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Sinulariols A–S, 19-oxygenated cembranoids from the Chinese soft coral *Sinularia rigida*

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ABSTRACT

Chemical examination of a Chinese soft coral *Sinularia rigida* resulted in the isolation and characterization of 19 new cembrane-type diterpenoids, designated as sinulariols A–S (**1**–**19**). Their structures were determined on the basis of extensive spectroscopic (2D NMR, IR, and MS) analysis in association with modified Mosher's method. All compounds featured unusual 19-oxygenated functionalities, which are rarely found from cembranoid family. The biogenetic transformation of the isolated compounds is postulated. Part of the isolated cembranoids showed significant anti-fouling activity.

1. Introduction

Soft coral genus *Sinularia* (Alcyoniidae) consists of 154 valid species, and is among the most widespread and commonly encountered octocorals on Indo-Pacific coral reefs.[1] Up to date, more than 50 *Sinularia* species have been examined chemically to elaborate a rich harvest of secondary metabolites, including sesquiterpenes, diterpenes, polyhydroxylated steroids, and polyamines. Cembranoid-type diterpenes are a family of typical secondary metabolites mainly distributed in coral genera *Sinularia*, *Sarcophyton*, and *Lobophytum*. Chemoecological investigation disclosed cembranoids to play a very important role in the survival of soft corals, such as anti-feeding, reproduction, anti-fouling, and allelopathy.[2,3] Cembranoids also displayed a wide range of potential biological activities, such as antimicrobial, anti-inflammatory, and cytotoxic activities.[1] Our previous investigation[4–7] revealed that a soft coral located in different geographic reefs dramatically varied the structural patterns of secondary metabolites. Genus *Sinularia* is widely distributed in South China Sea from the coral reefs of eastern coastline to western area near Vietnam, and is a rich source of cembrane diterpenoids. In our ongoing interest in the chemical diversity of soft corals inhabited in South China Sea, we collected a chemically unexamined soft coral *Sinularia rigida* in Sanya Bay, Hainan Island (China). Chemical examination resulted in the isolation of a class of unusual 19-oxygenated cembranoids, namely sinulariols A–S (**1**–**19**). Herein, the structural elucidation of the new compounds, along with the biological evaluation of part compounds are reported.

2. Results and discussion

The HPLC and ^{1}H NMR guided fractionation of EtOH extract of soft coral *S. rigida* informed the EtOAc solvable fraction containing the spectral features of terpenoids. Repeated column chromatography followed by reversed-phase HPLC separation of the EtOAc fraction led to the isolation of 19 cembrane diterpenoids.

Sinulariol A (**1**) was isolated as a colorless oil. Its molecular formula was established as $C_{20}H_{34}O_3$ based on HRESIMS (*m*/*z* 345.2402 $[M+Na]^+$) and NMR data, requiring 4° of unsaturation. The UV (248 nm) and IR (3736, 3320, and 1586 cm^{-1}) absorptions suggested the structure containing hydroxy and conjugated olefinic groups. The ^{13}C NMR and APT spectra exhibited a total of 20 carbon resonances, including six olefinic carbons (δ_C 117.9, 122.5, 130.1, 136.2, 138.1, and 148.6) representing three trisubstituted double

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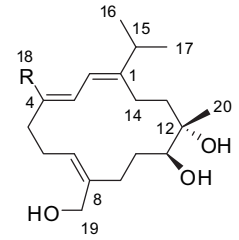

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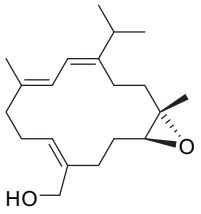

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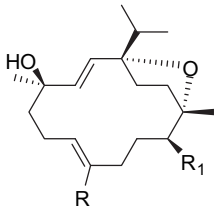
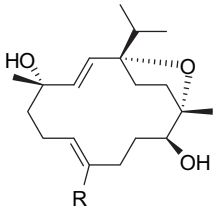


4. R = CH_2OH, R_1 = OH
5. R = CH_2OH, R_1 = Cl
19. R = COOMe, R_1 = OH



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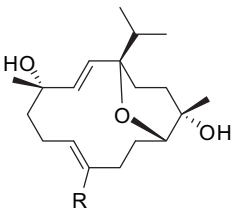

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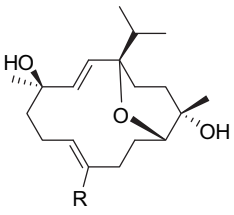

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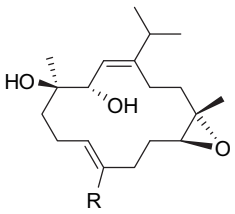

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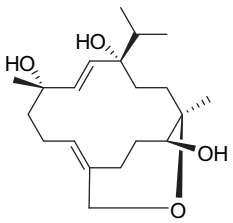

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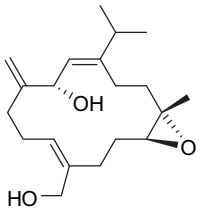

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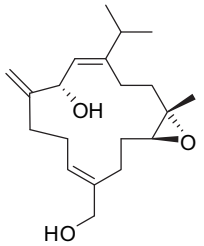

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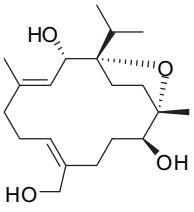

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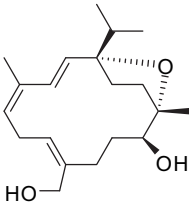

18

bonds, four methyl groups (δ_C 16.4, 21.3, 22.8, 23.0), and three hydroxy-bearing carbons (δ_C 71.0, 75.9, and 60.1), in addition to six aliphatic methylenes and an aliphatic methine. Apart from the sites of unsaturation accounted for three olefins, the molecule was judged to have a monocyclic pattern. Analysis of 2D NMR spectra (COSY, HMQC, and HMBC) led to the establishment of a 14-membered macrocyclic skeleton, featuring a cembrane diterpene closely related to cembrene-1,3,7-triene-11,12-diol, which was formerly isolated from an Indian soft coral *Lobophytum catalai*.[8] An isopropyl group was inferred from two methyl doublets resonated at δ_H 1.02 (3H, d, J=7.0 Hz, H_3-16) and 1.09 (3H, d, J=7.0 Hz, H_3-17) and their coupling to a methine proton (δ_H 2.51, dq, H-15) in COSY. The linkage of isopropyl group to an olefinic carbon C-1 (δ_C 148.6) was based on the HMBC correlations from H_3-16/H_3-17 to C-1, and thus C-1/C-2 was assigned to an olefinic group. The second olefin C-3/C-4 conjugated to C-1/C-2 was recognized from an AB olefinic spin system of H-2 (δ_H 6.05, d, J=11.0 Hz) and H-3 (δ_H 6.06, d, J=11.0 Hz), while a methyl group positioned to C-4 was based on the HMBC between H-3 and a vinyl methyl carbon (δ_C 16.4, C-18). The third olefin was assigned to C-7/C-8 as evident from the COSY correlations between H-7 (δ_H 5.32, dd, J=6.5, 8.0 Hz) and H_2-6 (δ_H 2.21, 2.34) and in turn between H_2-6 and H_2-5 (δ_H 2.10, 2.27), in association with the HMBC correlations of H_2-6 and H-2 to C-4. In addition, C-8 bearing a hydroxymethylene was evidenced from the HMBC relationships from the geminal protons H_2-19 (δ_H 3.99, 4.04) to C-7 (δ_C 130.1), C-8 (δ_C 138.1), and C-9 (δ_C 31.2). Additional HMBC relationships from H_3-20 (δ_H 1.08, s) to C-11 (δ_C 71.0, CH), C-12 (δ_C 75.5, qC), and C-13 (δ_C 36.5) ascertained the presence of a 11,12-diol functionality. Thus, the gross structure of **1** was determined as a 19-hydroxylated cembrene-1,3,7-triene-11,12-diol. Based on the NOE interactions from H-2 to H_3-17 (δ_H 1.12, d) and H_3-18 (δ_H 1.76, s) and from H-3 to H-5b (δ_H 2.10, m), the olefinic geometries were assigned to 1*E* and 3*E*. The NOE correlation between H_2-19 and H-6a (δ_H 2.34m) was ascribed to 7*Z*. The chemical shift of H_3-18 (<20 ppm) provided additional data to support 3*E* assignment.[9] The $J_{H\text{-}2/H\text{-}3}$ value (11.0 Hz) reflected a trans-diaxial coupling of the two olefinic protons, while the absence of NOE relationship of H-11 and H-10a (δ_H 1.43) in addition to the doublet H-11 coupled to H-10a ($J_{H\text{-}11/H\text{-}10a}$=10.0 Hz) indicated a trans-axial oriented H-11 against H-10a and the dihedral angle of H-11 and H-10b approximated to 90°. These findings implied the 14-membered backbone presenting a favorable conformation rather than flexible forms in solution. Thus, NOESY in association with coupling constants enabled to determine the relative configurations. When H-11 was assigned arbitrarily as α-orientation, H-10a was accordingly oriented in β-face. The NOE relationships between H-10a and H_3-20 (δ_H 1.08, s) and between H-10b (δ_H 1.99) and H-11 in combination with the absence of NOE between H-11 and H_3-20, clarified H_3-20 to be oriented in opposite to H-11 (Fig. 1). The absolute configuration of C-11 was determined using modified Mosher's method.[10,11] Firstly, the primary alcohol OH-19 was protected by *tert*-butyldimethylsilyl chloride (TBSCl) to form a TBS ether, which was then reacted with (*R*)- and (*S*)-*O*-methylmandelic acid (MPA) by a standard procedure to form (*R*)- and (*S*)-MPA esters. Analysis of $\Delta\delta^{RS}$ ($\Delta(\delta_R-\delta_S)$) values (Fig. 2) resulted in 11*S*, and C-12 was accordingly assigned to 12*S* configuration.

