Sinulaflexiolides A−K, Cembrane-Type Diterpenoids from the Chinese Soft Coral *Sinularia flexibilis*

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A bioassay-guided fractionation and chemical examination of the soft coral *Sinularia flexibilis* resulted in the isolation and characterization of sinulaflexiolides A−K (**1**−**11**), along with sinulariolone (**12**), 5-dehydrosinularolide (**13**), capillolide (**14**), sinulariolide (**15**), 5,8-epoxy-9-acetoxysinulariolide (**16**), flexibilide (**17**), dihydroflexibilide (**18**), and the enantiomer of 14-deoxycrassin (**19**). Their structures were determined on the basis of extensive spectroscopic (IR, MS, 2D NMR) data analysis and by comparison with spectroscopic data reported in the literature. Sinulaflexiolides D and E showed selective inhibitory activity against the gastric gland carcinoma cell line BGC-823 at 8.5 and 0.12 μM, respectively.

Cembranolides are a class of diterpenes containing a 14-membered carbocyclic nucleus, commonly fused to a five-, six-, or seven-membered lactone ring. There is a wide range of structural complexity within the series. They are characterized by methyl or methylene substitution at C-4, C-8, and C-12, while C-1 is substituted by an isopropyl group, any of which is easily oxidized during biogenetic transformation. So far, almost all cembranes isolated from Alcyonacean soft corals have *R* (α-series) configuration at C-1, whereas the analogues isolated from Gorgonacean animals possess *S*-configuration (*β*-series).[1] One hypothesis suggests that the cembrane skeleton originates from the cyclization of geranylgeranyl pyrophosphate.[1] The soft coral *Sinularia flexibilis* is a rich source of cembrane diterpenes,[2−12] and the relative ratios and chemical diversity of the cembranoids depend on the collection location; for example, lobane[13] and cladiellane-type[14] diterpenes are only isolated from the same specimen growing in the coral-rich habitat near Okinawa reef region (Japan). Previous chemical examination of *S. flexibilis* collected in different parts of the West-Pacific (Australia, Taiwan, the South China Sea, India, Japan) resulted in the isolation of more than 20 cembrane-type diterpenoids. Part of them exhibit interesting bioactivities including ichthyotoxic,[15] cytotoxic,[4,16,17] antimicrobial,[18] cardiotonic and vasorelaxant,[19] and antifouling properties.[20] As part of our continuing interest in chemical diversity of marine organisms from the South China Sea, a soft coral defined as *S. flexibilis* was collected from Sanya Bay, Hainan Island (P. R. China). Bioassay-guided fractionation revealed that the EtOAc fraction of the EtOH extract showed inhibitory activity against the tumor cell lines HL-60 (inhibitory ratio: 87% at 100 μg/mL), HeLa (inhibitory ratio: 92% at 100 μg/mL), BGC-823 (inhibitory ratio: 93% at 100 μg/mL), Bel-7402 (inhibitory ratio: 52% at 100 μg/mL), PC-3M-IE8 (inhibitory ratio: 83% at 100 μg/mL), and Hep-2 (inhibitory ratio: 83% at 100 μg/mL). This paper reports the structural elucidation of the new cembrane diterpenes sinulaflexiolides A−K (**1**−**11**). The cytotoxicity of several of these compounds is also evaluated.

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Results and Discussion

Repeated column chromatography of the bioactive EtOAc fraction resulted in the isolation and characterization of 11 new cembrane diterpenoids, sinulaflexiolides A−K (**1**−**11**), along with known cembranoid derivatives (**12**−**19**). The structures of known compounds were identified by analysis of the NMR spectroscopic data and by comparison with those reported in the literature. Sinulariolone (**12**) was isolated from Philippine *S. flexibilis*, and its stereochemistry was previously confirmed by X-ray crystallographic analysis.[7] 5-Dehydrosinularolide (**13**) was reported from the same organism collected in Taiwan,[2] Australia,[9] and the Red Sea.[21] Capillolide (**14**) originated from *S. microclavata*[22] and *S. capillosa*[15] collected in the South China Sea, while sinulariolide (**15**) and 9-acetoxy-5,8-tetrahydrofuransinulariolide (**16**) were previously isolated from the *S. flexibilis* growing in Okinawa[8] and also from the Chinese *S. capillosa.*[15] Flexibilide (sinularin, **17**) and dihydroflexibilide (dihydrosinularin, **18**) were previously obtained from the Japanese *S. flexibilis* collected in Okinawa.[16,23]

The spectroscopic data analysis and comparison of NMR and MS data revealed that the structure of **19** was identical to 14-deoxycrassin, a cembrane derivative originated from the gorgonian *Eunica mammosa*[1] and subsequently synthesized.[24] However, the antipodal rotation [$[\alpha]^{25}_{D} = -16.7$ (*c* 0.5, $CHCl_3$)] of **19** in comparison with that of 14-deoxycrassin [$[\alpha]^{25}_{D} = +21.0$ (*c* 0.6, $CHCl_3$)] suggested **19** to be the enantiomer of 14-deoxycrassin. This conclusion is in agreement with the observation that cembranoid diterpenes from Alcyonacean soft corals have opposite absolute configurations at C-1 compared to those obtained from gorgonians.[1]

Analysis of 1D and 2D NMR spectroscopic data (COSY, HMQC, and HMBC) in association with IR and MS spectroscopic data suggested that the gross structure of sinulaflexiolide A (**1**) was identical to sinuflexlin,[3] a biscembranoid formerly isolated from Taiwan *S. flexibilis*. The only noticeable difference was the *J* value associated with H-3′ (δ 4.02, brd, $J = 11.0$ Hz) of **1** in comparison with the latter (δ 4.07, dd, $J = 3.5$, 3.6 Hz). This finding indicated that H-3′ of **1** was axial−axial and axial−equatorial to H_2-2′, as previously reported for flexibilide (**17**) and dihydroflexibilide (**18**),[16] whereas H-3′ of sinuflexlin was equatorially oriented. The *J* values of H-3 and H-3′ were consistent with a *cis*-fusion of the δ-lactone,[15] which is characterized by a rather large coupling constant of H-3 ($J = 10.5$ Hz) and H-3′ ($J = 11.0$ Hz). Thus, compound **1** was determined to be a C-3′ epimer of sinuflexlin.

