7.0)		

[a]Recorded in $CDCl_3$ at 500 MHz. [b]Recorded in DMSO-d_6 at 600 MHz.

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Table 2. ^{1}H NMR Data for Sinulariols Z_2–Z_5 (9–12)

position	9[b]	10[a]	11[a]	12[a]
2	5.31 d (9.8)	5.08 d (8.0)	5.38 d (9.7)	4.93 dd (9.9, 1.8)
3	4.26 d (9.8)	4.67 d (8.0)	4.38 d (9.7)	2.55 t (9.5)
5	2.07 m	2.45 m	2.12 m	1.68 m
	1.48 m	2.08 m	1.64 m	2.25 m
6	2.30 m, 2.08 m	2.34 m, 2.25 m	2.38 m, 2.20 m	2.00 m, 1.80 m
7	5.37 dd (7.3, 5.6)	5.27 t (6.3)	5.54 br d (9.0)	2.28 m
9	2.32 m	2.32 m	2.31 m	1.97 m
	2.21 m	2.12 m	2.20 m	1.50 m
10	1.98 m, 1.54 m	2.10 m, 1.35 m	2.07 m, 1.47 m	2.29 m, 1.21 m
11	2.78 dd (9.2, 2.4)	2.67 dd (10.0, 3.0)	3.93 d (9.5)	3.22 dd (10.4, 5.2)
13	2.19 m	2.11 m	1.89 ddd (14.6, 9.3, 9.3)	2.16 m
	1.02 m	1.08 m	1.54 m	1.54 m
14	2.14 m	2.12 m	2.47 m	2.29 m
	1.94 m	1.87 ddd (13.0, 12.4, 5.8)	1.72 m	2.09 m
15	2.27 m	2.26 m	2.25 m	2.91 dq (7.0)
16	1.02 d (6.8)	1.041 d (6.8)	1.01 d (7.0)	0.99 d (7.0)
17	1.01 d (6.8)	1.037 d (6.8)	1.04 d (7.0)	1.03 d (7.0)
18	1.05 s	5.07 br s, 4.95 br s	1.05 s	1.133 s
19	1.66 s	1.66 s	4.26 d (12.2)	3.79 d (10.9)
			3.94 d (12.2)	3.46 d (10.9)
20	1.25 s	1.26 s	1.07 s	1.134 s
−OEt			3.47 dq (8.5, 7.0)	
			3.34 dq (8.5, 7.0)	
			1.16 t (7.0)	

[a]Recorded in $CDCl_3$ at 500 MHz. [b]Recorded in DMSO-d_6 at 600 MHz.

performed using 24-well polystyrene plates (Becton Dickinson 353047). Test compounds were first dissolved in a small amount of dimethyl sulfoxide (DMSO) and then diluted with filtered FSW to achieve final concentrations at 25 and 5 μg/mL. After the primary test of antifouling effects, the active compounds were diluted to 25.0, 10.0, 5.0, and 1.0 μg/mL for further test. About 15–20 competent larvae (cyprid stage *Ba. amphitrite* or newly released *Bu. neritina*) were added to each well with 1 mL of testing solution in three replicates, and wells containing larvae in FSW with DMSO only served as a control. Then the plates were incubated for 48 h at 23 °C. The effects of the test samples against biofouling were determined by examining the plates under a dissecting microscope to check for (1) attached larvae and (2) unattached larvae, as well as (3) any possible toxic effects of the treatments, such as death or paralysis of larvae, which were also recorded. The percentage of larval settlement was determined by counting the number of the attached individuals and expressed as a proportion of the total number of larvae added into each well. A concentration–response curve was then plotted, and a tread line was constructed for each compound. EC_{50} was calculated as the concentration where 50% of the larval population was inhibited to settle as compared to the control, while LC_{50} was calculated as the concentration where 50% of the larval population was dead.

RESULTS AND DISCUSSION

Repeated column chromatography of the EtOAc-soluble fraction obtained from the EtOH extract of the soft coral *S. rigida* led to the isolation and characterization of 13 cembranoids.

