

Article

Sinularones A–I, New Cyclopentenone and Butenolide Derivatives from a Marine Soft Coral *Sinularia* sp. and Their Antifouling Activity

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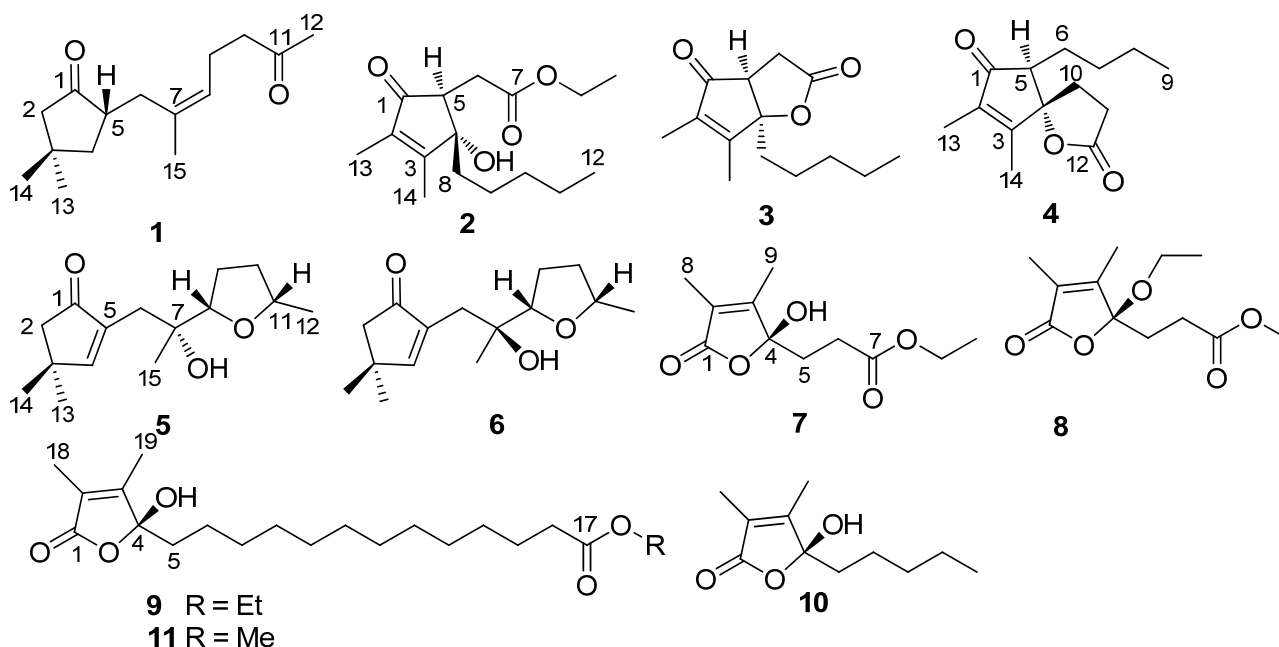
Abstract: Nine new compounds, namely sinularones A–I (**1–9**), characterized as cyclopentenone and butenolide-type analogues, were isolated from a soft coral *Sinularia* sp., together with a known butenolide (**10**). Their structures were elucidated by means of spectroscopic (IR, MS, 1D and 2D NMR, CD) analysis. The absolute configurations were determined on the basis of CD and specific rotation data in association with the computed electronic circular dichroism (ECD) by time dependent density functional theory (TD DFT) at 6-31+G(*d,p*)/DFT B3LYP/6-31+G(*d,p*) level. Compounds **1–2** and **7–10** showed potent antifouling activities against the barnacle *Balanus amphitrite*.

Keywords: soft coral; *Sinularia* sp.; sinularones A–I; structural elucidation; antifouling activity

1. Introduction

The genus *Sinularia* is a dominant biomass with about 100 known species widely distributed in the tropical reef environment [1–4], presenting a rich array of chemical diversity involving sesquiterpenes, diterpenes, polyhydroxylated steroids, and polyamine compounds. Several metabolites derived from *Sinularia* play an active role in chemical defense [5,6], and display a range of biological activities, such as antimicrobial [7], anti-inflammatory [8], and cytotoxic [9,10]. In the course of our search for bioactive natural products from marine invertebrates inhabited in the South China Sea, an octocoral *Sinularia* sp. was collected off Hainan Island. Chemical examination of the EtOAc soluble fraction of this specimen resulted in the isolation of the new cyclopentenone derivatives (**1–6**) and furanones (**7–9**), along with the known butenolide (**10**) (Figure 1). This paper reports the structural elucidation of the new compounds and the evaluation of their antifouling activities.