The molecular formula of sinulaflexiolide B (**2**) was determined to be $C_{20}H_{32}O_6$ by HRESIMS (*m*/*z* 391.2097 $[M + Na]^+$), implying

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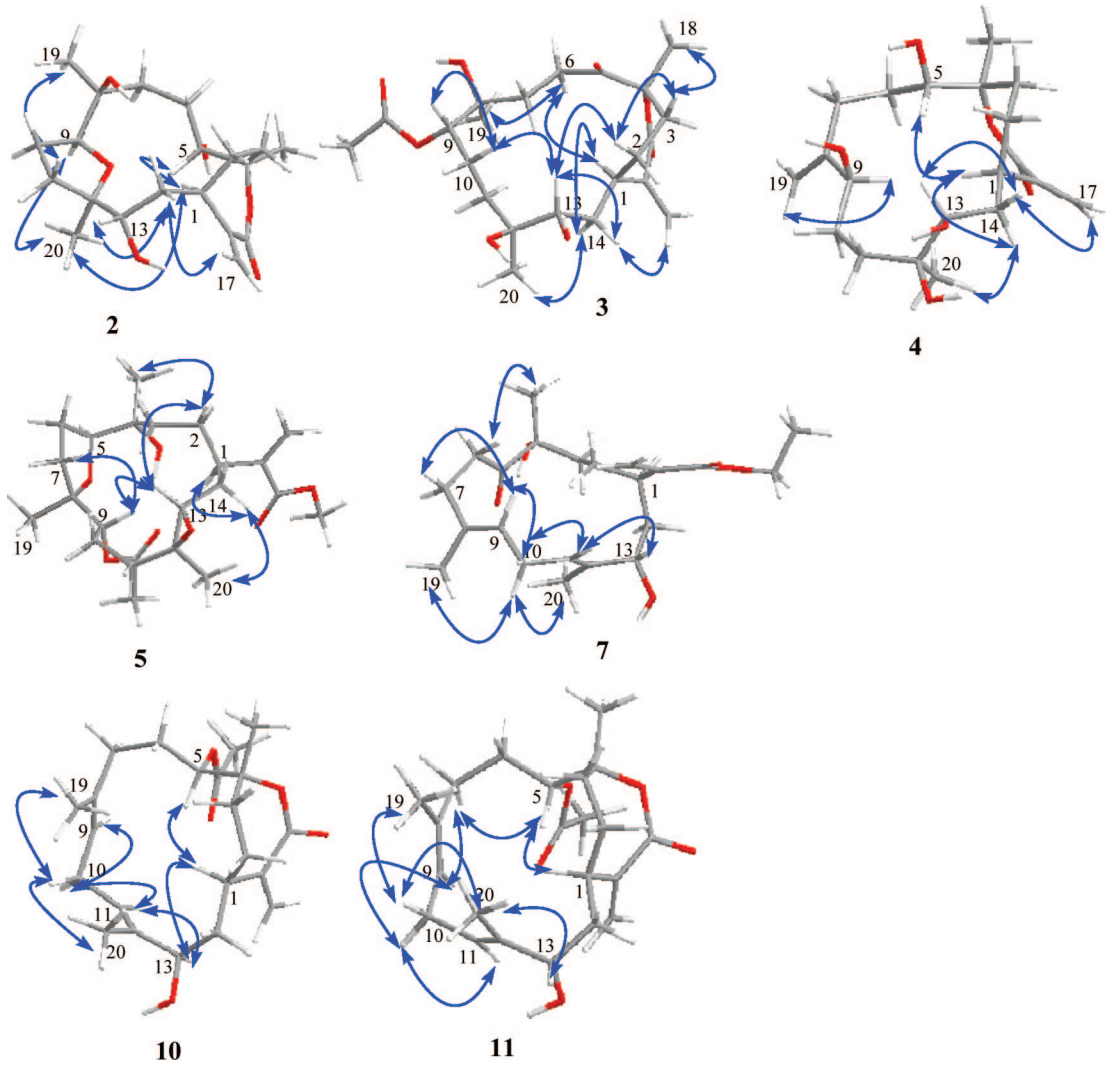


Figure 1. Computer-generated model for **2**−**4**, **5**, **7**, **10**, and **11** using MM2 force field calculations and key NOE correlations.

five degrees of unsaturation. IR absorption bands at 3421, 1711, and 1626 cm^{-1} suggested the presence of hydroxy, lactone, and olefinic groups. The analysis of 1D and 2D NMR spectroscopic data revealed that the gross structure of **2** was closely related to sinulariolone (**12**),[7] with the exception that C-5 was linked with a hydroxy group instead of a ketone (C-11 in the literature) in the latter. This was indicated by the presence of an additional hydroxymethine group (δ 4.50, dd; δ 71.9, CH), and its proton exhibiting a HMBC correlation to C-18 (δ 24.0, CH_3), and in turn the correlations of H_3-18 (δ 1.15, s) to C-4 (δ 91.3, qC), C-5 (δ 71.9, CH), and C-3 (δ 33.7, CH_2). The relative stereochemistry of **2** was determined by comparison of NMR and NOE data with that of sinulariolone[7] together with the assumption that the configuration of C-1 was *R* (α-series).[1] The stereogenic centers at C-1, C-4, C-8, C-9, C-12, and C-13 were in agreement with those of sinulariolone on the basis of the similar NOE values obtained (Figure 1) and NMR data in association with the chemical constants. The α-orientation of H-5 (δ 4.50, dd, J = 4.0, 11.0 Hz) in **2** is determined through a 1D GOESY experiment. Irradiation of H-5 resulted in the enhancement of H-1 (δ 2.91, m), while irradiation of H-1 caused the enhancement of H_3-20 and H-5. The NOESY interaction of OH-5 (δ 4.82, d, J = 4.0 Hz)/H_3-18 also supported the β-orientation of OH-5.