The molecular formula of sinulariol T (**1**) was determined as $C_{20}H_{34}O_4$ on the basis of the HRESIMS (*m*/*z* 361.2336 $[M + Na]^+$) and NMR data. Analysis of 1D and 2D NMR (COSY, HMQC, and HMBC) determined the gross structure of **1** to be identical to that of sinulariol J,[8] indicating a stereoisomer of the known analogue. Comparison of NMR data and NOE correlation ascertained that **1** shared the same relative configurations and olefinic geometries as those of sinulariol J regarding the region from C-1 to C-8. However, compound **1** showed NOE cross-peaks from H-11 to H-15, H_3-16, and H_3-18 (Figure 2), revealing H-11 of **1** to be oriented in the same face as the isopropyl group. Thus, compound **1** was determined as a C-11 epimer of sinulariol J.

Sinulariol U (**2**) had the same molecular formula as **1**, as deduced from HRESIMS and NMR data. Comparison of the NMR data revealed the structure of **2** to be closely related to that of **1**. However, compound **2** presented two olefinic protons at δ_H 5.48 (d, *J* = 10.9 Hz, H-3) and 5.20 (dd, *J* = 5.7, 10.5 Hz, H-7), which were attributed to two trisubstituted double bonds. The COSY coupling between H-3 and an oxymethine at δ_H 4.45 (d, *J* = 10.9 Hz, H-2) in addition to the HMBC interactions from H-3 to C-1 (δ_C 74.0, s) and C-18 indicated a double bond resided at C-3/C-4. An additional HMBC relationship between H-2 and C-12 (δ_C 75.7) ascertained that an ether bridge formed across C-2 and C-12. The observation of three D_2O exchangeable protons at δ_H 4.42 (d), 3.68 (s) and δ_H 4.46 (t) in the ^{1}H NMR spectrum (DMSO-d_6) in association with their HMBC and TOCSY correlations indicated the assignment of OH-1, OH-11, and OH-19. The geometries of the double bonds were determined as 3*E* and 7*Z* on the basis of the chemical shift of C-18 (δ_C 15.0)[10] and the NOE correlation between H_3-18 (δ_H 1.64, s) and H-2, and an NOE correlation between H-7 and H-9b (δ_H 2.02, m) was observed. The *trans* orientation of H-2 and H-3 was referred to the large coupling constant ($^3J_{H\text{-}2,H\text{-}3}$ = 10.9 Hz). Additional NOE correlations from H-11 (δ_H 3.84, d) to H-3 (δ_H 5.48, d), H-7, H-10a (δ_H 1.81, m), and H_2-13 (δ_H 1.93, 1.64, m) and between H_3-20 (δ_H 1.02, s) and H-10b (δ_H 1.36, m) and H-2 and H-15 (δ_H 1.81, m) allowed the assignment of the same face of H-2, H_3-20, and the isopropyl group, whereas H-11 was in the opposite orientation.

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Table 3. ^{13}C NMR Data for Sinulariols T−Z_5 in $CDCl_3$