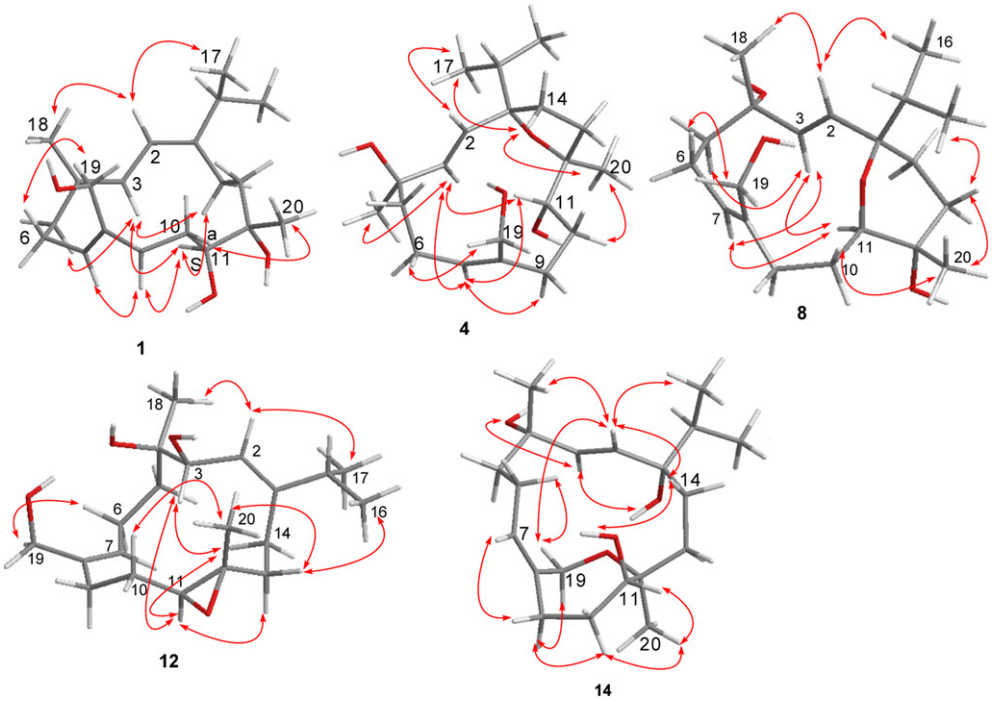

Fig. 1. Key NOE correlations of **1**, **4**, **8**, **12**, and **14**.

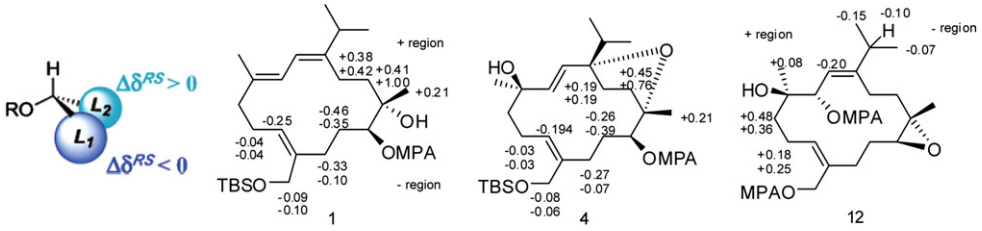

Fig. 2. The $\Delta\delta^{RS}$ ($\Delta(\delta_R-\delta_S)$) data for the MPA esters of **1**, **4**, and **12**.

The NMR data of sinulariol B (**2**) (Tables 1 and 2) were mostly compatible to those of **1**, except for the presence of an additional hydroxymethylene featured the geminal protons at δ_H 4.11 and 4.44 (each, d, J=11.8 Hz, H_2-18) and their corresponding carbon at δ_C 61.6 (CH_2). The ^{1}H NMR of **2** displayed three methyl resonances including two isopropyl methyl doublets H_3-16 and H_3-17 (δ_H 1.03 and 1.11), and a methyl singlet (δ_H 1.07) for H_3-20. Thus, compound **2** was assumed to be a 18-hydroxylated sinulariol A. This depiction was further evident from its molecular formula ($C_{20}H_{34}O_4$), which has 16 amu more than that of **1**, in addition to the HMBC correlations from H_2-18 to C-3 (δ_C 126.0, CH), C-4 (δ_C 138.2, qC), and C-5 (δ_C 37.4, CH_2). Based on the biogenetic consideration, the close similar NOE interactions of **2** and **1** led the assignment that the configurations of **2** were the same as **1** in regard to the corresponding stereogenic centers.

Sinulariol C (**3**) has a molecular formula of $C_{20}H_{32}O_2$ as determined by HRESIMS (m/z 327.2281 $[M+Na]^+$) and NMR data, implying 5° of unsaturation and lacking H_2O unit than that of **1**. Comparison of NMR data revealed its structure to be closely related to **1** in respect to the partial structure from C-1 to C-10 and from C-13 to C-14. Analysis of 1D and 2D NMR spectra revealed C-11 (δ_C 59.5) and C-12 (δ_C 60.9) as well as H-11 (δ_H 2.91) of **3** to be shifted to upfield in comparison with the corresponding signals of **1**. These data were in accordance with an 11,12-epoxide, as in the case of epoxyisoneocembrene A.[9] Thus, compound **3** was determined as a C-11/C-12 epoxidated analogue of **1**. The olefinic geometries of **3** were determined to be the same as those of **1** based on the similar NOE relationships, while the NOE correlations between H-11/H-13b (δ_H 1.64) and H_3-20/H-10b (δ_H 1.61) conducted to assign a *trans*-epoxide. Acidic hydrolysis of **3** (5% acetic acid) derived a major product whose NMR and MS spectroscopic data were identical to **1**. This fact indicated that the acid-catalyzed hydrolysis likely followed a borderline S_N2 mechanism,[12] in which H_2O preferred to attach at more substituted carbon C-12. Accordingly, the configuration of C-11 in **3** maintained the same form as **1**, while *S* configuration was assigned to C-12.

The molecular formula of sinulariol D (**4**) was determined as $C_{20}H_{34}O_4$ based on HRESIMS (m/z 361.2350, $[M+Na]^+$) data, requiring 4° of unsaturation. Its ^{1}H and ^{13}C NMR data (Tables 1 and 2) featured a cembrane-based skeleton and closely related to a known cembranoid, 4,11-dihydroxy-1,12-oxidocembra-2,7-diene.[13] The difference was due to C-19 where a hydroxymethylene (δ_H 4.42, 3.82; J=11.6 Hz for each) of **4** instead of a methyl group was observed. A *trans*-disubstituted olefin at C-2/C-3 flanked by quaternary carbons was recognized by the doublet coupling constant (16.0 Hz) of H-2 (δ_H 5.65) and H-3 (δ_H 5.88), while the NOE cross-peak between H-7 (δ_H 5.44, t) and H-9b (δ_H 2.08) inferred to 7*Z* geometry. The relative configurations of the stereogenic centers at C-1, C-4, C-11, and C-12 were determined by additional NOE relationships and J values. The absolute configuration of C-11 was determined by modified Mosher's method.[10,11] Following the same protocol as used for **1**, the (*R*)- and (*S*)-MPA esters of 19-TBS ether of **4** were prepared. Calculation of $\Delta\delta^{RS}$ data resulted in 11*S* configuration. In MM2 energy-minimized conformation (Fig. 1), H-11 was suggested to be α-oriented. The NOE correlations between H-11(δ_H 3.44, d)/H-7, H-11/H-3, and H-3/H-7 were indicative of spatial

Table 1
^{13}C NMR data ($CDCl_3$) for sinulariols A–L and N–R (**1**–**12** and **14**–**18**)

Compds	1[a]	2[a]	3[a]	4[b]	5[b]	6[b]	7[b]	8[b]	9[b]	10[b]	11[a]	12[b]	14[a]	15[b]	16[b]	17[b]	18[a]
C-1	148.6	150.2	147.9	88.1	88.9	88.2	88.1	78.5	77.9	78.1	77.0	152.0	76.5	148.4	148.7	90.0	89.5
CH-2	117.9	118.0	117.9	131.9	130.0	131.5	131.2	129.3	128.3	127.9	128.5	121.4	132.8	123.4	124.1	72.3	133.1
CH-3	122.5	126.0	122.4	136.9	137.7	136.8	136.4	139.8	140.4	140.6	140.8	70.7	135.2	69.4	68.2	126.2	129.5
C-4	136.2	138.2	135.0	73.1	73.7	72.4	72.0	72.7	72.8	73.5	72.7	74.6	73.9	148.7	149.5	138.6	134.8
CH_2-5	39.9	37.4	39.2	42.5	43.2	41.9	41.2	43.9	44.0	43.3	42.2	36.6	41.9	33.5	33.4	38.0	126.1
CH_2-6	25.5	27.2	25.4	23.4	23.9	23.5	25.4	22.7	24.8	23.7	25.0	23.5	22.0	25.0	25.6	24.5	27.1
CH-7	130.1	131.7	131.0	131.1	132.8	131.9	144.8	135.0	146.8	134.1	143.6	131.7	124.5	129.9	128.0	130.1	131.6
C-8	138.1	137.7	137.3	136.3	136.2	136.1	130.9[c]	134.5	129.4	135.2	130.4	137.6	140.4	137.1	137.8	136.6	136.2
CH_2-9	31.2	33.7	33.3	29.0	30.4	31.4	29.8	36.0	33.0	35.2	31.9	35.4	28.9	33.0	24.8	28.6	32.8
CH_2-10	28.6	29.0	25.1	30.4	32.0	29.9	30.5[d]	27.2	26.7	26.2	25.2	25.2	33.2	24.2	25.0	31.4	31.1
CH-11	71.0	70.9	59.5	74.1	67.4	73.7	73.2	75.5	74.5	74.9	75.0	62.9	75.2	62.5	62.5	74.2	76.9
C-12	75.5	76.0	60.9	85.1	85.5	85.2	84.7	70.5	70.8	70.6	70.2	62.3	80.3	61.5	61.7	84.9	85.2
CH_2-13	36.5	36.0	34.9	36.5	37.7	36.5	36.5	36.6	37.3	36.9	37.0	41.6	26.4	37.9	38.3	35.8	36.3
CH_2-14	24.1	24.0	23.7	33.1	34.1	31.0	30.4[d]	27.8	29.4	27.9	27.7	26.5	32.8	26.8	24.8	29.7	35.0
CH-15	31.5	31.5	33.1	38.6	38.9	38.2	38.2	40.1	39.4	39.2	37.8	36.4	38.5	32.2	34.1	32.8	38.6
CH_3-16	23.0	23.0	22.6	18.3	18.3	18.1	18.0	17.3	17.0	17.4	17.5	21.8	17.5	22.5	22.0	19.4	18.4
CH_3-17	21.3	21.2	22.2	17.7	17.5	17.8	17.8	16.5	16.8	16.6	16.6	21.5	16.6	21.4	21.8	19.2	17.7
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CH_3-20	22.8	22.8	18.9	20.1	20.3	19.6	19.3	19.5	19.5	19.3	19.2	15.8	23.1	16.6	17.6	20.5	20.0
OMe							51.9		51.4		51.5						

[a] Recorded at 125 MHz.
[b] At 100 MHz.
[c] Data deduced from HMBC spectrum.
[d] Assignments within a column could be interchanged.

proximity among these protons, implying the olefinic substituents favoring 'up' or 'down'-orientation toward the 14-membered ring. Thus, H-3 and H-7 were also oriented to 'down' face. The coupling constant $J_{H\text{-}11/H\text{-}10a}$ (10.8 Hz) along with the absence of NOE relationship between H-10a (δ_H 1.47) and H-11 suggested their trans-diaxial relationship. Therefore, H-10a was depicted to be β-oriented. The obvious NOE relationships between H-10a/H_3-20 (δ_H 1.10, s) and from H_3-20 to H_3-16 and H_3-17 in association with the absence of H-11/H_3-20 relationship ascertained β-faces of isopropyl group and H_3-20. Additional NOE interactions between H_3-16/H_3-17 and H-2 and between H-3 and H_3-18 suggested H_3-18 and H-3 to be α-oriented. In order to support these assignments, 1D gNOESY experiments (in DMSO-d_6) were measured. Irradiation of H-2 led to the enhancement of OH-4 (δ_H 4.37, s) and H_3-16 (δ_H 0.78, d), and irradiation of H-3 resulted in the enhancement of H_3-18 and H-11, confirming α-face of H_3-18. Additional NOE irradiation of H_3-20 (δ_H 0.95, s) resulted in the enhancement of H_3-16/H_3-17 and OH-11 (δ_H 4.46, d, J=6.3 Hz), providing further data to support H_3-20 spatially approximated to OH-11 and isopropyl group. Accordingly, the absolute configurations of C-1, C-4, and C-12 enabled to be assigned as 1*S*, 4*R*, and 12*S*, respectively.