The molecular formula of sinulaflexiolide C (**3**) was determined as $C_{22}H_{34}O_8$ on the basis of negative HRESIMS (*m*/*z* 425.2188 [M − H]$^-$), corresponding to six degrees of unsaturation. A comparison of the NMR spectroscopic data revealed that the structure of **3** was closely related to sinulariolone (**12**), possessing a ketone (δ 210.0, qC, C-5) and a seven-membered lactone. However, in the spectra of **3** an acetyl group (δ 2.07, s; 21.3, CH_3; 171.0, qC) was recognized, and the signals for the 9,12-tetrahydrofuran system were missing and replaced by two relatively high-field resonances of oxygenated carbons at δ 77.6 (CH, C-9) and 77.0 (qC, C-12). This suggested that the tetrahydrofuran ring of **12** was opened, while C-9 and C-12 of **3** were still substituted by oxygen atoms. The HMQC spectrum assigned the resonance at δ 4.72 (dd) to C-9, which in turn showed a HMBC correlation to an acetyl carbonyl carbon (δ 171.0, qC), thus establishing the position of the acetoxy group at C-9, while C-8, C-12, and C-13 were substituted by hydroxy groups. Although NOE effects can be controversial in the assignment of the relative configuration of conformationally flexible cembranoids, the correlations observed for compound **3** provided reasonable evidence to support the relative configurations depicted in Figure 1. An NOE correlation between H-17a (δ 5.51, brs) and H_2-14 assigned H-1 to have the α-orientation, as observed previously in all other known analogues. The NOE interactions of H_3-20/H-14a (δ 1.96, m), H-14a/H-1, H-13/H-2a (δ 2.12, m), and H-13/H-14b (δ 1.55, m) allowed the assignment of H_3-20α and OH-13α. The correlations H-2a/H-3a (δ 2.47, dd) and H-3a/H_3-18 assigned H_3-18 to the β-face. In addition, the NOESY cross-peaks of H-1/H-6a (δ 3.40, dd), H-6a/H_3-19, H_3-18/H-6b (δ 2.20, dd), H-9/H-10b (δ 1.78, m), and H-13/H-10b (Figure 1) confirmed the assignment of H_3-19α and H-9β.

Table 1. ^{1}H and ^{13}C NMR Data of Sinulaflexiolide A (**1**)[a]

position	δ_H (ppm)	δ_C (ppm)	HMBC (H→C)	NOESY
1	1.92 m	33.1 CH		H-3, H-13β
2	1.65 m	30.6 CH_2		
	1.90 m			
3	3.82 d (10.5)	82.8 CH	C-1, C-2, C-4, C-18	H-18, H-1
4		73.3 C		
5	1.62 m	40.0 CH_2		
	1.80 m			
6	1.94 m	23.5 CH_2		
	2.10 m			
7	5.30 m	127.4 CH	C-6, C-9, C-19	
8		133.3 C		
9	2.10 m	35.0 CH_2		
10	1.50 m	25.3 CH_2		
	1.90 m			
11	2.70 dd (6.0, 6.5)	62.3 CH	C-9, C-10, C-12, C-20	H-13β
12	59.1 s			
13	1.09 m	34.7 CH_2		
	1.94 m			
14	1.08 m	24.9 CH_2		
	1.73 m			
15	2.05 m	47.5 CH		
16		173.9 C		
17	1.72 m	25.5 CH_2		
	1.81 m			
18	1.25 s	24.7 CH_3	C-3, C-4, C-5	
19	1.61 s	16.5 CH_3	C-7, C-8, C-9	
20	1.14 s	15.6 CH_3	C-11, C-12, C-13	
1′	1.80 m	40.0 CH		H-3′, H-13′β
2′	1.55 m	31.2 CH_2		
	1.90 m			
3′	4.02 d (11.0)	84.0 CH	C-1′, C-2′, C-4′, C-18′	H-18′, H-1′
4′		73.3 C		
5′	1.55 m	40.0 CH_2		
6′	1.95 m	23.6 CH_2		
	2.10 m			
7′	5.30 m	126.4 CH	C-6′, C-9′, C-19′	
8′		133.6 C		
9′	2.08 m	35.1 CH_2		
10′	1.60 m	25.2 CH_2		
	1.90 m			
11′	2.82 dd (6.0, 6.5)	62.2 CH	C-9′, C-10′, C-12′, C-20′	H-13′ β
12′	59.1 s			
13′	1.10 m	34.8 CH_3		
	1.90 m			
14′	1.60 m	25.5 CH_2		
	1.78 m			
15′		74.9 C		
16′		174.9 C		
17′	1.52 m	27.4 CH_2		
	1.82 m			
18′	1.25 s	24.9 CH_3	C-3′, C-4′, C-5′	
19′	1.61 s	16.4 CH_3	C-7′, C-8′, C-9′	
20′	1.21 s	15.7 CH_3	C-11′, C-12′, C-13′	
OH-4	4.60 s		C-3, C-4, C-5, C-18	
OH-4′	4.58 s		C-3′, C-4′, C-5′, C-18′	
OH-15′	5.45 s		C-1′, C-15′, C-16′, C-17′	H-1′

[a] Measured in DMSO-d_6.

The HRESIMS and NMR spectroscopic data indicated that sinulaflexiolide D (**4**) shared the same molecular formula as **2**. The ^{1}H and ^{13}C NMR spectroscopic features of **4** were very compatible with those of **3**, indicating the presence of a seven-membered α-*exo*-methylene lactone ring. The difference was due to the presence of an additional *exo*-methylene group as observed in the ^{1}H NMR data at δ 4.85 (1H, brs) and 4.92 (1H, brs) and the corresponding carbons at δ 109.2 (CH_2) and 149.9 (qC), but the absence of a ketone group at C-5 as in **3**, which was replaced by a hydroxymethine (δ 3.90, brd; 66.4, CH). The fact that **4** had two methyl groups (δ 1.22, s; 1.08, s) rather than three suggested an *exo*-methylene group in **4** to replace one methyl group of **3**. The COSY and HMBC correlations allowed the location of two methyl groups at C-4 and C-12, respectively; thus, C-8 featured an *exo*-methylene group. The presence of a hydroxy group at C-5 was confirmed through the HMBC correlations from H_3-18 (δ 1.22, s) to C-4 (δ 89.3, qC), C-3 (δ 31.8, CH_2), and C-5 (δ 66.4, CH) and in turn from H-5 (δ 3.90) to C-18. The relative configuration of **4** could be assigned using NOESY data and by comparison with the NOE data of **3**. Since H-1 was biogenetically assumed to be α-oriented, the NOE relationships H-1/H-14a (δ 1.38, m), H_3-20/H-14a, H-13/H-14b (δ 2.10, m), and H-14b/H-17a (δ 5.72) suggested H-13β and H_3-20α. A further NOE interaction of H-5/H-1 but absence of H-5/H_3-18 assigned the ε-lactone ring to be *trans*-fused, as observed in other analogues, while H-5 was assignable to α. A cross-peak of H-19a (δ 4.92, brs)/H-9 (δ 3.88, m) was indicative of spatial proximity with respect to H-9 and H-19a. This was in agreement with the MM_2-optimized structure (Figure 1), which concluded the β-orientation of OH-9.