position	1	2	3	4	5	6	7	8	9	10	11	12
1	76.3 C	74.0 C	78.3 C	144.2 C	77.8 C	78.3 C	77.9 C	79.5 C	151.8 C	148.8 C	152.7 C	141.2 C
2	126.4 CH	74.7 CH	75.3 CH	119.3 CH	128.1 CH	129.5 CH	125.0 CH	127.20 CH	121.1 CH	124.3 CH	119.7 CH	128.0 CH
3	140.0 CH	126.6 CH	121.8 CH	121.3 CH	139.7 CH	140.0 CH	140.9 CH	133.1 CH	70.5 CH	70.2 CH	70.7 CH	49.5 CH
4	73.2 C	139.5 C	140.3 C	139.3 C	72.8 C	72.8 C	74.2 C	134.4 C	74.3 C	150.5 C	75.3 C	81.9 C
5	44.1 CH_2	40.3 CH_2	39.1 CH_2	34.5 CH_2	43.7 CH_2	44.1 CH_2	44.5 CH_2	127.22 CH	37.7 CH_2	32.4 CH_2	39.3 CH_2	40.6 CH_2
6	23.6 CH_2	25.1 CH_2	25.0 CH_2	26.0 CH_2	22.8 CH_2	22.9 CH_2	23.6 CH_2	27.3 CH_2	23.9 CH_2	27.2 CH_2	23.2 CH_2	24.3 CH_2
7	132.3 CH	128.8 CH	128.2 CH	133.1 CH	129.8 CH	134.5 CH	129.1 CH	127.8 CH	127.0 CH	125.7 CH	133.5 CH	51.4 CH
8	135.8 C	138.9 C	139.5 C	137.5 C	132.6 C	135.3 C	133.1 C	132.5 C	133.5 C	134.2 C	137.4 C	75.1 C
9	33.3 CH_2	30.8 CH_2	23.1 CH_2	26.0 CH_2	38.0 CH_2	35.8 CH_2	37.5 CH_2	40.5 CH_2	37.4 CH_2	36.9 CH_2	33.1 CH_2	31.8 CH_2
10	24.9 CH_2	30.4 CH_2	26.4 CH_2	24.3 CH_2	25.9 CH_2	27.1 CH_2	25.8 CH_2	27.0 CH_2	24.1 CH_2	24.5 CH_2	27.9 CH_2	22.3 CH_2
11	78.2 CH	75.4 CH	74.8 CH	83.7 CH	75.1 CH	74.1 CH	74.8 CH	76.7 CH	62.9 CH	62.1 CH	71.5 CH	68.3 CH
12	70.3 C	75.7 C	74.4 C	79.2 C	70.9 C	74.2 C	70.8 C	71.1 C	61.8 C	61.3 C	79.0 C	59.3 C
13	33.3 CH_2	28.5 CH_2	38.3 CH_2	49.3 CH_2	37.1 CH_2	32.0 CH_2	37.2 CH_2	37.2 CH_2	41.4 CH_2	39.9 CH_2	34.7 CH_2	35.0 CH_2
14	28.2 CH_2	27.7 CH_2	30.3 CH_2	77.3 CH	30.0 CH_2	27.4 CH_2	30.3 CH_2	31.7 CH_2	26.0 CH_2	26.5 CH_2	22.2 CH_2	23.8 CH_2
15	30.2 CH	37.2 CH	38.0 CH	27.9 CH	39.7 CH	40.0 CH	39.5 CH	40.4 CH	35.5 CH	34.6 CH	35.3 CH	29.4 CH
16	17.5 CH_3	16.3 CH_3	17.3 CH_3	21.9 CH_3	17.2 CH_3	17.3 CH_3	17.1 CH_3	17.4 CH_3	21.9 CH_3	22.1 CH_3	22.5 CH_3	21.5 CH_3
17	16.7 CH_3	16.2 CH_3	16.8 CH_3	21.3 CH_3	17.0 CH_3	16.6 CH_3	17.0 CH_3	16.9 CH_3	21.8 CH_3	21.9 CH_3	22.0 CH_3	19.9 CH_3
18	28.4 CH_3	15.0 CH_3	17.0 CH_3	25.1 CH_3	27.8 CH_3	27.9 CH_3	29.0 CH_3	19.4 CH_3	22.8 CH_3	111.4 CH_2	23.1 CH_3	24.0 CH_3
19	60.6 CH_2	60.3 CH_2	66.7 CH_2	59.7 CH_2	14.8 CH_3	61.1 CH_2	14.8 CH_3	14.7 CH_3	14.7 CH_3	14.7 CH_3	61.1 CH_2	66.5 CH_2
20	23.5 CH_3	19.6 CH_3	21.4 CH_3	21.1 CH_3	19.6 CH_3	15.8 CH_3	19.5 CH_3	19.3 CH_3	16.5 CH_3	16.4 CH_3	18.5 CH_3	15.7 CH_3
−OEt						55.9 CH_2					56.7 CH_2	
						16.1 CH_3					15.7 CH_3	