The HRESIMS of sinulariol E (**5**) presented the pseudo molecular ion peaks at m/z 379.2010 and 381.1986 with a ratio of 1:0.33, containing a chlorine atom in the molecular formula of $C_{20}H_{33}ClO_3$. The close similarity of NMR spectroscopic data indicated **5** to be structurally closely related to **4**. Further NMR examination revealed C-11 of **5** to be shifted upfield (δ_C 67.4, CH), around 6.7 ppm higher than that of **4**, whereas C-10 and C-12 were slightly shifted downfield. These findings allowed a chlorine atom to be linked at C-11 of **5** to replace hydroxy group of **4** in corresponding position. The relative configurations of **5** were the same as **4** based on the close similar NMR and NOE data of both compounds.

Sinulariol F (**6**) has the same molecular formula as **4**, as established by HRESIMS and NMR data. The NMR data of **6** virtually resembled those of **4**, while 2D NMR analysis indicated both compounds possessing the same gross structure. Examination of NOESY interactions ascertained that **6** maintained the same geometries in respect to C-2/C-3 and C-7/C-8 olefins, while the relative configurations of the chiral centers at C-1, C-11, and C-12 were identical to those of **4**. However, the slight difference of chemical shifts (in DMSO-d_6) was found in the segment from C-2 to C-5, where H-2 (δ_H 5.37), C-3 (δ_C 137.5), C-4 (δ_C 70.9), and C-5 (δ_C 43.1) shifted to upfield, in contrast to H-3 (δ_H 5.64), H_3-18 (δ_H 1.16), C-2 (δ_C 129.8), and C-18 (δ_C 30.3), which shifted slightly to downfield in comparison with the corresponding resonances of **4**. These findings suggested **6** to be a C-4 epimer of **4**.[14] The NOE correlations between H_3-18 and H-2 rather than H_3-18 and H-3 as found in **4** confirmed this assignment.

Sinulariol G (**7**) has a molecular formula of $C_{21}H_{34}O_5$ as determined by HRESIMS data. The NMR spectroscopic features of **7** mostly resembled those of **6**. For instance, the oxygenated tertiary carbons C-1 (δ_C 88.1) and C-12 (δ_C 84.7) were characteristic of an 1,12-epoxide, while a *trans*-disubstituted olefin C-2/C-3 flanked by quaternary carbons was assigned by the coupling constant (16.0 Hz) of H-2 (δ_H 5.55) and H-3 (δ_H 5.66). A C-7/C-8 trisubstituted olefin along with 4, 11-dihydroxy and 4,12-dimethyl-1-isopropyl functionalities were also recognized in NMR spectra, and the gross structure was further concluded by 2D NMR analysis. The presence of a carbonyl (δ_C 169.1) and a methoxy (δ_H 3.78, δ_C 51.9) groups was observed in NMR spectra, and their HMBC relationship indicated **7** containing a methyl ester. The linkage of the methyl ester to C-8 to replace a hydroxymethylene of **6** was deduced by the HMBC correlation from H-7 (δ_H 5.90, dd, J=6.4, 11.1 Hz) to the carbonyl carbon. The similar NOE relationships of **7** and **6** assumed **7** maintaining the same configurations as **6**.

Sinulariol H (**8**) possesses the same molecular formula as **6**, as established by HRESIMS and NMR data. The close similar ^1H and ^{13}C

Table 2
^{1}H NMR data ($CDCl_3$) of sinulariols A–H (**1**–**8**)

	1[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]
2	6.05d (11.0)	6.25d (11.5)	5.93d (11.0)	5.65d (16.0)	5.63d (15.6)	5.58d (16.0)	5.55d (16.0)	5.44d (16.4)
3	6.06d (11.0)	6.17d (11.5)	5.87d (11.0)	5.88d (16.0)	5.81d (15.6)	5.84d (16.0)	5.66d (16.0)	6.18d (16.4)
5	2.27m	2.45ddd (1.5, 7.5, 12.0)	2.24ddd	1.84m	1.86m	1.85m	1.88m	1.97m
	2.10m	2.17ddd (1.0, 12.0, 12.0)	(6.5, 9.0, 11.5)	1.72m	1.66m	1.63ddd	1.68m	1.53m
			2.11dd			(2.4, 12.5, 12.5)		
			(10.0, 11.5)					
6	2.34m	2.56ddd (10.2, 12.0, 12.0)	2.34ddd	2.53m	2.34dddd	2.68dddd	3.10ddd	2.64ddd
	2.21m	2.25ddd (5.4, 7.5, 12.0)	(9.0, 9.0, 11.5)	2.15m	(2.5, 4.0, 7.9, 14.5)	(2.0, 10.0, 12.5, 12.5)	(11.1, 13.0, 13.0)	(10.0, 11.2, 14.0)
			2.23ddd		2.38ddd	2.09m	2.17m	2.02m
			(5.0, 6.5, 11.5)		(7.7, 12.0, 14.5)			
7	5.32dd (6.5, 8.0)	5.36dd (5.4, 10.2)	5.40dd (5.0, 9.0)	5.44t (7.2)	5.41dd (7.7, 7.9)	5.36dd (6.0, 10.0)	5.90dd (6.4, 11.1)	5.32dd (4.4, 10.0)
9	2.41ddd (2.0, 3.2, 13.5)	2.34ddd (3.0, 3.0, 12.0)	2.35m	2.34m	2.44dd (3.6, 12.8)	2.19ddd	2.70ddd (2.0, 4.0, 13.0)	2.38ddd (4.7, 4.7, 13.5)
	2.19dd (12.0, 13.5)	2.24dd (11.5, 12.0)	2.32m	2.08m	2.14ddd	(2.0, 5.6, 12.0)	2.16dd (13.0, 13.0)	2.08ddd (6.0, 11.0, 13.4)
					(1.6, 12.8, 13.2)	2.09ddd		
						(1.8, 12.0, 12.0)		
10	1.99ddd (2.0, 12.0, 15.0)	1.95ddd (3.0, 11.5, 12.0)	1.80m	2.02dd (16.0, 16.5)	2.24 brd (13.2)	2.01ddd	1.76m	1.94dddd
	1.43dddd (3.2, 10.0, 15.0)	1.43ddd (3.0, 9.7, 12.0)	1.61m	1.47dddd	1.75ddd	(2.0, 12.0, 14.0)	1.38m	(1.4, 5.0, 5.0, 12.8)
				(2.0, 7.0, 10.8, 16.5)	(2.0, 10.8, 13.2)	1.39dddd		1.50m
						(1.8, 5.6, 9.8, 14.0)		
11	3.68d (10.0)	3.76d (9.7)	2.91dd (5.0, 7.3)	3.44d (10.8)	3.87d (10.8)	3.50d (9.8)	3.38d (10.1)	3.31dd (1.4, 6.9)
13	1.95ddd (4.5, 4.5, 14.5)	1.96dd (6.0, 14.7)	1.99ddd	1.99m	1.94m	2.03m	2.05ddd (7.0, 12.0, 12.0)	1.70m
	1.74ddd (2.0, 4.5, 13.0)	1.78ddd (4.0, 12.3, 14.7)	(5.5, 9.0, 14.0)	1.67m	1.81m	1.68m	1.67m	1.59m
			1.64m					
14	2.65ddd (4.5, 10.5, 13.0)	2.76ddd (3.4, 11.2, 14.6)	2.29m	1.85m	1.90m	1.87m	1.94m	1.67m
	2.12ddd (2.0, 13.0, 13.0)	2.11ddd (3.4, 6.0, 14.6)	1.91ddd		1.83m		1.82ddd (6.0, 13.0, 13.0)	1.61m
			(6.0, 7.0, 13.5)					
15	2.51dq (7.0)	2.51dq (6.8)	2.31dq (6.9)	1.66dq (6.8)	1.66dq (6.8)	1.67dq (7.0)	1.67dq (6.8)	1.81dq (6.8)
16	1.02d (7.0)	1.03d (6.8)	1.02d (6.9)	0.86d (6.8)	0.84d (6.8)	0.86d (7.0)	0.86d (6.8)	0.76d (6.8)
17	1.09d (7.0)	1.11d (6.8)	1.03d (6.9)	0.81d (6.8)	0.80d (6.8)	0.80d (7.0)	0.78d (6.8)	0.91d (6.8)
18	1.76s	4.44d (11.8)	1.74s	1.28s	1.29s	1.27s	1.26s	1.36s
		4.11d (11.8)						
19	4.04d (11.8)	4.18d (11.8)	4.11d (12.0)	4.42d (11.6)	4.34d (11.8)	4.28d (12.0)		4.45d (11.6)
	3.99d (11.8)	3.86d (11.8)	4.05d (12.0)	3.82d (11.6)	4.06d (11.8)	4.11d (12.0)		4.01d (11.6)
20	1.08s	1.07s	1.26s	1.10s	1.21s	1.11s	1.10s	1.14s
OMe							3.78s	

[a] Recorded at 500 MHz.
[b] At 400 MHz.