Table 2. ^{1}H NMR Data for Sinulaflexiolides B−I (**2**−**9**)

	2[a]	**3**[b]	**4**[a]	**5**[b]	**6**[c]	**7**[a]	**8**[c]	**9**[c]
1	2.91 m	3.16 ddd (5.5,10.0,10.0)	2.18 m	2.85 m	3.13 m	2.63 m	2.60 m	
2	0.95 m	1.05 m	1.13 m	1.73 m	1.70 m	1.35 m	1.28 m	6.40 d (10.0)
	2.31 m	2.12 m	1.37 m	1.82 m	1.96 m	1.44 m	1.40 m	
3	1.75 m	1.85 m	1.61 m	1.60 m	1.66 m	1.90 m	2.10 m	6.20 d (10.0)
	1.77 m	2.47 dd (4.0,11.5)	2.08 m	2.00 m	1.93 m	2.07 m	2.38 m	
5	4.50dd(4.0,11.0)		3.90 brd (9.0)	4.02 dd (5.5,10.5)	3.98 dd (5.5,11.0)			1.80 m 2.81 ddd (2.5,11.0,11.0)
6	1.16 m	2.20 dd (8.5,19.2)	1.00 m	1.83 m	1.77 m,	2.28 m	2.40 m	2.10 m
	1.32 m	3.40 dd (8.0,19.2)	1.32 m	1.86 m	1.85 m	2.82 m	3.16 dd (7.5,15.6)	2.30 m
7	1.50 m	1.60 m	2.10 m	1.65 m	1.80 m	2.35 m	2.05 m	5.24 dd (7.0,7.0)
	1.92 m	2.40 dd (8.5,11.0)	2.12 m	1.91 m	2.24 m	2.40 m	2.40 m	
9	3.90 dd (4.0,9.0)	4.72 dd (4.5,11.5)	3.88 m	5.03 brd (11.0)	5.25 d (10.5)	5.40 t (7.5)	4.98 dd (6.0, 7.0)	2.02 dd (5.5,12.0)2.30 m
10	1.90 m	1.78 m	2.00 m	1.70 m	1.62 m	2.70 m	1.57 m	1.20 m
	1.92 m	1.91 m	2.05 m	1.93 m	1.98 m	2.80 m	2.03 m	2.30 m
11	1.45 m	1.80 m	1.40 m	0.91 dd (10.0,11.0)	1.27 m	5.48 t (7.5)	1.15 m	3.65 d (11.0)
	1.65 m	1.82 m	1.98 m	2.14 m	2.14 dd (8.5, 14.0)		1.90 m	
13	3.50 m	3.45 brd (10.0)	2.90 brd (9.0)	3.03 brd (13.0)	3.41 dd (2.3, 10.0)	4.09 dd (2.0,8.5)	2.58 m	1.90 m
								2.30 m
14	1.50 m	1.55 m	1.38 m	1.62 m	1.82 m	1.92 m	1.04 m	2.30 m
	1.65 m	1.96 m	2.10 m	1.97 m	1.96 m	2.10 m	1.98 m	2.76 ddd (6.5,6.5,10.0)
16								1.48 s
17	6.02 br.s	6.36 br.s	6.19 brs	6.43 brs	6.64 brs	6.50 brs	6.18 brs	1.48 s
	5.46 br.s	5.51 br.s	5.72 brs	5.83 brs	5.98 brs	5.42 brs	5.61 brs	
18	1.15 s	1.48 s	1.22 s	1.10 s	1.07 s	1.16 s	1.13 s	1.83 s
19	0.96 s	1.27 s	4.92 brs	1.24 s	1.25 s	1.70 s	1.60 s	1.70 s
			4.85 brs					
20	0.94 s	1.15 s	1.08 s	1.38 s	1.39 s	1.66 s	1.20 s	1.22 s
Ac		2.07 s		2.08 s	1.68 s			
MeO				3.79 s			3.69 s	
EtO					1.05 t (7.0)	1.08 t (7.0)		
					4.09 q (7.0)	4.10 q (7.0)		

[a] Measured in DMSO-d_6. [b] In $CDCl_3$. [c] In C_6D_6.

Table 3. ^{13}C NMR Data for Sinulaflexiolides B−I (**2**−**9**) (δ in ppm, J in Hz)

position	**2**[a]	**3**[b]	**4**[a]	**5**[b]	**6**[c]	**7**[a]	**8**[c]	**9**[c]
1	33.2 CH	32.7 CH	36.6 CH	37.9 CH	38.3 CH	37.5 CH	36.8 CH	139.4 qC
2	29.7 CH_2	31.1 CH_2	30.9 CH_2	27.4 CH_2	27.0 CH_2	25.7 CH_2	26.0 CH_2	119.8 CH
3	33.7 CH_2	34.2 CH_2	31.8 CH_2	36.0 CH_2	36.2 CH_2	39.2 CH_2	37.0 CH_2	122.1 CH
4	91.3 qC	90.0 qC	89.3 qC	73.8 qC	73.4 qC	79.5 qC	79.0 CH	139.3 qC
5	71.9 CH	210.0 qC	66.4 CH	87.8 CH	88.2 CH	213.0 qC	216.0 qC	31.5 CH_2
6	27.0 CH_2	29.4 CH_2	28.0 CH_2	26.7 CH_2	27.2 CH_2	33.8 CH_2	33.7 CH_2	25.2 CH_2
7	40.0 CH_2	34.4 CH_2	31.8 CH_2	36.3 CH_2	36.3 CH_2	30.9 CH_2	31.6 CH_2	124.2 CH
8	73.4 qC	76.0 qC	149.9 qC	83.8 qC	83.5 qC	134.0 qC	135.0 qC	136.0 qC
9	85.3 CH	77.6 CH	77.9 CH	75.9 CH	75.9 CH	122.4 CH	124.5 CH	35.2 CH_2
10	25.4 CH_2	21.7 CH_2	25.5 CH_2	24.5 CH_2	24.7 CH_2	26.3 CH_2	23.5 CH_2	27.6 CH_2
11	37.5 CH_2	34.5 CH_2	35.5 CH_2	34.8 CH_2	35.3 CH_2	124.8 CH	38.5 CH_2	69.8 CH
12	87.5 qC	77.0 qC	74.2 qC	61.3 qC	60.5 qC	138.0 qC	60.0 qC	76.3 qC
13	73.8 CH	75.3 CH	72.1 CH	59.8 CH	59.3 CH	75.0 CH	59.5 CH	30.5 CH_2
14	37.7 CH_2	38.2 CH_2	37.2 CH_2	32.4 CH_2	33.4 CH_2	38.7 CH_2	33.0 CH_2	20.4 CH_2
15	145.9 qC	144.0 qC	142.5 qC	143.0 qC	144.3 qC	142.0 qC	142.8 qC	74.3 qC
16	169.2 qC	169.0 qC	169.2 qC	169.0 qC	166.9 qC	169.0 qC	167.5 qC	29.1 CH_3
17	123.2 CH_2	124.9 CH_2	124.8 CH_2	124.5 CH_2	123.6 CH_2	124.0CH_2	126.0 CH_2	30.0 CH_3
18	24.0 CH_3	29.6 CH_3	23.6 CH_3	25.3 CH_3	25.1 CH_3	25.4 CH_3	25.1 CH_3	23.2 CH_3
19	19.6 CH_3	20.3 CH_3	109.2 CH_2	20.9 CH_3	20.7 CH_3	16.8 CH_3	17.1 CH_3	16.2 CH_3
20	16.1 CH_3	18.8 CH_3	26.3 CH_3	17.3 CH_3	17.3 CH_3	10.8 CH_3	17.0 CH_3	22.5 CH_3
Ac		171.0 qC	171.4 qC	170.7 qC				
	21.3 CH_3	20.9 CH_3	20.2 CH_3					
MeO				52.0 CH_3			52.5 CH_3	
ErO					13.9 $CH_3$60.5 CH_2	13.9 $CH_3$60.6 CH_2		