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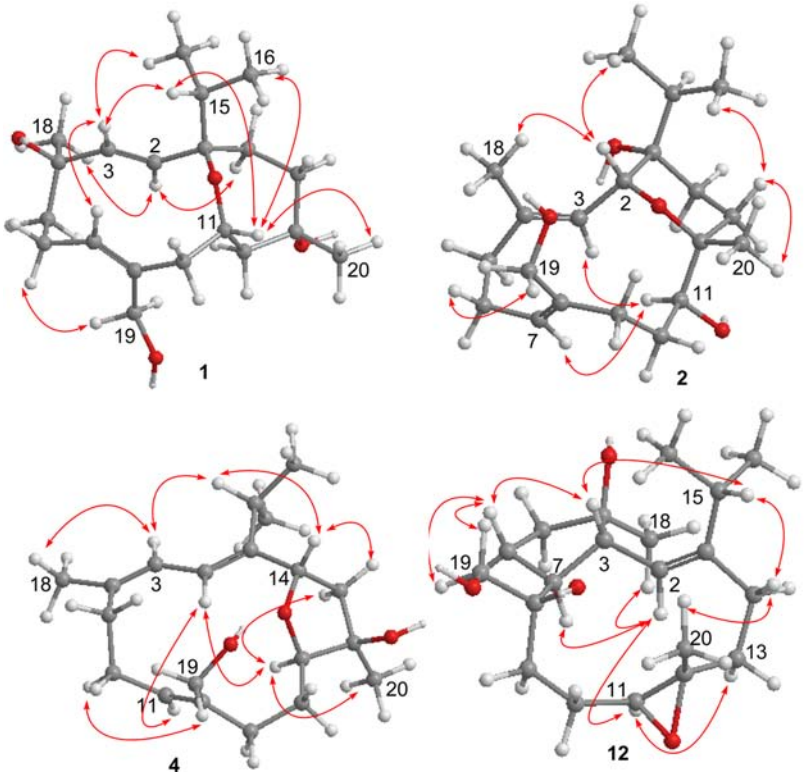

Figure 2. Key NOE correlations of **1**, **2**, **4**, and **12**.

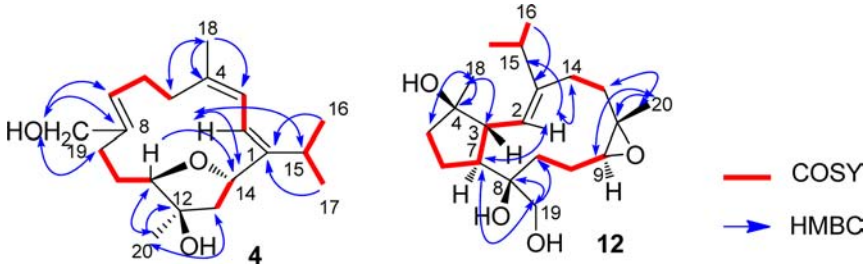

Figure 3. Key COSY and HMBC correlations of **4** and **12**.

The 2D NMR spectroscopic analyses revealed the gross structure of sinulariol V (**3**) to be identical to that of **2**. The difference was attributed to the *trans* geometry of the olefinic bond at C-7 and C-8 due to the NOE correlation observed between H-7 and H_2-19, whereas the remaining NOE interactions and *J* values of **3** were the same as those of **2**. Thus, **3** was determined as a geometric isomer of **2**.

Sinulariol W (**4**) had a molecular formula of $C_{20}H_{32}O_3$, which was inferred from the pseudomolecular ion peak observed at *m*/*z* 343.2240 ($[M + Na]^+$) in the HRESIMS spectrum. The UV absorption at 239 nm suggested the presence of a conjugated olefinic unit. The ^1H and ^{13}C NMR data (Tables 1 and 3) indicated a cembranoid-type derivative, while 2D NMR data led to the establishment of a 14-carbocyclic backbone bearing a 1,3,7-triene. Apart from a hydroxymethylene group linked to C-8, three oxygenated carbons resonating at δ_C 83.7 (d), 79.2 (s), and 77.3 (d) were assigned to C-11, C-12, and C-14 on the basis of HMBC correlations. The formation of an ether bridge across C-11 and C-14 was deduced from the HMBC relationships between H-11 (δ_H 3.45, d) and C-14 and between H-14 (δ_H 4.62, t) and C-11 (Figure 3). Thus, 5 degrees of unsaturation were fully covered by the mentioned functional groups and cyclic units, suggesting C-12 to be hydroxylated. The 1*E*, 3*Z*, and 7*Z* geometries were ascertained through the NOE correlations from H-3 to H_3-18 (δ_H 1.84, s), H-15 (δ_H 2.69, dq), and H_3-17 (δ_H 1.08, d) and between H_2-19 and H_2-6. Additional NOE interactions from H-11 to H-2 and H_3-20 (δ_H 1.25, s) and from H-15 to H-3 and H-14 (Figure 2) resulted in the *trans* geometry of the epoxy group, while H_3-20 was oriented in the same face as H-11.