Table 3
^{1}H NMR data ($CDCl_3$) for sinulariols I–L and N–R (**9**–**12** and **14**–**18**)

	9[b]	**10**[b]	**11**[a]	**12**[b]	**14**[a]	**15**[b]	**16**[b]	**17**[b]	**18**[a]
2	5.39d (16.4)	5.68d (16.0)	5.84d (16.0)	5.18d (10.0)	5.57d (16.0)	5.15d (7.2)	5.11d (8.0)	4.29d (7.7)	5.49d (15.8)
3	6.07d (16.4)	6.06d (16.0)	6.00d (16.0)	4.28d (10.0)	5.67d (16.0)	4.79d (7.2)	4.74d (8.0)	5.44d (7.7)	6.89d (15.8)
5	2.00ddd	1.94m	1.92m	1.81ddd (2.0, 8.4, 14.4)	1.76m	2.69ddd	2.52m	2.20m	5.54dd (4.0, 9.0)
	(2.4, 7.6, 13.9)	1.69m	1.79m	1.54ddd (2.3, 10.2, 14.4)	1.48ddd	(5.0, 5.0, 14.0)	2.09m	2.17m	
	1.74m				(5.0, 5.5, 12.5)	2.19m			
6	3.01dddd	2.38ddd	2.80dddd	2.70dddd	1.95ddd	2.55dddd	2.51m	2.31m	3.25 br dd
	(2.0, 9.4, 11.4, 14.5)	(7.4, 12.0, 12.0)	(2.6, 9.0, 13.6, 14.4)	(2.0, 10.0, 10.2, 11.2)	(5.0, 9.0, 13.0)	(5.0, 10.5, 10.5, 12.0)	2.22m		(13.1, 14.4)
	2.35ddd	2.19ddd	2.34m	1.96dddd	1.88m	2.31m			2.66dddd
	(7.0, 7.4, 14.0)	(7.4, 8.5, 12.0)		(2.3, 5.6, 8.4, 11.2)					(2.0, 3.5, 12.0, 14.0)
7	6.03dd (7.2, 9.6)	5.33t (7.4)	5.84dd[d]	5.47dd (5.6, 10.0)	5.14 brd (11.5)	5.36dd (5.2, 8.4)	5.46dd	5.24t (7.2)	5.50dd (3.5, 10.5)
							(5.0, 5.4)		
9	2.55ddd	2.35ddd	2.44m	2.39ddd (2.0, 4.0, 14.4)	2.39dd (13.5, 13.5)	2.35m	2.47m	2.33ddd	2.43ddd
	(4.3, 7.9, 13.5)	(5.6, 7.2, 14.0)	2.35m	2.13m	2.01ddd			(2.8, 11.6, 13.0)	(5.0, 8.0, 13.0)
	2.22ddd	2.14ddd			(2.0, 5.5, 13.5)			2.12ddd	2.06m
	(4.6, 8.8, 13.4)	(1.5, 5.6, 14.0)						(3.2, 3.6, 13.0)	
10	1.88dddd	1.96ddd	1.85m	2.31m	1.89dddd	1.90m	1.99m	1.86ddd	2.02ddd
	(3.6, 4.4, 8.0, 14.6)	(1.5, 7.2, 13.0)	1.33m	1.40dddd	(2.0, 4.0, 5.5, 14.0)	1.62m	1.58m	(3.2, 11.6, 14.0)	(8.0, 8.0, 12.0)
	1.34m	1.48dddd		(2.0, 4.4, 10.0, 13.2)	1.64dddd			1.43dddd	1.46dddd
		(1.5, 5.6, 8.4, 13.0)			(2.5, 2.5, 13.5, 14.0)			(2.8, 3.6, 11.2, 14.0)	(5.0, 8.5, 8.5, 12.0)
11	3.21dd (3.6, 6.6)	3.40dd (1.5, 8.4)	3.00dd (1.5, 10.0)	3.03dd (3.6, 10.0)	3.40 br s	2.66dd (4.0, 10.0)	2.78dd	3.28d (10.3)	3.54d (8.5)
							(2.7, 9.5)		
13	1.69m	1.69m	1.70m	2.21m	1.84dd (5.5, 11.5)	2.12m	1.93m	2.09m	2.10m
	1.56m	1.67m	1.68m	1.06m	1.28dd (11.5, 12.0)	0.87m	1.37m	1.74m	1.74m
14	1.59m	1.66m	1.69m	2.26m	1.86dd (5.5, 12.0)	2.00m	2.29m	1.84m	1.90ddd
		1.52ddd	1.50ddd	1.97m	1.47dd (12.0, 12.0)	1.96m	1.95m	1.75m	(6.5, 12.0, 14.0)
		(3.0, 5.0, 12.0)	(3.4, 3.4, 13.0)						1.78m
15	1.77dq (7.0)	1.85dq(6.8)	1.88dq (7.0)	2.28dq (6.8)	1.77dq (7.0)	2.26dq (6.8)	2.25dq (6.8)	2.08dq (6.8)	1.71dq (7.0)
16	0.77d (6.8)	0.77d (6.8)	0.76d (7.0)	1.00d (6.8)[c]	0.96d (7.0)	1.03d (6.8)	1.03d (6.8)	1.06d (6.8)	0.86d (7.0)
17	0.87d (6.8)	0.92d (6.8)	0.91d (7.0)	1.01d (6.8)[c]	0.94d (7.0)	1.06d (6.8)	1.03d (6.8)	1.00d (6.8)	0.83d (7.0)
18	1.39s	1.30s	1.30s	1.10s	1.30s	4.90 br s	5.08 br s	1.63s	1.77s
						4.87 br s	4.93 br s		
19		4.16 br s		4.49d (12.0)	4.32d (15.7)	4.22d (11.8)	4.07d (12.5)	4.31d (11.8)	4.30d (12.0)
				3.87d (12.0)	3.97d (15.7)	4.16d (11.8)	4.01d (12.5)	4.09d (11.8)	4.24d (12.0)
20	1.15s	1.14s	1.11s	1.28s	1.24s	1.23s	1.20s	1.12s	1.06s
OMe	3.75s		3.74s						

[a] Recorded at 500 MHz.
[b] At 400 MHz.
[c] Assignments in columns could be interchanged.
[d] Signal partially obscured.

NMR data of **8** in comparison with those of **6** indicated **8** to be an analogue of **6**. Detailed examination of 2D NMR data revealed downfield-shifted C-11 (δ_C 75.0, CH) and upfield-shifted C-12 (δ_C 69.1, qC) and C-1 (δ_C 77.3, qC). These distinctions indicated an alternative array of ether and hydroxyl functionalities. The HMBC correlations from H-11 (δ_H 3.07, dd, J=2.2, 7.0 Hz) to C-1, together with the observation of OH-12 (δ_H 4.19, s) and its HMBC relationships, confirmed the presence of 1,11-epoxy group, while C-4 and C-12 were hydroxylated. The 2*E* and 7*Z* geometries were referred to $J_{H\text{-}2/H\text{-}3}$ value (16.4 Hz) and the NOE relationships between H-7 and H-9 and between H_2-19 and H_2-6. In addition, the NOE interactions between H-11/H-3, H-11/H-7, and H-3/H-7 clarified these protons approximated spatially and oriented in the same face. Additional NOE interactions between H-11/OH-12, H_3-20/H_3-17, H_3-18/H-2, and H_3-16/H-2 ascertained isopropyl group spatially approximated to H_3-20, which was in opposite face toward H-11, while H_3-18 showed the same orientation as that of **6**. In biogenetic consideration, compound **8** was likely to be an ether rearranged isomer of **6**.

The molecular formula of sinulariol I (**9**) was determined as $C_{21}H_{34}O_5$ based on its HRESIMS and NMR data. A comparison of NMR data revealed the structural features of **9** in respect to the substructures from C-1 to C-6 and from C-10 to C-14 resembled those of **8**, whereas the segment from C-7 to C-9 together with C-19 functionality was compatible to that of **7**. The presence of a methyl ester was evident from a carbonyl absorption (1698 cm^{-1}) in IR and the HMBC relationship between a carbonyl carbon (δ_C 168.4, C-19) and the methoxy protons (δ_H 3.75, s). In addition, the HMBC correlations from H-7 (δ_H 6.03) and OMe to C-19 confirmed the location of methyl ester group at C-8. The relative configurations of the substituents at chiral centers C-1, C-4, C-11, and C-12 were supposed to be the same as **8** due to the similar NOE and NMR data.

Sinulariol J (**10**) was determined to be a C-4 epimer of **8**, based on the comparison of their NMR data and 2D NMR spectroscopic analysis. Like epimers **4** and **6**, the distinct ^1H and ^{13}C NMR differences **10** and **8** were attributed to the resonances surround C-4 where the upfield-shifted C-2 (δ_C 127.9) and C-5 (δ_C 43.3) and the downfield-shifted C-3 (δ_C 140.6), C-18 (δ_C 29.8), and C-4 (δ_C 73.5) were observed. Key NOE relationships (in DMSO-d_6) between H_3-18 (δ_H 1.14)/H-3 (δ_H 5.69) and H-2 (δ_H 5.36)/OH-4 (δ_H 4.43) indicated OH-4 spatially close to H-2 as in the case of **4**. Since C-4 of **4** was assigned to *R* configuration, compound **10** was thus assumed to be a 4*R* isomer of **8**.

Sinulariol K (**11**) has the same molecular formula as **9** as determined by HRESIMS and NMR data. Interpretation of IR and NMR data indicated that **11** is a stereoisomer of **9**. Similar to the NMR differences observed in isomers **10** and **8**, H-2 (δ_H 5.84, d) and C-18 (δ_C 30.2) of **11** shifted to downfield in comparison with the corresponding signals of **9**, implying the distinction occurred at the stereogenic center C-4. The NOE correlations observed between H_3-18 and H-3 instead of H_3-18/H-2 along with the similar NOE relationships of other protons led to the assignment of **11** to be a C-4 epimer of **9**.