[a] Measured in DMSO-d_6. [b] In $CDCl_3$. [c] In C_6D_6.

Table 4. ^1H and ^{13}C NMR Data for Sinulaflexiolides J and K (**10**, **11**) (δ in ppm, J in Hz)[a]

	10		**11**	
1	31.7 CH	2.63 m	31.6 CH	2.70 m
2	29.3 CH_2	0.95 m	28.1 CH_2	0.90 m
		1.75 m		1.75 m
3	32.7 CH_2	1.95 m	32.3 CH_2	
		1.93 m		
4	85.6 qC		85.6 qC	
5	71.2 CH	5.46 d (10.0)	70.6 CH	5.39 d (10.0)
6	27.3 CH_2	1.10 m	27.8 CH_2	1.15 m
		1.55 m		1.60 m
7	34.6 CH_2	1.82 m	33.9 CH_2	1.82 m
		1.85 m		1.98 m
8	132.7 qC		132.7 qC	
9	128.0 CH	5.27 brd (10.0)	125.5 CH	5.53 m
10	26.8 CH_2	2.45 m	25.7 CH_2	2.63 m
		3.10 ddd (10.0, 10.0, 13.5)		2.71 m
11	129.1 CH	5.69 dd (6.5, 11.0)	127.2 CH	5.50 m
12	134.7 qC		134.1 qC	
13	65.9 CH	4.52 dd (3.0, 11.5)	76.6 CH	3.96 dd (8.0, 8.0)
14	39.2 CH_2	1.52 m	37.5 CH_2	1.76 m
		2.00 m		1.86 m
15	145.2 qC		144.4 qC	
16	168.5 qC		168.5 qC	
17	124.0 CH_2	5.35 brs	124.5 CH_2	5.39 brs
		6.21 brs		6.26 brs
18	23.5 CH_3	1.22 s	24.2 CH_3	1.21 s
19	15.7 CH_3	1.54 s	16.1 CH_3	1.59 s
20	16.7 CH_3	1.64 s	9.2 CH_3	1.70 s
Ac	20.6 CH_3	1.96 s	20.7 CH_3	1.98 s
	170.3 qC		170.6 qC	

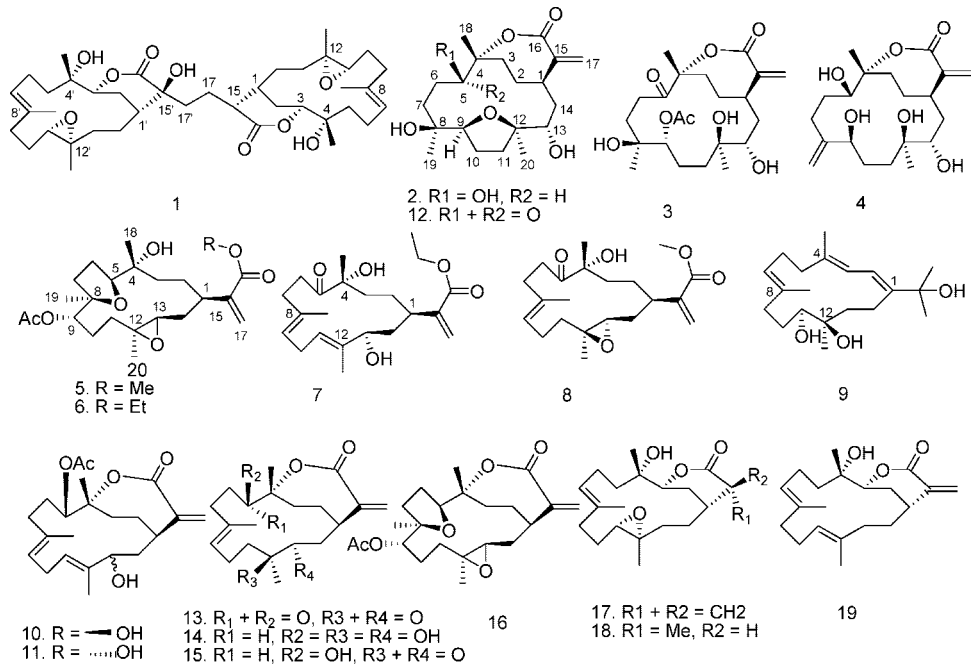
[a] Measured in C_6D_6.

Sinulaflexiolide E (**5**) had a molecular formula of $C_{23}H_{36}O_7$ as determined by HRESIMS (m/z 425.2536 $[M + Na]^+$). Its NMR spectral data were closely related to those of 9-acetoxy-5,8-tetrahydrofuransinulariolide (**16**),[15] as defined by the NMR data. The difference was due to the exhibition of a methoxy group (δ 52.0, CH_3; δ 3.79, s), whose protons showed HMBC correlation with the carbonyl carbon C-16, indicating the formation of a methyl ester. Moreover, C-4 of **5** displayed a signal at δ 73.8 (qC) instead of δ 88.7 (qC, C-4) of the known seven-membered lactones, implying a hydroxy group present at C-4. Analyses of NOESY and NMR data revealed that the relative stereochemistry of **5** was the same as **16**.