The HRESIMS and 2D NMR data (Tables 1 and 3) established the structure of sinulariol X (**5**) as 19-dehydroxysinulariol H, and this assignment was supported by the molecular formula $C_{20}H_{34}O_3$ of **5** with one less oxygen atom than that of sinulariol H[8] in association with the presence of an olefinic methyl group (δ_H 1.65, s; δ_C 14.8) instead of 19-hydroxymethylene of the latter compound.

Comparison of the NMR data revealed sinulariol Y (**6**) to be structurally closely related to sinulariol H,[8] except for the presence of an ethoxy group, whose oxymethylene protons at δ_H 3.43 (1H, dq, *J* = 8.5, 7.0 Hz) and 3.39 (1H, dq, *J* = 8.5, 7.0 Hz) coupled with the methyl protons at δ_H 1.13 (3H, t, *J* = 7.0 Hz) were observed in the COSY spectrum. This unit was

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determined to be located at C-12 through the HMBC correlation of the oxymethylene protons with C-12 (δ_C 74.2). Compound **6** was suggested to be an artifact derived from sinulariol H during the extraction process.

Sinulariol Z (**7**) had the same molecular formula as **5** as determined through its HRESIMS and NMR data (Tables 1 and 3), whereas its NMR data were mostly identical to those of sinulariol J. The distinction was attributed to the NMR data of **7** presenting an olefinic methyl group (δ_H 1.61, s, H_3-19) to replace a hydroxymethylene of the known analogue.

Sinulariol Z_1 (**8**) had a molecular formula of $C_{20}H_{33}O_2$, losing a H_2O unit in comparison with that of **5**, as deduced by the HRESIMS (m/z 305.2462 $[M + Na]^+$) data. The NMR data of **8** were closely similar to those of **5**, except for the presence of three olefinic bonds instead of two and the absence of an oxygenated carbon at C-4. Interpretation of the 2D NMR spectra indicated the additional double bond to reside at C-4 (δ_C 134.4) and C-5 (δ_C 127.2), whereas the remaining signals were closely similar to those of **7**. The geometry of C-4 and C-5 was in accordance with 4*Z* on the basis of the NOESY interactions between H_3-18 and H-5. Chemical conversion of **5** under acidic conditions (0.5% HOAc) yielded **8** (Figure 4), confirming **8** to be a 4-dehydroxylated derivative of **5**.

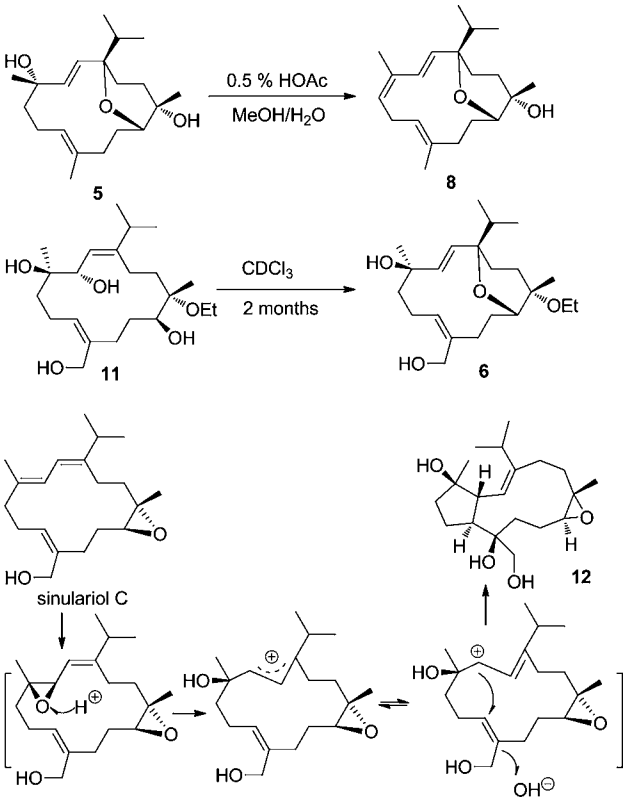

Figure 4. Chemical conversions of **5**, **11**, and sinulariol C.