HRESIMS (m/z 361.2336, $[M+Na]^+$) data of sinulariol L (**12**) afforded a molecular formula of $C_{20}H_{34}O_4$, containing 4° of unsaturation. Interpretation of 2D NMR data conducted to a conclusion that the partial structure from C-5 to C-14 was identical to that of **3**. In regard to the subunit from C-1 to C-4, a trisubstituted olefin to be located at C-1/C-2 was determined by the HMBC relationships of isopropyl methyl protons (δ_H 1.00, 1.01) and C-1 (δ_C 152.0). An AB spin system for an olefinic proton H-2 (δ_H 5.18, d, J=10.0 Hz) and a hydroxymethine H-3 (δ_H 4.28, d, J=10.0 Hz) reflected C-4 to be a tertiary carbon. In addition, the tertiary methyl protons H_3-18 showed HMBC interactions with C-3 (δ_C 70.7, CH), C-4 (δ_C 74.6, qC), and C-5 (δ_C 36.6, CH_2), confirming the presence of a 3,4-diol unit. Accordingly, **12** was determined as a 3,4-dihydroxylated analogue of **3**. The NOE correlations between H-2/H_3-16 (δ_H 1.00) and H-7 (δ 5.47, dd, J=5.6, 10.0 Hz)/H-9b (δ 2.13, m) were in accordance with 1*E* and 7*Z*. The absolute configuration of C-3 was deduced as *S* by modified Mosher's method to calculate the $\Delta\delta^{RS}$ values of its (*R*)-and (*S*)-MPA esters. A *trans*-epoxide was evident from the NOE interaction between H-11 (δ_H 3.03)/H-13a (δ_H 1.06) and H_3-20 (δ_H 1.28)/H-10a (δ_H 2.31). Since a NOE correlation between H-11 and H-3 was observed obviously, the absolute configurations of C-11 and C-12 were assigned to *S*. In addition, the large $J_{H\text{-}2/H\text{-}3}$ value (10.0 Hz) in association with the absence of their NOE relationship implied a trans-diaxial orientation of H-2 and H-3. In regard to the stereogenic center C-4, the obvious NOE interaction of H_3-18 and H-2 rather than H_3-18 and H-3 disclosed H_3-18 to be close to H-2 but to be far from H-3. Therefore, C-4 was assumed to be *R*.

Sinulariols M (**13**) and S (**19**) are a pair of inseparable analogues with a ratio of 2/3 as detected by ^1H NMR. Both compounds shared the same molecular formula ($C_{21}H_{34}O_5$) based on HRESIMS (m/z 389.2283 $[M+Na]^+$) data. Detailed analysis of ^1H and ^{13}C NMR spectroscopic data revealed C-8 of both compounds to be located by a methyl ester as deduced by the HMBC correlations from H-7 (δ_H 6.09 of **13** and 6.01 of **19**) and methoxy protons (δ_H 3.79 of **13** and 3.76 of **19**) to carbonyl carbon (δ_C 168.5 of **13**, and 169.5 of **19**), respectively. Further interpretation of COSY, HMQC, HMBC, as well as 1D gTOCSY confirmed the basic skeleton of **13** to be compatible to **12** with the exception of the substitution at C-8, whereas **19** closely resembled the basic structure of **4** apart from C-19 functionality. Further interpretation of NOESY spectra indicated the configurations of **13** to be identical to **12**, while those of **19** were the same as **4**.

Sinulariol N (**14**) has the same molecular formula as **8** as established from its HRESIMS and NMR data. Comparison of NMR data and interpretation of 2D NMR spectra (COSY, HMQC, and HMBC) resulted in the structure of **14** being closely related to **8**, except for the signals C-12 and C-19 of **14**, which shifted dramatically to downfield at δ_C 64.8 and 80.3, respectively. In addition, a COSY correlation between H-11 (δ_H 3.40, br) and a OH (δ_H 2.48, d, J=6.5 Hz) in $CDCl_3$ indicated C-11 to be substituted by a hydroxyl group. The HMBC relationship between H_2-19 and C-12 confirmed a new ether bridge connected to C-12 and C-19. Thus, C-1 was supposed to be linked by a hydroxyl group. These assignments were further confirmed by the observation of two tertiary OH groups at δ_H 3.83 (s, OH-1) and 4.45 (s, OH-4) and a OH doublet at δ_H 4.22 (d, J=6.5 Hz) in association with their HMBC and COSY relationships when the NMR spectra were measured in DMSO-d_6. Accordingly, compound **14** was a 1,4,11-trihydroxy-12,19-epoxy analogue. The olefinic geometries were determined as 2*E* and 7*Z* according to the $J_{H\text{-}2/H\text{-}3}$ value (16.0 Hz) and the enhancement of H-9b (δ_H 2.01) when H-7 (δ_H 5.14) was irradiated in 1D gNOESY. The MM2 energy-minimized conformation of **14** was in accordance with the NOE relationships (Fig. 2), of which the NOE interactions from H-2 to H_3-18 and H_3-17 and from H-3 to OH-1 and OH-4 were indicative of the same face of isopropyl group and H_3-18, as the case of **8**. A weak NOE correlation between H-19a/H-2 indicated the ether bond oriented in the same face as H-2. Thus, the NOE correlation between H_3-20 and H-11 in addition to the NOE relationships between OH-11/H-14a (δ_H 1.47) and H-2/H-14a (Fig. 2) suggested C-11 maintaining the same configuration as that of **8** and **6**. In a biogenetic view, the structure of **14** was regarded to be derived from **6** through ether rearrangement.

Sinulariol O (**15**) has a molecular formula of $C_{20}H_{32}O_3$ as determined by HRESIMS, indicating 5° of unsaturation. The NMR data of **15** were mostly compatible to those of **12**, except for the presence of an exocyclic olefin, which was evident from the olefinic methylene signals (δ_H 4.90, 4.87; δ_C 113.0) in addition to a quaternary carbon at δ_C 148.7. The HMBC correlation from H-3 (δ_H 4.79d, J=7.2 Hz) to the carbon of olefinic methylene, and in turn from the methylene protons to C-3 (δ_C 69.4, CH), C-4, and C-5 (δ_C 33.5, CH_2)

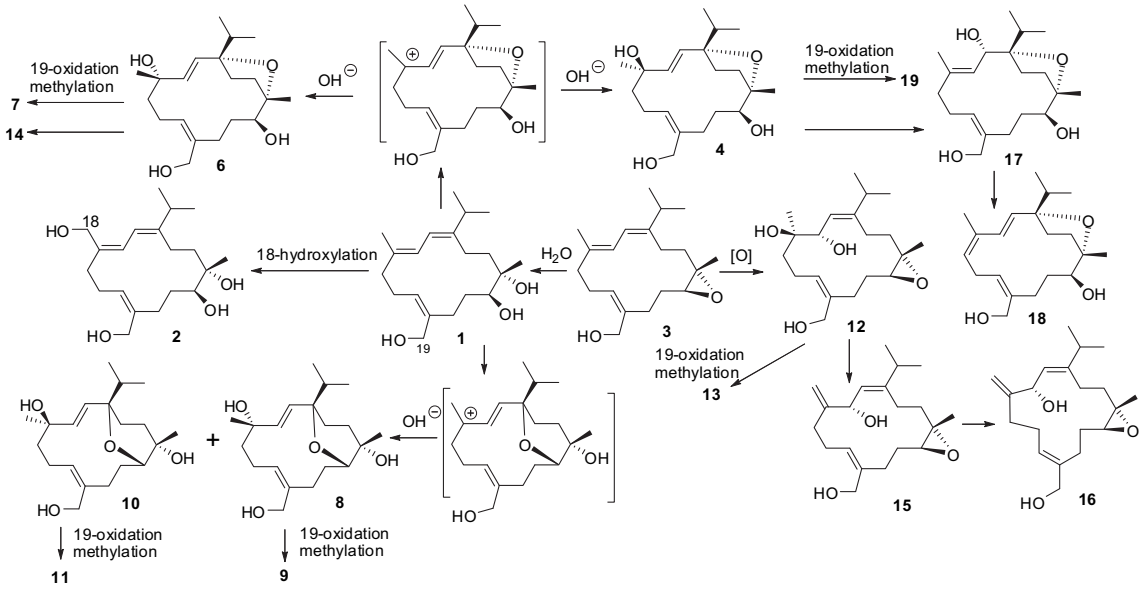
confirmed the location of exocyclic olefin at C-4. The similar NOE relationships between **15** and **12** suggested **15** being a 4-dehydroxylated analogue of **12**.

Analysis of NMR and HRESIMS data conducted the gross structure of sinulariol P (**16**) to be the same as **15**. However, C-19 of **16** shifted significantly to downfield at δ_C 66.0 (CH_2) in comparison with the corresponding carbon of **15**. The obvious NOE correlation between H-7 (δ_H 5.46, dd, J=5.0, 5.4 Hz) and H_2-19 (δ_H 4.01, 4.07) instead of H_2-19 and H-6 relationship indicated **16** to be an 7*E* isomer of **15**.

The NMR data of sinulariol Q (**17**) (Tables 1 and 3) indicated its partial structure in respect to the macrocyclic skeleton from C-5 to C-13 was in good agreement with that of **6**. Although an AB spin system of H-2 (δ_H 4.29, d, J=7.7 Hz) and H-3 (δ_H 5.44, d, J=7.7 Hz) was similar with those of **6**, the HMQC spectrum deduced C-2 to be a hydroxymethine (δ_C 72.3), while an olefin presented at C-3/C-4 was determined by the olefinic methyl H_3-18 (δ_H 1.63, s) showing HMBC corrections to C-3 (δ_C 126.2, CH), C-4 (δ_C 138.6, qC), and C-5 (δ_C 38.0, CH_2). Thus, **17** was assumed to be derived from **6** by the OH migration from C-4 to C-2 following olefinic rearrangement. The upfield resonance of H_3-18 (δ_C 16.9) was indicative of 3*E* geometry.[15] The NOE correlations between H-11 (δ_H 3.28, d)/H-3 and H-11/H-7 (δ_H 5.24, t) indicated the macrocyclic conformation of **17** being in accordance with that of **6**, while the opposite orientation of H-2 and H-3 was recognized by their coupling constants and the absence of their NOE relationship. Thus, the NOE interactions between H-2/H_3-18 and H-2/isopropyl methyls (δ_H 1.00, 1.06) in addition to the close similar NOE relationships of **17** and **6** established H-2 to be in the same face as isopropyl group.

Sinulariol R (**18**) has a molecular formula of $C_{20}H_{32}O_3$ as determined by HRESIMS and NMR data, requiring 5° of unsaturation. Its NMR data were very compatible to these of **6**, except for the presence of an additional trisubstituted olefin at δ_C 134.8 (s, C-4) and 126.1 (d, C-5). The HMBC correlations of the olefinic methyl protons H_3-18 (δ_H 1.77, s) to C-3 (δ_C 129.5), C-4 (δ_C 134.8), and C-5 (δ_C 126.1) confirmed the presence of an olefin at C-4/C-5. Thus, **18** was determined as a 4-dehydroxylated analogue of **6**. The NOESY cross-peak between H-5 (δ_H 5.54, dd, J=4.0, 9.0 Hz) and H_3-18 in association with the downfield-shifted C-18 (δ_C 20.2) allowed the assignment of 4*Z* geometry. The large coupling constant (15.8 Hz) of H-2 and H-3, together with NOESY correlation between H-7 (δ_H 5.50, dd, J=3.5, 10.5 Hz) and H-9b (δ_H 2.06, m) were consistent with 2*E* and 7*Z* geometries. The stereogenic centers of C-1, C-11, and C-12 were the same as **17** due to the similar NOE and NMR data. Compound **17** was partly derived to **18** under NMR magnetic effect in $CDCl_3$ supported the stereogenic assignment.