The NMR data of sinulaflexiolide F (**6**) were similar to those of **5**, with the exception that the methyl ester of the latter was replaced by an ethyl ester based on the presence of the ethoxy resonances at δ 4.09 (2H, q, J = 7.0 Hz) and 1.05 (3H, t, J = 7.0 Hz) along with their corresponding carbons at δ 60.5 (CH_2) and 13.9 (CH_3). The molecular formula of **6**, $C_{24}H_{38}O_7$ as established by HRESIMS (m/z 439.2686 $[M + H]^+$), was 14 amu higher than that of **5** and further supported this structure assignment. The relative stereochemistry of **6** was in agreement with that of **5** as revealed by the similar NOESY correlations.

The molecular formula of sinulaflexiolide G (**7**) was found to be $C_{22}H_{34}O_5$ by HRESIMS (m/z 401.2300 $[M + Na]^+$), indicating

Chart 1



six degrees of unsaturation. The IR absorptions at 3384, 1710, and 1654 cm^{-1} suggested the presence of hydroxy and carbonyl groups. With the exception of the NMR data at δ 6.50 (1H, brs, H-17a) and 5.42 (1H, brs, H-17b) and δ 124.0 (t, C-17), 142.0 (s, C-15), and 169.0 (s, C-16), which were assigned to the α-*exo*-methylene ester, the backbone contained two trisubstituted double bonds, as indicated by ^{13}C values at δ 138.0 (qC), 134.0 (qC), 124.8 (CH), and 122.4 (CH), a ketone at δ 213.0 (qC), and two hydroxylated carbons at δ 79.5 (qC) and 75.0 (CH). The ^1H NMR spectrum exhibited the resonances for four methyl groups at δ 1.08 (3H, t, $J = 7$ Hz), 1.16 (3H, s), 1.66 (3H, s), and 1.70 (3H, s), and one of which (δ 1.08) showed COSY correlation with an oxymethylene (δ 4.10), indicating the presence of an ethoxy group. The methyl singlet at δ 1.16 (H_3-18) showed HMBC correlations with δ 213.0 (qC, C-5), 79.5 (qC, C-4), and 39.2 (CH_2, C-3), locating a ketone and a hydroxy group at C-5 and C-4, respectively. Two olefinic methyl groups at δ 1.66 (3H, s) and 1.70 (3H, s) could be assigned to H_3-20 at C-12 and H_3-19 at C-8. Since the COSY correlations were observed from both olefinic protons at δ 5.48 (t, $J = 7.5$ Hz, H-11) and 5.40 (t, J=7.5 Hz, H-9) to the methylene protons at δ 2.70 (1H, m) and 2.80 (1H, m), the positions of double bonds were thus assigned to C-8/C-9 and C-11/C-12. The HMBC cross-peaks between H_3-20 and C-11, C-12 (δ 138.0, qC), and C-13 (δ 75.0, CH), and in turn between H-13 (δ 4.09, dd) and C-11, C-12, allowed the assignment of a hydroxy group at C-13. In addition, the ethoxy group was annexed to C-16 to form an ethyl ester, as evidenced by the HMBC correlation between the methylene protons and C-16. The geometries of the trisubstituted double bonds were determined to be *E* due to the NOESY correlations of H-9/H-7a (δ 2.35, m) and H-11/H-13 (Figure 1). The latter correlation also suggested the β-face of H-13. H_3-18 was determined to be β-oriented on the basis of NOE relationships between H-13/H-2a (δ 1.44, m) and H_3-18/H-2a.

The NMR spectroscopic data of sinulaflexiolide H (**8**) were closely related to those of 5-dehydrosinulariolide (**13**), except for the presence of an additional methoxy group (δ 3.69, s; 52.5, CH_3), which was deduced to form a methyl ester to C-16 (δ 167.5, qC) on the basis of HMBC correlations. In addition, C-4 of **8** appeared at δ 79.0 (qC), replacing the significant downfield ^{13}C NMR value of C-4 as observed in other seven-membered lactone derivatives, implying C-4 was substituted by a hydroxy group. This was also supported by the molecular weight of **8**, which was 32 amu higher than that of **13**, as indicated by the HRESIMS data. The relative stereochemistry of **8** was in agreement with 5-dehydrosinulariolide due to the similar NMR and NOE relationships of both compounds. NOE interactions were observed from both compounds at H-1/H-14a (δ 1.98, m), H_3-20/H-14a, H-13/H-14b (δ 1.04, m), H-13/H-3b (δ 2.38, m), H-13/H-9, H_3-18/H-3b, and H_3-19/H-10a (δ 2.03, m), which were consistent with the 9*E* geometry, the α-face of H-1 and H_3-20, and the β-orientation of H-13 and H_3-18.

Sinulaflexiolide I (**9**) had a molecular formula of $C_{20}H_{34}O_2$, as determined by HRFABMS, indicating four degrees of unsaturation. The ^1H NMR spectrum exhibited five methyl singlets at δ 1.22 (3H, s), 1.48 (6H, s), 1.70 (3H, s), and 1.83 (3H, s), two of which were attributed to a 2-hydroxyisopropane group based on the HMBC correlation from δ 1.48 (6H, s) to a hydroxylated quaternary carbon at δ 74.3 (qC) and to the methyl carbons at δ 29.1 (CH_3) and 30.0 (CH_3). The ^{13}C NMR spectrum showed in total 20 carbons including six olefinic resonances at δ 139.3 (qC), 136.0 (qC), 136.0 (qC), 124.2 (CH), 122.1 (CH), and 119.8 (CH) for three trisubstituted double bonds. Thus, the basic backbone of **9** was determined to be a 14-membered cembrane. A comparison of NMR spectroscopic data revealed that the structure of **9** was closely related to 11,12-epoxy-1(*E*),3(*E*),7(*E*)-cembratrien-15-ol.[24] The difference was observed by the presence of two hydroxylated carbons at δ 76.3 (qC) and 69.8 (CH) that replaced the 11,12-epoxy carbons of the known analogue. The HMBC correlations of H_3-20 (δ 1.22, s) with C-12 (δ 76.3, qC), C-11 (δ 69.8, CH), C-10 (δ 27.6, CH_2), and H-11 (δ 3.65, brd, $J = 11.0$ Hz) and of H_3-19 (δ 1.70, s) with C-9 (δ 35.2, CH_2) located hydroxy groups at C-11 and C-12, respectively. The similar NOE and NMR data of **9** and 11,12-epoxy-1(*E*),3(*E*),7(*E*)-cembratrien-15-ol suggested that the geometries of double bonds of **9** were 1*E*, 3*E*, and 7*E*. With respect to the orientation of hydroxy groups at C-11 and C-12, the NOESY cross-peaks between H-11/H-13a (δ 1.90, m), H-11/H-7, H-11/H-9 (δ 2.02, dd), H_3-20/H-14a (δ 2.76, ddd), and H_3-20/H-10a (δ 2.30, m) suggested the opposite orientation of H-11 and H_3-20. Since the cembranoids isolated from this specimen showed exclusively α-orientation of H_3-20, biogenetically, H-11 was thus β-oriented.