Figure 1. Structures of compounds (**1–10**).



2. Results and Discussion

Sinularone A (**1**) has a molecular formula of $C_{15}H_{24}O_2$ as established by the HRESIMS (m/z 259.1674 $[M + Na]^+$, calcd. 259.1674) and NMR data, indicating 4° unsaturation. The IR absorptions at 1738 and 1717 cm^{-1} suggested the presence of carbonyl groups. The ^1H NMR spectrum exhibited the signals for a single olefinic proton at δ_{H} 5.12 (1H, t, $J = 7.2$ Hz, H-8), four methyl singlets at δ_{H} 1.01 (3H, s, H₃-13), 1.13 (3H, s, H₃-14), 1.61 (3H, s, H₃-15) and 2.07 (3H, s, H₃-12), together with 11 aliphatic protons. APT spectra displayed 15 carbon resonances, involving two ketones at δ_{C} 220.0 (C-1) and 208.5 (C-11), and two olefinic carbons at δ_{C} 125.7 (CH, C-8) and 133.9 (C, C-7). The proton signals and their associated carbons were assigned by HMQC. The COSY cross-peaks allowed to establish the proton-proton spin systems from C-4 to C-6 and from C-8 to C-10, while the HMBC interactions from the olefinic H₃-15 to C-6 (δ_{C} 32.3), C-7, and C-8, and from H₃-12 to C-11 and C-10 (δ_{C} 43.4) led to the connectivity of the subunits to form a linear chain defined as a

7-methylhept-7-en-11-one group (Figure 2). Additional HMBC interactions from the geminal protons $H_{\alpha,\beta}$ -2 (δ_H 2.08, 2.16) to C-3 (δ_C 34.0), C-4 (δ_C 43.5), C-5 (δ_C 46.7) and C-1, along with the correlations from both H_3 -13 and H_3 -14 to C-2 (δ_C 52.8), C-3, and C-4, and from H-5 (δ_H 2.49, m) to C-1 and C-3 indicated the presence of a 3,3-dimethylcyclopentanone nucleus. Subsequently, the linear side chain was determined to be linked to the nucleus at C-5 on the basis of the COSY relationships in addition to the HMBC interactions from H_2 -4 (δ_H 1.41, 1.84) to C-6 and from H_2 -6 to C-1, C-4, and C-5. The geometry of Δ^7 was assigned to 7Z according to the NOE interaction between H_3 -15 and H-8 in addition to the chemical shift of C-15 (δ_C 23.5), higher than 20 ppm [11]. In regard to the configuration of the stereogenic center C-5, the CD spectrum showed a negative Cotton effect at 212 nm for $n-\pi^*$ transition in agreement with the electronic circular dichroism (ECD) data which was calculated for 5S configuration by time dependent density functional theory (TD DFT) at 6-31+G(d,p)/DFT B3LYP/6-31+G(d,p) level [12]. This assignment was also supported by the octant rule which allowed the side chain to be located at the negative CD region (Figure 3) [13].

Figure 2. Key COSY, HMBC and NOE relationships of (1).