Cembrane-type diterpenoids are commonly found in *Sinularia* genus and are considered as the chemotaxonomic markers of *Sinularia* species. Cembranoids containing 19-oxygenated functionalities were rarely reported from marine organisms previously. It is also noteworthy that cembranoids possessing C-1/C-11 epoxide such as **8**–**11** are reported from nature for the first time, while the analogues containing C-1/C-12 epoxide were only found from Japanese *Lobophytum schoedei*[16] and Indian *Sinularia ovispiculata*.[13] It is interesting to note that the structural patterns from this soft coral specimen are closely related to those isolated from plant tobacoo,[14] although they inhabit in great different ecological locations and belong to different species kingdom. In general, it is assumed that most cembrane diterpenes are generated from enzymatic cyclization of geranylgeranyl pyrophosphate,[17] while cembrene C[18,19] is considered as an initial metabolite to derive diverse cembranes via various olefinic migration and oxidation as induced by ecological and geographic conditions, involving symbiotic association between polyps and zooxanthellae. A plausible biogenetic relationship of the isolated cembranoids was proposed (Scheme 1). Although the biogenetic pathway of the unusual 19-oxygenated functionalities is uncertain, compound **3** is likely a precursor to generate other analogues through oxidation, epoxidation, and olefinic migration. These depictions are partly supported by the chemical conversion of the isolated analogues. For example, compound **1** is stable in MeOH solution, but it partly converted to compounds **4** and **10** when it exposed to air in two weeks. Compound **17** was partly derived to form **18** by 500 MHz NMR magnetic effect in $CDCl_3$, while the latter compound was also able to be derived from **4** under trace acid (AcOH). The environmental sensitive cembranoids evoked us to investigate whether part of the cembrane analogues are derived during separation process. Therefore, we detected the MeOH extract of the frozen soft coral by HPLC/ESI-MS. Fortunately, all isolated compounds were presented in HPLC fingerprint, and they are checked by HPLC-ESIMS data. These facts indicated that the isolated cembranoids may originate from soft coral. The co-occurrence of epimers such as **8** and **10** in soft coral was considered to be derived by ecological condition rather than enzymic reaction.



Scheme 1. Plausible biogenetic relationship of the isolated cembranoids.

In bioassays, the isolated compounds showed weak cytotoxicity against human tumor cell lines Bel-7402 (hepatocellular carcinoma), CNE-2 (nasopharyngeal carcinoma), and HT-1080 (fibrosarcoma) in a dose of 50 μg/ml, and also weak inhibition toward a profile of pathogenetic microorganisms (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Saccharomyces cerevisia*, Aspergillus fumigatus, *Aspergillus flavus*, *Fusarium oxysporum*) in a dose of 20 μg/ml. In order to detect whether they possess chemical ecological property, the anti-fouling activity of the isolated compounds were tested against the larval settlement of barnacle *Balanus amphitrite* and *Bugula 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. The results showed that compound **10** was significantly inhibited the larval settlement of *B. amphitrite*, while compound **16** showed moderate inhibition against the adhesion of *B. neritina* (Table 4). These data informed the isolated cembranes partly taking part in anti-fouling functions.

Table 4
The effects of compounds against larval settlement of fouling species

Compds	*B. amphitrite*		*B. neritina*	
	EC_{50} (μg/ml)	LC_{50}/EC_{50}	EC_{50} (μg/ml)	LC_{50}/EC_{50}
1	22.5	>2.22	>25	UD[a]
10	5.65	>8.85	22.50	>2.22
16	>25	UD	14.03	>3.56

[a] UD: undetectable.

Present work provided additional structural patterns to enrich cembranoid family. Although numerous cembranoids have been isolated from marine organisms, searching for more novel structural patterns and their pharmaceutical and chemoecological usage are unlimited. Rich diverse *Sinularia* species inhabited in South China Sea provided a promising pool for the discovery of novel bioactive marine natural products.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. IR spectra were recorded on Thermo Nicolet Nexus 470 FT-IR spectrometer. ^1H, ^{13}C, and 2D NMR spectra were recorded on Bruker Avance 400 NMR and Bruker Avance 500 NMR spectrometers (400/500 MHz for ^1H, and 100/125 MHz for ^{13}C). Chemical shifts expressing in δ (ppm) referred to the solvent peaks δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 2.50 and δ_C 40.0 for DMSO-d_6, respectively, and coupling constants in hertz. ESIMS and HRESIMS were obtained from 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 Alltech uvis-200 detector at 210 nm and semipreparative reversed-phased columns (YMC-packed C_8, 5 μm, 250×10 mm; and Kromasil-ODS, 10 μm, 250×10 mm).

3.2. 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 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, and also deposited at the National Museum of Natural History Naturalis, The Netherlands.

3.3. Extraction and isolation

The frozen sample (2.5 kg, wet weight) was homogenized and then extracted with MeOH. The concentrated extract was desalted by 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 *n*-BuOH to yield the corresponding fractions. The EtOAc fraction (5.5 g) was subjected to Si gel vacuum liquid chromatography (VLC) eluting with a gradient of PE/EtOAc (10:1, 5:1, 3:1, 1:1, 0:1) to obtain six portions (P1-P6). Each portion was detected by ^1H NMR spectrum, and cembranoids were recognized to exist in portions P2–P5. P2 (516.7 mg) was subsequently subjected to Si gel column (200–300 mesh) chromatography eluting with a gradient of petroleum ether (PE)/acetone (10:1 to 0:1) and followed by ODS column chromatograph with a gradient of MeOH/H_2O (from 65% to 80%) to yield **3** (2.4 mg), **9** (1.9 mg), **7** (1.3 mg), and **5** (5.2 mg). P5 (700.1 mg) was chromatographed on an ODS column eluting with a gradient of MeOH/H_2O (65%, 70%, 75%, 80%, 85%) to afford a mixture of **13** and **19** (3.8 mg), **1** (38.2 mg), and **10** (3.9 mg). P4 (911.3 mg) was subjected to flash chromatography on a Si gel column (160–200 mesh) eluting with acetone/PE (4:7) and followed by RP-C18 column using MeOH/H_2O (77:23) as an eluant to yield **11** (3.0 mg), **14** (3.5 mg), and **18** (1.3 mg). P3 (87 mg) was separated upon semipreparative HPLC with 38% CH_3CN/H_2O as a mobile phase to yield **8** (19.7 mg), **6** (10.1 mg), **4** (48.5 mg), **12** (27.7 mg), and **17** (3.1 mg). Compounds **2** (4.6 mg), **15** (3.7 mg), and **16** (2.8 mg) were purified from P6 (65 mg) by the same protocol as for P5.

*3.3.1. Sinulariol A (**1**).* Colorless oil; $[\alpha]_D^{24}$ +17.1 (*c* 0.79, $CHCl_3$); UV (MeOH) λ_{max} 248 nm; IR (KBr) ν_{max} 3736, 3320, 2961, 2934, 2872, 1714, 1586, 1450, 1376, 1295, 1138 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 345.2402 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2400).

*3.3.2. Sinulariol B (**2**).* Colorless oil; $[\alpha]_D^{22}$ +53.0 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} 202, 242 nm; IR (KBr) ν_{max} 3305, 2958, 2925, 2857, 1716, 1609, 1448, 1299, 1176, 1134, 1080 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 361.2343 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*3.3.3. Sinulariol C (**3**).* Colorless oil; $[\alpha]_D^{22}$ −8.8 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} 243 nm; IR (KBr) ν_{max} 3402, 2958, 2874, 1549, 1451, 1379, 1134, 1032 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 327.2281 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_2Na$, 327.2295).

*3.3.4. Sinulariol D (**4**).* Colorless oil; $[\alpha]_D^{24}$ +7.5 (*c* 1.35, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3340, 2962, 2929, 2873, 1717, 1456, 1373, 1310, 1169, 1078, 1048, 1014 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2 (^1H and ^{13}C NMR data in DMSO-d_6, see Supplementary data); HRESIMS *m/z* 361.2350 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*3.3.5. Sinulariol E (**5**).* Colorless oil; $[\alpha]_D^{24}$ −81.5 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} 202 nm; IR (KBr) ν_{max} 3333, 2963, 2931, 2872, 1720, 1456, 1373, 1313, 1275, 1072, 1053, 1003 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 379.2011 (100%), 381.1982 (32.5%) $[M+Na]^+$ (calcd for $C_{20}H_{33}{}^{35}ClO_3Na$, 379.2010; $C_{20}H_{33}{}^{37}ClO_3Na$, 381.1981).

*3.3.6. Sinulariol F (**6**).* Colorless oil; $[\alpha]_D^{24}$ −9.8 (*c* 0.38, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3734, 3383, 2966, 2931, 2875,

1452, 1375, 1079, 1014 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2 (^1H and ^{13}C NMR data in DMSO-d_6, see Supplementary data); HRESIMS *m/z* 361.2334 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*3.3.7. Sinulariol G (**7**).* Colorless oil; $[\alpha]_D^{27}$ −21.7 (*c* 0.24, MeOH); UV (MeOH) λ_{max} 201, 228 nm; IR (KBr) ν_{max} 3449, 2964, 2931, 1692, 1627, 1512, 1453, 1154, 1011 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 389.2296 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_5Na$, 389.2298).

*3.3.8. Sinulariol H (**8**).* Colorless oil; $[\alpha]_D^{24}$ −71.3 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} 200 nm; IR (KBr) ν_{max} 3343, 2962, 2931, 2874, 1700, 1652, 1458, 1378, 1133, 1084 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2 (^1H and ^{13}C NMR Data in DMSO-d_6, see Supplementary data); HRESIMS *m/z* 361.2336 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*3.3.9. Sinulariol I (**9**).* Colorless oil; $[\alpha]_D^{26}$ −18.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 202, 228 nm; IR (KBr) ν_{max} 3735, 3414, 2933, 2870, 1698, 1624, 1442, 1381, 1246, 1141, 1061 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 389.2298 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_5Na$, 389.2298).

*3.3.10. Sinulariol J (**10**).* Colorless oil; $[\alpha]_D^{24}$ −40.5 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3343, 2961, 2932, 2874, 1712, 1672, 1443, 1376, 1130, 1058 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3 (^1H and ^{13}C NMR Data in DMSO-d_6, see Supplementary data); HRESIMS *m/z* 361.2349 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*3.3.11. Sinulariol K (**11**).* Colorless oil; $[\alpha]_D^{24}$ −47.1 (*c* 0.15, MeOH); UV (MeOH) λ_{max} 201, 229 nm; IR (KBr) ν_{max} 3320, 2961, 2873, 1709, 1444, 1378, 1248, 1200 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 389.2299 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_5Na$, 389.2298).