Sinulaflexiolides J (**10**) and K (**11**) were a pair of inseparable isomers with a ratio of 4:5. Both compounds shared the same molecular formula of $C_{22}H_{32}O_5$ as determined by HRFABMS. The NMR data of both compounds showed close similarity and were comparable to those of 13-acetylsandensolide.[6] However, an

acetoxy group of **10** (δ 1.96, 20.6, 170.3) and **11** (δ 1.98, 20.7, 170.6) was substituted at C-5 rather than at C-13 of the latter. This was clear from the HMBC correlations of H-5 (δ 5.46, d, J = 10.0 Hz, **10**) and H-5 (δ 5.39, d, J = 10.0 Hz, **11**) to acetyl carbonyl carbons at δ 170.3 (qC) and 170.6 (qC) and to C-4 (δ 85.6, 2 × C) and C-18 (δ 23.5, 24.2), respectively. The NMR data of **10** that differed in comparison to those of **11** were due to C-13, where the resonances at δ 4.52 (1H, dd, J = 3.0, 11.5 Hz) for H-13 of the former and its corresponding carbon resonated at δ 65.9 (CH), whereas H-13 of **11** was at δ 3.96 (1H, dd, J = 8.0, 8.0 Hz) and its corresponding carbon was at δ 76.6 (CH). This finding suggested **10** and **11** to be a pair of C-13 epimers. The NOE interaction between H-13/H-1 was indicative of 13-Hα for **10**; therefore, H-13 of **11** was β-oriented. The fact that C-20 (δ 9.2) of **11** shifted significantly upfield compared to that of **10** (δ 16.7) due to the γ-gauche effect also supported the assignment of the H-13 configuration.

The compounds containing a methyl or an ethyl ester group are potential artifacts generated during extraction and/or isolation; for example, **5** and **6** may be derived from 9-acetoxy-5,8-tetrahydrofuran-12,13-epoxysinularolide (**16**) by methylation or ethylation, while **8** was potentially converted from 5-dehydrosinularolide.

Compounds **5**, **6**, **10**, **14**, **16**, and **19** representing six-membered and seven-membered lactones, containing methyl and ethyl esters (without lactone groups), were tested against the tumor cell lines HCT-8 (human intestinal epithelial adenocarcinoma), Bel-7402 (hepatocellular carcinoma), BGC-823 (human gastric gland carcinoma), A549 (human lung carcinoma), and A2780 (human ovarian carcinoma). Compounds **5** and **6** showed selective activity against the BGC-823 cell line (IC_{50}, 8.5 and 0.12 μM, respectively), while the rest showed no activity (IC_{50} > 10 μg/mL). This finding suggested that the methyl or ethyl ester derivatives possess stronger cytotoxicity against the BGC-823 cell line than the six-membered or seven-membered lactones.

The cembrane-type derivatives are a class of diterpene commonly found from specimens of *S. flexibilis* growing in different localities of the Indian and Pacific Oceans. They are considered to be reliable chemotaxonomic markers. The present work implies that the ecological nature of the coral habitat in the South China Sea is closely related to other South-Asian regions. In addition to the abundant cembranoids, we also isolated a sesquiterpene alismoxide[25] as a minor component, and this was found in the genus *Sinularia* for the first time. This suggests that the biosynthetic pathway for producing metabolites in *S. flexibilis* not only follows the geranylgeranyl pyrophosphate route but also utilizes the farnesyl diphosphate pathway to produce sesquiterpenes.

Experimental Section

General Experimental Procedures. Optional rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were determined on a Thermo Nicolet Nexus 470 FTIR spectrometer. ^{1}H and ^{13}C NMR and 2D NMR spectra were recorded on an Avance-500 FT 500 MHz NMR spectrometer using TMS as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants (J) are reported in hertz (Hz). EIMS was performed on a Bruker APEX II mass spectrometer, and ESIMS were recorded in the MDS-SCIEX-QSTAR (ABI, USA). HRFABMS were obtained from GCT-MS instruments. Column chromatography was carried out with Si gel (160−200 mesh and 200−300 mesh), and GF_{254} Si gel for TLC was provided by Qingdao Marine Chemistry Co. Ltd.

Animal Material. The soft coral *Sinularia flexibilis* was collected from the inner coral reef at a depth of around 8 m in Hainan Island of China in May 2004, and the samples were frozen immediately after collection. The specimen was identified by one of the authors (L.v.O.), and the voucher specimens (HSF-11) are deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China.

Extraction and Isolation. The frozen soft coral *S. flexibilis* (6.0 kg) was homogenized, then extracted with MeOH. The MeOH extract (143.2 g) was partitioned between H_2O and petroleum ether (PE), EtOAc, and *n*-butanol. The PE fraction (7.5 g) was subjected to vacuum liquid chromatography using 160−200 mesh Si gel and eluted with a gradient of EtOAc/PE to obtain seven fractions (FA−FG). Fraction FE (4:1, 1.5 g) was fractionated on a Sephadex LH-20 column by eluting with methanol to yield four subfractions (ESF1−ESF4). ESF2 (120 mg) was chromatographed on an ODS column and eluted with a gradient of MeOH/H_2O (60%−80%) to obtain **12** (26.5 mg), **15** (8.5 mg), and **9** (15.3 mg). Fraction FF (3:1, 1.2 g) followed the same protocol as FE on a Si gel column by eluting with hexane/acetone (2:1) to obtain 13 subfractions (FSF1−FSF13). Fraction FSF2 (210 mg) was purified on a Si gel-H column and eluted with PE/acetone (3:1) to obtain **13** (168.9 mg). FSF11 (0.4 g) was separated by using semipreparative HPLC (RP-18 column, 60% MeOH in H_2O as a mobile phase) to obtain **12** (10.0 mg) and **7** (3.0 mg). FSF13 (75 mg) was separated by using semipreparative HPLC (RP-18 column, 75% MeOH in H_2O as a mobile phase) to yield **6** (10.0 mg), **4** (4.5 mg), and a mixture of **10** and **11** (7.5 mg). The EtOAc fraction (10.0 g) was fractionated on a Si gel column eluted with a gradient of PE/acetone to obtain four fractions (Et1−Et4). Fraction Et-1 (3:1, 2.5 g) was chromatographed using an ODS column and eluted with 65% MeOH/H_2O to yield three subfractions (SBEt1−SBEt3). SBEt1 (2:1, 100 mg) was fractionated using semipreparative HPLC (RP-18 column, 60% MeOH in H_2O as a mobile phase) to obtain **1** (4.3 mg), **2** (2.5 mg), **3** (2.0 mg), and **8** (3.0 mg), while SBEt3 (87 mg) followed the same protocol as SBEt1 eluting with 55% MeOH/H_2O as a mobile phase to obtain **5** (10.5 mg), **14** (3.7 mg), **18** (5.3 mg), **19** (2.2 mg), and **16** (8.6 mg).