Sinulariols Z_2 (**9**) and Z_3 (**10**) were determined to be the 19-dehydroxylated analogues of sinulariols L and O,[8] respectively, on the basis of a comparison of the NMR and NOE data in addition to the 2D NMR and HRESIMS data analyses.

The 2D NMR analyses of sinulariol Z_4 (**11**) indicated its structure was closely related to that of **6**, except for a double bond residing at C-1 and C-2 and the presence of a 3,11-dihydroxy group. The close similarity of the NOE correlations of both compounds indicated the same relative configurations of **11** and **6**. This assignment was supported by **11** being partly converted to **6** in $CDCl_3$ solution standing in a refrigerator (2 °C) for two months (Figure 4).

Sinulariol Z_5 (**12**) had a molecular formula of $C_{20}H_{34}O_4$ as determined by HRESIMS and NMR data, requiring 4 degrees of unsaturation. The ^{1}H NMR spectrum of **12** exhibited four methyl signals, including two singlets overlapped at δ 1.13 (s, H_3-18, H_3-20) and two methyl doublets at δ 1.03 and 0.99 (each d) for an isopropyl group due to the coupling with a methine (δ 2.91, sept, H-15) in the COSY spectrum. Detailed interpretation of the 2D NMR spectra revealed **12** to be a capnosane-based cembranoid.[10] A 3,7-bonded cyclopentane ring was inferred from the COSY relationships from methine proton H-3 (δ_H 2.55, t, J = 9.5 Hz) to H-7 (δ_H 2.28) and olefinic proton H-2 (δ_H 4.93, d, J = 9.5 Hz). The coposition of a hydroxymethylene and a hydroxy group at C-8 (δ_C 75.1, qC) was deduced through the geminal protons H_2-19 at δ_H 3.79 (1H, d, J = 10.9 Hz) and 3.46 (1H, d, J = 10.9 Hz) correlated to C-7 (δ_C 51.4, CH), C-8, and C-9 (δ_C 31.8, CH_2) in its HMBC spectrum. Additional HMBC correlations from H_3-20 to C-11 (δ_C 68.3, CH), C-12 (δ_C 59.3, qC), and C-13 (δ_C 35.0, CH_2) and from H-11 to C-20 indicated that an epoxy group resided at C-11 and C-12 (Figure 3). The relative configurations of **12** were determined by the J values and NOE correlations. The *anti* orientation of H-3 and H-7 was assigned through the NOE interaction between H-7 and H-2. The NOE correlation between H-3 (δ_H 2.55, t) and H-15 (δ_H 2.91, dq) with the absence of the NOE interaction from H-2 to isopropyl protons clearly demonstrated the 1*Z* geometry. The NOE interactions between H-2 and H-11 (δ_H 3.22, dd), H-11 and H-13b (δ_H 1.54, m), and H-2 and H_3-18 were indicative of the *trans* geometry for the epoxy group, while H-11 and H_3-18 were oriented in the same face but opposite H-3. Treatment of sinulariol C by tracing HCl in $CHCl_3$ solution partly generated a product which was identical to **12** on the basis of the comparison of their NMR and MS data. The mechanism of chemical conversion is depicted in Figure 4, which is supposed to be induced through transannular cyclization.[11,12]

Compound **13** was determined as (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra- 2,7-diene on the basis of the MS and NMR data analyses and comparison with NMR data reported in the literature.[13]