*3.3.12. Sinulariol L (**12**).* Colorless oil; $[\alpha]_D^{24}$ −9.0 (*c* 0.49, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3338, 2960, 2929, 2871, 1726, 1655, 1554, 1462, 1382, 1261, 1098, 1021 cm^{-1}; ^1H 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).

*3.3.13. Sinulariols M (**13**) and S (**19**).* Colorless oil; UV (MeOH) λ_{max} 203, 229 nm; IR (KBr) ν_{max} 3487, 2963, 2873, 1716, 1573, 1440, 1381, 1246, 1205, 1134, 1072 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.26 (d, *J*=9.7 Hz, H-2, **13**), 5.69 (d, *J*=15.5 Hz, H-2, **19**), 4.17 (d, *J*=9.7 Hz, H-3, **13**), 5.78 (d, *J*=15.5 Hz, H-3, **19**), 2.00 (m, H-5a, **13**), 1.87 (m, H-5a, **19**), 1.63 (m, H-5b, **13**), 1.77 (m, H-5b, **19**), 2.55 (m, H_2-6, **13**), 2.77 (m, H-6a, **19**), 2.57 (m, H-6b, **19**), 6.09 (dd, *J*=7.5, 8.0 Hz, H-7, **13**), 6.01 (dd, *J*=8.0, 8.0 Hz, H-7, **19**), 2.55 (m, H_2-9, **13**), 2.40 (m, H_2-9, **19**), 1.97 (m, H-10a, **13**), 1.56 (m, H-10b, **13**), 1.94 (m, H-10a, **19**), 1.52 (m, H-10b, **19**), 2.73 (dd, *J*=2.8, 9.6 Hz, H-11, **13**), 3.21 (d, *J*=10.3 Hz, H-11, **19**), 2.20 (m, H-13a, **13**), 1.06 (m, H-13b, **13**), 2.04 (m, H-13a, **19**), 1.62 (m, H-13b, **19**), 2.20 (m, H-14a, **13**), 1.92 (m, H-14b, **13**), 1.94 (m, H-14a, **19**), 1.76 (m, H-14b, **19**), 2.28 (dq, *J*=7.0 Hz, H-15, **13**), 1.63 (dq, *J*=7.0 Hz, H-15, **19**), 1.02 (d, *J*=7.0 Hz, H_3-16, **13**), 0.84 (d, *J*=7.0 Hz, H_3-16, **19**), 1.01 (d, *J*=7.0 Hz, H_3-17, **13**), 0.80 (d, *J*=7.0 Hz, H_3-17, **19**), 1.11 (s, H_3-18, **13**), 1.29 (s, H_3-18, **19**), 1.19 (s, H_3-20, **13**), 1.11 (s, H_3-20, **19**), 3.79 (s, OMe, **13**), 3.76 (s, OMe, **19**); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.0 (qC, C-1, **13**), 88.0 (qC, C-1, **19**), 121.1 (CH, C-2, **13**), 131.8 (CH, C-2, **19**), 70.7 (CH, C-3, **13**), 136.7 (CH, C-3, **19**), 74.6 (qC, C-4, **13**), 73.1 (qC, C-4, **19**), 37.0 (CH_2, C-5, **13**), 42.6 (CH_2, C-5, **19**), 25.7 (CH_2, C-6, **13**), 25.4 (CH_2, C-6, **19**), 144.1 (CH, C-7, **13**), 145.7 (CH, C-7, **19**), 130.9 (qC, C-8, **13**), 130.9 (qC, C-8, **19**), 33.3 (CH_2, C-9, **13**), 28.3 (CH_2, C-9, **19**), 24.9 (CH_2, C-10, **13**), 32.2 (CH_2, C-10, **19**), 62.6 (CH, C-11, **13**), 73.3 (CH, C-11, **19**), 62.0 (qC, C-12, **13**), 85.0 (qC, C-12, **19**), 41.2 (CH_2, C-13, **13**), 35.8 (CH_2, C-13, **19**), 25.8 (CH_2, C-14, **13**), 33.7 (CH_2, C-14, **19**), 35.8 (CH, C-15, **13**), 38.9 (CH, C-15, **19**), 21.9 (CH_3, C-16, **13**), 18.5 (CH_3, C-16, **19**), 21.6 (CH_3, C-17, **13**), 17.4 (CH_3, C-17, **19**), 22.6 (CH_3, C-18, **13**), 30.9 (CH_3, C-18, **19**), 168.5 (qC, C-19, **13**), 169.5 (qC, C-19, **19**), 16.3 (CH_3, C-20, **13**), 19.8 (CH_3, C-20, **19**), 51.6 (OCH_3, **13**), 51.7 (OCH_3, **19**); HRESIMS *m/z* 389.2283 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_5Na$, 389.2298).

*3.3.14. Sinulariol N (**14**).* Colorless oil; $[\alpha]_D^{22}$ +4.3 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} 204 nm; IR (KBr) ν_{max} 3447, 2964, 2930, 2873, 1580, 1447, 1376, 1285, 1163, 1087 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 361.2357 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*3.3.15. Sinulariol O (**15**).* Colorless oil; $[\alpha]_D^{20}$ +72.8 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} 202 nm; IR (KBr) ν_{max} 3340, 2960, 2873, 1710, 1459, 1385, 1249, 1074, 1008 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 343.2241 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2244).

*3.3.16. Sinulariol P (**16**).* Colorless oil; $[\alpha]_D^{23}$ +27.6 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} 202 nm; IR (KBr) ν_{max} 3735, 3415, 2961, 2932, 2872, 1712, 1459, 1383, 1058, 1006 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 343.2230 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2244).

*3.3.17. Sinulariol Q (**17**).* Colorless oil; $[\alpha]_D^{23}$ −11.1 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} 201 nm; IR (KBr) ν_{max} 3735, 3327, 2924, 2855, 1558, 1454, 1089, 1014 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 361.2351 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

*3.3.18. Sinulariol R (**18**).* Colorless oil; $[\alpha]_D^{22}$ +26.5 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} 200, 239 nm; IR (KBr) ν_{max} 3405, 2966, 2876, 1710, 1626, 1450, 1379, 1081 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 343.2236 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2244).

3.4. Preparation of TBS ether of 1[20]

To a CH_2Cl_2 solution (400 μL) containing **1** (2.6 mg, 0.008 mmol), 4-dimethylaminopyridine (DMAP, 1.0 mg, 0.008 mmol), and imidazole (2.2 mg, 0.032 mmol), *tert*-butyldimethylsilyl chloride (TBSCl, 4.8 mg, 0.032 mmol) was added to stir for 9 h at rt under argon atmosphere. After filtrated and concentrated under reduced pressure, the residue was purified by an ODS column chromatography eluting with 61% and 100% MeOH/H_2O to obtain TBS ether (2.6 mg, 73.8% yield).

*3.4.1. TBS ether of **1**.* ^1H NMR (400 MHz, $CDCl_3$) δ 6.00 (1H, d, *J*=11.2 Hz, H-2), 6.06 (1H, d, *J*=11.2 Hz, H-3), 2.22 (2H, m, H-5a and H-6b), 2.12 (1H, m, H-5b), 2.31 (1H, m, H-6a), 5.24 (1H, dd, *J*=7.2, 6.4 Hz, H-7), 2.42 (1H, br d, *J*=14.0 Hz, H-9a), 2.14 (1H, ddd, *J*=14.2, 12.8, 3.6 Hz, H-9b), 1.87 (1H, br t, *J*=14.4 Hz, H-10a), 1.42 (1H, m, H-10b), 3.68 (1H, d, *J*=10.8 Hz, H-11), 1.91 (1H, m, H-13a), 1.70 (1H, m, H-13b), 2.62 (1H, ddd, *J*=14.0, 10.2, 5.6 Hz, H-14a), 2.08 (1H, m, H-14b), 2.49 (1H, sept, *J*=6.8 Hz, H-15), 1.10 (3H, d, *J*=6.8 Hz, Me-16), 1.03 (3H, d, *J*=6.8 Hz, Me-17), 1.74 (3H, s, Me-18), 4.22 (1H, d, *J*=11.6 Hz, H-19a), 3.92 (1H, d, *J*=11.6 Hz, H-19b), 1.09 (3H, s, Me-20), 0.91 (9H, s, $(CH_3)_3CSi(CH_3)_2$), 0.08 (6H, s, $(CH_3)_3CSi(CH_3)_2$). HRESIMS *m/z* 459.3254 $[M+Na]^+$ (calcd for $C_{26}H_{48}O_3SiNa$, 459.3265).

3.5. Preparation of (*R*)- and (*S*)-MPA esters[21]

To a CH_2Cl_2 solution (360 μL) containing TBS ether of **1** (1.3 mg, 0.0030 mmol), (*R*)-MPA (1.0 mg, 0.0060 mmol), and DMAP, *N*,*N*′-dicyclohexylcarbodiimide (DCC, 1.3 mg, 0.0063 mmol) was added

to react for 24 h at rt. The crude products were separated by ODS column chromatography, eluting with 60%, and 100% MeOH/H_2O, successively, and the 100% fraction was purified by RP-HPLC (94% MeOH/H_2O as mobile phase) to afford (*R*)-MPA ester (0.5 mg). In a similar manner, (*S*)-MPA ester (0.6 mg) was prepared from (*S*)-MPA.

3.5.1. (R)-MPA ester of TBS ether of ***1****.* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.083 (1H, d, *J*=11.0 Hz, H-2), 6.402 (1H, d, *J*=11.0 Hz, H-3), 2.200 (1H, m, H-5a), 2.082 (1H, m, H-5b), 2.255 (2H, m, H_2-6), 5.121 (1H, br t, *J*=7.8 Hz, H-7), 2.149 (1H, m, H-9a), 1.915 (1H, br t, *J*=13.5 Hz, H-9b), 1.278 (1H, m, H-10a), 1.101 (1H, m, H-10b), 5.043 (1H, d, *J*=10.0 Hz, H-11), 1.713 (1H, m, H-13a), 1.470 (1H, dt, *J*=14.8, 4.0 Hz, H-13b), 2.926 (1H, ddd, *J*=14.0, 12.6, 3.8 Hz, H-14a), 1.787 (1H, m, H-14b), 2.469 (1H, sept, *J*=6.8 Hz, H-15), 1.095 (3H, d, *J*=6.8 Hz, Me-16), 0.974 (3H, d, *J*=6.8 Hz, Me-17), 1.702 (3H, s, Me-18), 4.000 (1H, d, *J*=12.0 Hz, H-19a), 3.743 (1H, d, *J*=12.0 Hz, H-19b), 1.035 (3H, s, Me-20), 0.835 (9H, s, $(CH_3)_3CSi(CH_3)_2$), −0.012, −0.018 (each 3H, s, $(CH_3)_3CSi(CH_3)_2$−).