Bioassays. A tetrazolium-based colorimetric assay (MTT assay) was used for the *in vitro* test against HCT-8, Bel-7402, BGC-823, A549, and A2780 tumor cell lines.

Sinulaflexiolide A (1): colorless oil; $[\alpha]^{20}_D$ −16.0 (*c* 0.30, MeOH); IR (KBr) γ_{max} 3327, 2926, 1722, 1634, 1452, 1335, 1214, 1150, 1041 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1; HRESIMS *m/z* 687.4458 (calcd for $C_{40}H_{63}O_9$, 687.4472).

Sinulaflexiolide B (2): colorless oil; $[\alpha]^{20}_D$ +6.0 (*c* 0.50, MeOH); IR (KBr) γ_{max} 3421, 2929, 1711, 1626, 1454, 1377, 1254, 1152, 1049 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 368.2097 (calcd for $C_{20}H_{32}O_6Na$, 391.2091).

Sinulaflexiolide C (3): colorless oil; $[\alpha]^{20}_D$ +9.1 (*c* 0.20, MeOH); IR (KBr) γ_{max} 3410, 3247, 2927, 1710, 1639, 1413, 1116 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; (negative) HRESIMS *m/z* 425.2188 (calcd for $C_{22}H_{33}O_8$, 425.2181).

Sinulaflexiolide D (4): colorless oil; $[\alpha]^{20}_D$ +5.1 (*c* 0.40, MeOH); IR (KBr) γ_{max} 3397, 2936, 1705, 1629, 1459, 1379, 1257,1145,1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 391.2104 (calcd for $C_{20}H_{32}O_6Na$, 391.2091).

Sinulaflexiolide E (5): colorless oil; $[\alpha]^{20}_D$ +5.1 (*c* 0.80, MeOH); IR (KBr) γ_{max} 3436, 2927, 2868, 1722, 1628, 1460, 1375, 1237, 1039 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 425.2536 (calcd for $C_{23}H_{37}O_7$, 425.2534).

Sinulaflexiolide F (6): colorless oil; $[\alpha]^{20}_D$ +5.8 (*c* 0.40, MeOH); IR (KBr) γ_{max} 3511, 3416, 2929, 2858, 1727, 1626, 1462, 1375, 1238, 1038 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 439.2686 (calcd for $C_{24}H_{39}O_7$, 439.2690).

Sinulaflexiolide G (7): colorless oil; $[\alpha]^{20}_D$ −4.2 (*c* 0.20, MeOH); IR (KBr) γ_{max} 3422, 3384, 2925, 1710, 1654, 1454, 1027 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 401.2300 (calcd for $C_{22}H_{34}O_5Na$, 401.2298).

Sinulaflexiolide H (8): colorless oil; $[\alpha]^{20}_D$ −6.2 (*c* 0.20, MeOH); IR (KBr) γ_{max} 3365, 2928, 1715, 1692, 1634, 1444, 1357, 1029 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 365.2330 (calcd for $C_{21}H_{33}O_5$, 365.2328).

Sinulaflexiolide I (9): colorless oil; $[\alpha]^{20}_D$ −4.3 (*c* 0.15, MeOH); IR (KBr) γ_{max} 3421, 2930, 1712, 1652, 1456, 1377, 1248, 1058 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 345.2052 (calcd for $C_{20}H_{34}O_3Na$, 345.2036).

Sinulaflexiolides J (10) and K (11). colorless oil; $[\alpha]^{20}_D$ +6.6 (*c* 0.23, MeOH); IR (KBr) γ_{max} 3441, 2942, 1735, 1714, 1627, 1454, 1375, 1239, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; HRESIMS *m/z* 377.2326 (calcd for $C_{22}H_{33}O_5$, 377.2328).

(−)-14-Deoxycrassin (18): colorless oil; $[\alpha]^{20}_D$ −8.7 (*c* 0.10, MeOH); $[\alpha]^{25}_D$ −16.7 (*c* 0.5, $CHCl_3$); IR (KBr) γ_{max} 3435, 1725, 1458, 1382, 1251, 1082 cm^{-1}; ^{1}H NMR (C_6D_6) δ 2.16 (1H, m, H-1), 1.40 (1H, m, H-2a), 2.07 (1H, m, H-2b), 3.80 (1H, brd, J = 11.0 Hz, H-3), 1.67 (1H, m, H-5a), 1.82 (1H, m, H-5b), 1.64 (1H, m, H-6a), 2.16

(1H, m, H-6b), 4.90 (1H, dd, $J = 3.0, 5.0$ Hz, H-7), 1.88 (1H, m, H-9a), 2.11 (1H, m, H-9b), 2.02 (1H, m, H-10a), 2.24 (1H, m, H-10b), 5.07 (1H, m, H-11), 1.99 (2H, m, H-13), 1.10 (1H, m, H-14a), 1.80 (1H, m, H-14b), 5.38 (1H, brs, H-17a), 6.72 (1H, brs, H-17b), 1.40 (3H, s, H-18), 1.43 (3H, s, H-19), 1.49 (3H, s, H-20); ^{13}C NMR (C_6D_6) δ 33.4 (CH, C-1), 26.2 (CH_2, C-2), 82.7 (CH, C-3), 73.7 (qC, C-4), 38.7 (CH_2, C-5), 22.3 (CH_2, C-6), 125.6 (CH, C-7), 134.1 (qC, C-8), 39.9 (CH_2, C-9), 24.2 (CH_2, C-10), 126.2 (CH, C-11), 131.9 (qC, C-12), 35.9 (CH_2, C-13), 31.1 (CH_2, C-14), 140.0 (qC, C-15), 166.0 (qC, C-16), 125.7 (CH_2, C-17), 24.6 (CH_3, C-18), 14.5 (CH_3, C-19), 13.5 (CH_3, C-20); HRESIMS *m*/*z* 319.2267 (calcd for $C_{20}H_{31}O_3$, 319.2268).

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