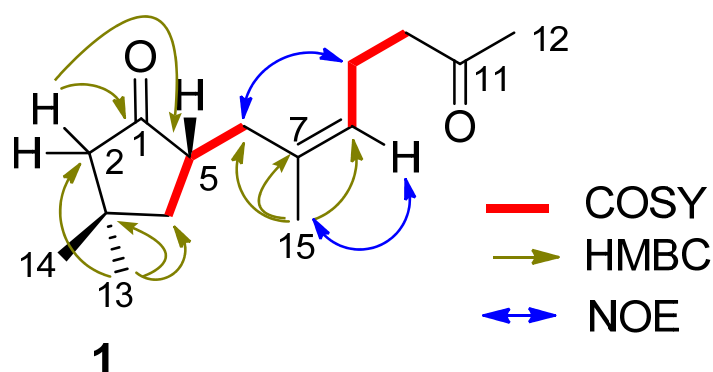
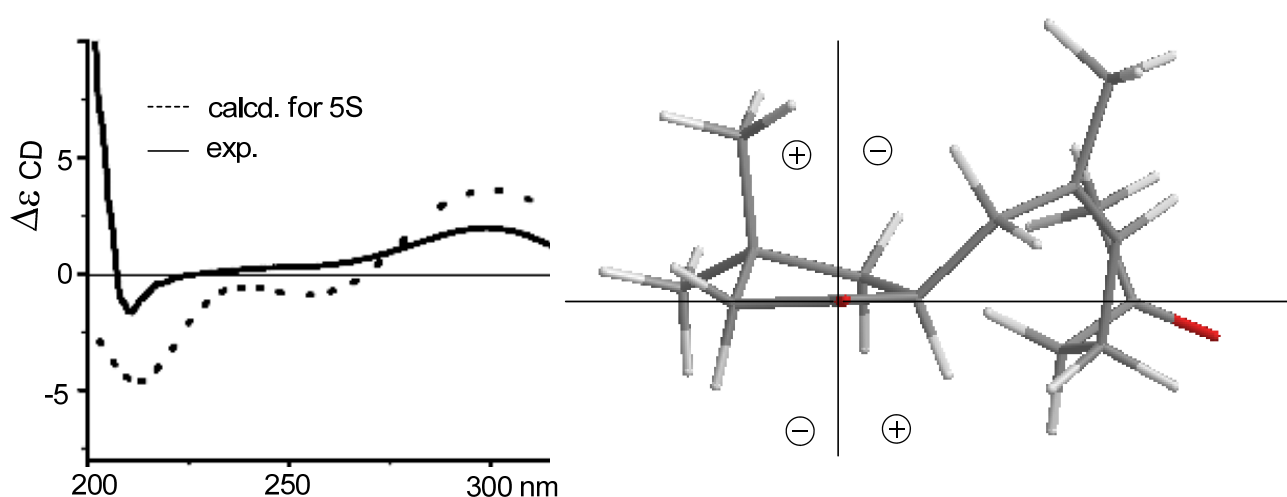


Figure 3. CD, electronic circular dichroism (ECD) and octant rule of (1).



Sinularone B (2) has a molecular formula of $C_{16}H_{26}O_4$ as determined by the HRESIMS (m/z 305.1732 $[M + Na]^+$, calcd. 305.1729) and NMR data, indicating 4° unsaturation. The IR absorptions at 3489, 1737, 1707 and 1653 cm^{-1} suggested the presence of hydroxy, carbonyl, and olefinic groups. The 1H NMR spectrum exhibited two terminal methyls appearing as doublets at δ_H 0.80 (3H, t, $J = 7.0$ Hz, H_3 -12)

and 1.21 (3H, t, $J = 7.1$ Hz, H₃-1'), and two olefinic methyl singlets at δ_{H} 1.62 (3H, s, H₃-13) and 1.90 (3H, s, H₃-14), in addition to 13 aliphatic protons (Table 1). APT spectrum displayed a total of 16 carbon resonances, of which two carbonyl carbons, two olefinic carbons, an oxygen-bearing sp³ carbon, and an oxymethylene were observed (Table 2). The presence of a *n*-pentyl unit was recognized by the COSY and HMBC relationships to establish a side chain from C-8 to C-12, while an ethoxy group was ascribed to the COSY coupling between H₃-1' and δ_{H} 4.10 (2H, q, $J = 7.1$ Hz, H₂-2'). In HMBC spectrum, the interactions from H₃-13 to the carbons at δ_{C} 203.8 (C-1), 134.3 (C-2), and 170.0 (C-3), and from H₃-14 to C-2, C-3, and C-4 (δ_{C} 80.1), in addition to the interaction from a methine proton δ_{H} 2.89 (1H, dd, $J = 5.6, 8.4$, H-5) to C-1, C-2, C-3, and C-4, conducted to establish a 2,3-dimethylcyclopent-2-enone nucleus. The COSY coupling between H-5 and the methylene protons H₂-6 at δ_{H} 2.44 (1H, dd, $J = 5.6, 16.0$ Hz) and 2.35 (1H, dd, $J = 8.4, 16.0$ Hz) in association with the HMBC interaction from the carbonyl carbon (δ_{C} 172.5, C-7) to H-5, H₂-6, and the oxymethylene H₂-2', ascertained an ethylacetate unit linked to C-5 (δ_{C} 55.3). The oxygenated quaternary carbon C-4 was determined to be co-positioned by a *n*-pentyl unit and a hydroxy group on the basis of the HMBC interactions between H-5 and the methylene carbon C-8 (δ_{C} 35.9), and in turn from H₂-8 (δ_{H} 1.54, 1.58) to C-3, C-4, and C-5, in addition to a D₂O exchangeable proton (δ_{H} 5.28, s) showing HMBC relationship with C-4. The NOE interaction between H₂-6 and H₂-8 (δ_{H} 1.54, 1.58) was indicative of their *cis* relationship. Thus, the relative configurations of the stereogenic centers were depicted to be 4*S** and 5*S**. The measured CD curve of **2** was closely similar to the calculated ECD for 4*S*,5*S*-isomer in opposite to the data for 4*R*,5*R*-isomer (Figure 4), indicating **2** to be in agreement with 4*S* and 5*S*.