3.5.2. (S)-MPA ester of TBS ether of ***1****.* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.034 (1H, d, *J*=11.0 Hz, H-2), 6.329 (1H, d, *J*=11.0 Hz, H-3), 2.212 (1H, m, H-5a), 2.124 (1H, m, H-5b), 2.297 (2H, m, H_2-6), 5.373 (1H, br t, *J*=7.3 Hz, H-7), 2.476 (1H, br dd, *J*=12.8, 2.4 Hz, H-9a), 2.010 (1H, br t, *J*=12.9 Hz, H-9b), 1.736 (1H, br t, *J*=12.8 Hz, H-10a), 1.455 (1H, m, H-10b), 5.024 (1H, d, *J*=10.0 Hz, H-11), 1.300 (1H, m, H-13a), 0.469 (1H, dt, *J*=14.2, 4.7 Hz, H-13b), 2.548 (1H, td, *J*=13.8, 3.0 Hz, H-14a), 1.365 (1H, m, H-14b), 2.324 (1H, partly overlapped, H-15), 1.037 (3H, d, *J*=6.8 Hz, Me-16), 0.893 (3H, d, *J*=6.8 Hz, Me-17), 1.698 (3H, s, Me-18), 4.093 (1H, d, *J*=12.0 Hz, H-19a), 3.842 (1H, d, *J*=12.0 Hz, H-19b), 0.825 (3H, s, Me-20), 0.868 (9H, s, $(CH_3)_3CSi(CH_3)_2$), 0.025 (6H, s, $(CH_3)_3CSi(CH_3)_2$−).

3.6. Preparation of TBS ether of 4

Following the same protocol as mentioned for **1**, TBS ether of **4** (8.7 mg, 76.5% yield) was prepared.

3.6.1. TBS ether of ***4****.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.62 (1H, d, *J*=15.6 Hz, H-2), 5.84 (1H, d, *J*=15.6 Hz, H-3), 1.83 (1H, m, H-5a), 1.65 (1H, m, H-5b), 2.32 (1H, m, H-6a), 2.19 (1H, m, H-6b), 5.36 (1H, t, *J*=7.6 Hz, H-7), 2.36 (1H, br d, *J*=15.8 Hz, H-9a), 2.02 (1H, m, H-9b), 2.02 (1H, m, H-10a), 1.43 (1H, m, H-10b), 3.44 (1H, d, *J*=10.0 Hz, H-11), 1.90 (1H, m, H-13a), 1.71 (1H, m, H-13b), 1.90 (1H, m, H-14a), 1.79 (1H, m, H-14b), 1.65 (1H, m, H-15), 0.85 (3H, d, *J*=6.8 Hz, Me-16), 0.82 (3H, d, *J*=6.8 Hz, Me-17), 1.28 (3H, s, Me-18), 4.35 (1H, d, *J*=11.6 Hz, H-19a), 4.05 (1H, d, *J*=11.6 Hz, H-19b), 1.09 (3H, s, Me-20), 0.91 (9H, s, $(CH_3)_3CSi(CH_3)_2$), 0.10 and 0.09 (each 3H, s, $(CH_3)_3CSi(CH_3)_2$). HRESIMS *m*/*z* 475.3214 $[M+Na]^+$ (calcd for $C_{26}H_{48}O_4SiNa$, 475.3216).

3.7. Preparation of (*R*)- and (*S*)-MPA esters

Followed by the same method as indicated for **1**, TBS ether of **4** was converted to its *R*-MPA ester of TBS ether (1.4 mg) and *S*-MPA ester of TBS ether (1.2 mg), respectively.

3.7.1. (R)-MPA ester of TBS ether of ***4****.* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.553 (1H, d, *J*=15.5 Hz, H-2), 5.780 (1H, d, *J*=15.5 Hz, H-3), 1.843 (1H, ddd, *J*=12.0, 8.8, 1.8 Hz, H-5a), 1.743 (1H, td, *J*=12.0, 1.7 Hz, H-5b), 2.291 (1H, dddd, *J*=15.5, 9.9, 5.9, 4.0 Hz, H-6a), 2.152 (1H, m, H-6b), 5.422 (1H, dd, *J*=11.0,4.5 Hz, H-7), 2.107 (1H, ddd, *J*=14.2, 3.4, 1.5 Hz, H-9a), 1.875 (1H, td, *J*=14.0,1.5 Hz, H-9b), 1.409 (1H, m, H-10a), 1.167 (1H, br t, *J*=13.5 Hz, H-10b), 5.041 (1H, d, *J*=10.0 Hz, H-11), 1.665 (1H, ddd, *J*=11.7,11.7,7.5 Hz, H-13a), 1.341 (1H, ddd, *J*=11.9, 5.8, 3.5 Hz, H-13b), 1.777 (2H, m, H_2-14), 1.611 (1H, sept, *J*=7.0 Hz, H-15), 0.807 (3H, d, *J*=7.0 Hz, Me-16), 0.773 (3H, d, *J*=7.0 Hz, Me-17), 1.240 (3H, s, Me-18), 4.203 (1H, d, *J*=11.5 Hz, H-19a), 4.109 (1H, d, *J*=11.5 Hz, H-19b), 1.056 (3H, s, Me-20), 0.871 (9H, s, $(CH_3)_3CSi(CH_3)_2$−), 0.036, 0.025 (each 3H, s, $(CH_3)_3CSi(CH_3)_2$−).

3.7.2. (S)-MPA ester of TBS ether of ***4****.* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.487 (1H, d, *J*=15.0 Hz, H-2), 5.767 (1H, d, *J*=15.0 Hz, H-3), 1.845 (1H, dd, *J*=13.0, 8.0 Hz, H-5a), 1.747 (1H, br t, *J*=12.0 Hz, H-5b), 2.319 (1H, m, H-6a), 2.178 (1H, m, H-6b), 5.616 (1H, dd, *J*=11.0,4.0 Hz, H-7), 2.374 (1H, m, H-9a), 1.945 (1H, m, H-9b), 1.668 (1H, m, H-10a), 1.552 (1H, m, H-10b), 5.021 (1H, d, *J*=9.5 Hz, H-11), 1.217 (1H, m, H-13a), 0.579 (1H, ddd, *J*=12.1, 4.5, 4.5 Hz, H-13b), 1.587 (2H, m, H_2-14), 1.540 (1H, m, H-15), 0.753 (3H, d, *J*=7.0 Hz, Me-16), 0.733 (3H, d, *J*=7.0 Hz, Me-17), 1.236 (3H, s, Me-18), 4.282 (1H, d, *J*=12.0 Hz, H-19a), 4.164 (1H, d, *J*=12.0 Hz, H-19b), 0.844 (3H, s, Me-20), 0.895 (9H, s, $(CH_3)_3CSi(CH_3)_2$−), 0.065, 0.059 (each 3H, s, $(CH_3)_3CSi(CH_3)_2$−).

3.8. Preparation of (*R*)- and (*S*)-MPA esters of 12

Followed by the same procedure as for the preparation of MPA esters of **1**, (*R*)-MPA ester of **12** (1.4 mg), and (*S*)-MPA ester of **12** (1.7 mg) were obtained.

3.8.1. (R)-MPA ester of ***12****.* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.840 (1H, d, *J*=9.5 Hz, H-2), 5.302 (1H, d, *J*=9.5 Hz, H-3), 1.675 (1H, m, H-5a), 1.467 (1H, m, H-5b), 2.172 (2H, m, H_2-6), 5.227 (1H, t, *J*=6.7 Hz, H-7), 2.113 (2H, m, H_2-9), 1.695 (1H, m, H-10a), 1.219 (1H, m, H-10b), 3.073 (1H, br d, *J*=9.0 Hz, H-11), 1.913 (1H, m, H-13a), 1.029 (1H, m, H-13b), 1.953 (1H, m, H-14a), 1.913 (1H, m, H-14b), 2.097 (1H, m, H-15), 0.895 (3H, d, *J*=7.0 Hz, Me-16), 0.815 (3H, d, *J*=7.0 Hz, Me-17), 0.967 (3H, s, Me-18), 4.751 (1H, d, *J*=12.0 Hz, H-19a), 4.507 (1H, d, *J*=12.0 Hz, H-19b), 1.122 (3H, s, Me-20).

3.8.2. (S)-MPA ester of ***12****.* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.044 (1H, d, *J*=9.5 Hz, H-2), 5.258 (1H, d, *J*=9.5 Hz, H-3), 1.191 (1H, m, H-5a), 1.111 (1H, m, H-5b), 1.993 (1H, m, H-6a), 1.926 (1H, m, H-6b), 5.077 (1H, t, *J*=5.0 Hz, H-7), 2.170 (2H, m, H_2-9), 1.811 (1H, m, H-10a), 1.402 (1H, m, H-10b), 3.148 (1H, br d, *J*=9.5 Hz, H-11), 2.199 (1H, m, H-15), 0.969 (3H, d, *J*=6.8 Hz, Me-16), 0.966 (3H, d, *J*=6.8 Hz, Me-17), 0.884 (3H, s, Me-18), 4.673 (1H, d, *J*=12.4 Hz, H-19a), 4.525 (1H, d, *J*=12.4 Hz, H-19b), 1.171 (3H, s, Me-20).

3.9. Biological assays

The cytotoxic properties of the isolated compounds were tested in vitro using human tumor cell lines including Bel-7402, CNE-2, and HT-1080 at 100 μg/ml, and 50 μg/ml by WST-8 method as mentioned in literature.[22]

The antimicrobial activities were tested against *S. aureus*, *S. pneumoniae*, *P. aeruginosa*, *E. coli*, *C. albicans*, *S. cerevisia*, Aspergillus fumigatus, *A. flavus*, *F. oxysporum* by the agar disc diffusion assay.[23]

3.10. Larval settlement bioassays[24,25]

Adults of the barnacle *B. amphitrite* Darwin were exposed to air for more than 6 h, and then were placed in a container filled with fresh 0.22-μm filtered sea water (FSW) to release nauplii. The collected nauplii were reared to cyprid stage according to the method described by Thiyagarajan et al.[24] 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 *B. neritina* were collected from submerged rafts at the fish farmsin Yung Shue-O,

Hong Kong (114°21′E, 22°24′N) and larvae were obtained according to the method described by Dobretsov.[25]

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Supplementary data

1D and 2D NMR, IR, and MS spectra for Sinulariols A–S (**1**–**19**). This material is available free of charge via the internet at http://pubs.acs.org. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.06.029.

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