In the antifouling assay, 10 cembranoids, including those previously isolated from the same specimen, were selected to test for the inhibition of barnacles *Ba. amphitrite* and *Bu. neritina*. The effects of the test samples against biofouling were determined by examining the plates under a dissecting microscope to check for (1) attached larvae, (2) unattached larvae, and (3) dead larvae. Compounds **13** and **7** displayed dose dependency (Figure 5) and showed significant inhibition against the larval settlement of the barnacle *Ba. amphitrite* with EC_{50} = 4.86 and 4.57 μg/mL and moderate inhibition against *Bu. neritina* with EC_{50} = 12.34 and 13.48 μg/mL, respectively (Table 4). In addition, compounds **13** and **7** showed a high therapeutic ratio with $LC_{50}/EC_{50} > 10$ for the inhibition of *Ba. amphitrite*. Previously, we reported sinulariol J possessed potent inhibition against the larval settlement of *Ba. amphitrite*, while sinulariol P showed moderate inhibition against the adhesion of *Bu. neritina*.[8] Analyses of the primary structure−activity relationship revealed functional groups and olefinic geometry directly affecting the inhibitory activity. Active compound **13** is a C-19 dehydroxylated sinulariol D, while sinulariol D and its C-4 epimer sinulariol F showed weak activity against *Ba.*

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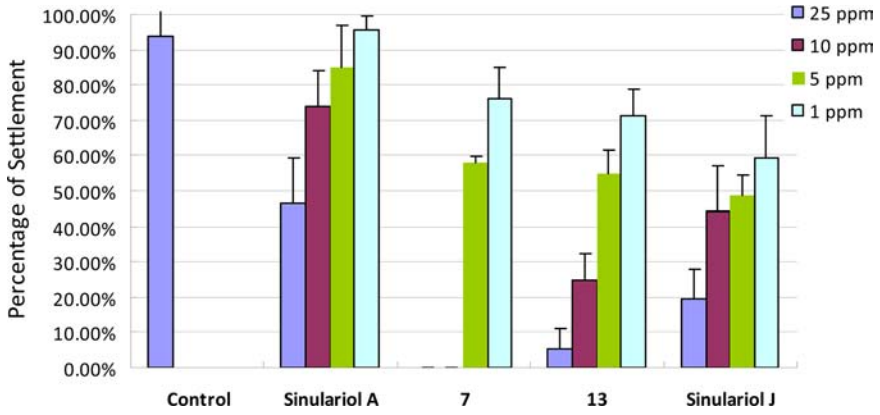

Figure 5. Inhibition of the larval settlement of the barnacle *Ba. amphitrite*.

Table 4. Effects of Compounds against Larval Settlement of *Ba. amphitrite* and *Bu. neritina*[a]

	Ba. amphitrite		*Bu. neritina*	
compd	EC_{50} (μg/mL)	LC_{50}/EC_{50}	EC_{50} (μg/mL)	LC_{50}/EC_{50}
13	4.86	>10.29	12.34	>4.05
7	4.57	>10.94	13.48	>3.71
sinulariol A	22.5	>2.22	>25	UD
sinulariol J	5.65	>8.85	22.50	>2.22
sinulariol P	>25	UD	14.03	>3.56
sinulariol F	>25	UD	>25	UD
sinulariol O	>25	UD	>25	UD
sinulariol H	>25	UD	>25	UD
sinulariol D	>25	UD	>25	UD
sinulariol L	>25	UD	>25	UD

[a]UD = undetectable.

amphitrite and *Bu. neritina*, indicating OH-19 dramatically decreased the inhibition in a 1,12-epoxy pattern. The similar data were found in comparison with the inhibitory activity of **7** and sinulariols H and J. In 11,12-epoxy analogues, sinulariol P exhibited stronger inhibition against *Bu. neritina* than sinulariols L and O, suggesting the 7*E* geometry to be better than 7*Z*. Unfortunately, the rest of the compounds remain uncertain for inhibitory activity due to the minor amount of samples.

The present work provided additional antifouling cembranoids derived from *Sinularia* species, implying compounds **7** and **13** to be the potential candidates for the development of nontoxic antifoulants.[12] Testing the isolated compounds against a panel of tumor cell lines revealed all compounds had weak cytotoxicity. This finding in addition to the antifouling testing results indicated the cembranoid derivatives possessing a 1-isopropyl-14-carbocyclic backbone played a chemoecological role other than toxic, while the substitutionary groups directly affected their ecological function.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR, IR, and HRESIMS spectra for compounds **1**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org..

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Notes

The authors declare no competing financial interest.

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