The NMR spectroscopic data of sinularone C (**3**) were closely related to those of **2**, except for the absence of the signals for an ethoxy group. Analysis of 1D and 2D NMR (COSY, HMQC and HMBC) disclosed a 2,3-dimethylcyclopent-2-enone nucleus, and a *n*-pentyl group being linked to the nucleus at C-4, showing the partial structure to be the same as that of **2**. The COSY cross-peaks between H-5 (δ_{H} 3.11) and H₂-6 (δ_{H} 2.49, 3.08) and the HMBC interactions of these protons with a carbonyl carbon C-7 (δ_{C} 174.9) and the ketone C-1 (δ_{C} 204.9) allowed to link C-6 of an acetyl unit to cyclopent-2-enone nucleus at C-5. In addition, a quaternary carbon observed at δ_{C} 92.4 was assigned to C-4 on the basis of the HMBC interactions of C-4 to H-5 and H₂-6. The molecular formula (C₁₄H₂₀O₃) (HRESIMS m/z 259.1315 [M + Na]⁺) of **3** showing a C₂H₆O unit less than that of **2** and requiring 5° unsaturation, in association with the obvious downfield shifted C-4 in comparison with that of **2**, allowed to connect C-4 and C-7 to form a γ -lactone, which was fused to the cyclopentenone ring across C-4 and C-5. The observed NOE interactions from H-5 to H₂-8 (δ_{H} 1.79, 1.97) and H₂-9 (δ_{H} 1.10, 1.16) indicated H-5 to be oriented in the same face as *n*-pentyl group. This assignment was supported by the irradiation of H₂-8 causing the NOE enhancement of H-5 (1.7%, 1.2%). Compound **3** is likely a precursor to generate **2** by ethoxylation. Thus, the absolute configuration of C-5 in **3** is suggested to be the same as that of **2**. Accordingly, the chiral centers of **3** were assumed to be 4*R* and 5*S*.

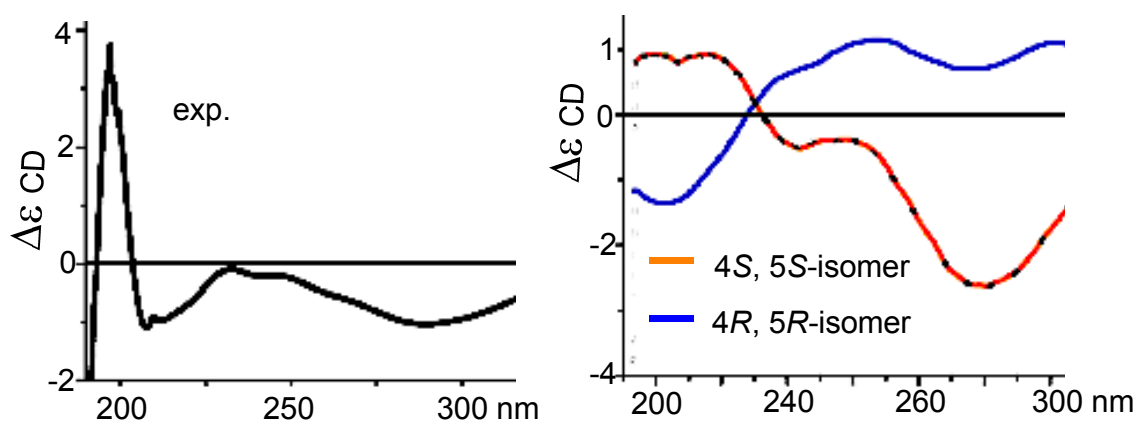
Table 1. ^1H NMR data of sinulactones A–H (1–8) (500 MHz, δ_{H} , J in Hz).

No.	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	7 ^a	8 ^b
2	2.08 d (16.4)				2.18 s	2.18 d (18.0)		
	2.16 d (16.4)				2.18 s	2.22 d (18.0)		
4	1.41 dd (11.5, 12.4)				7.35 s	7.35 s		
	1.84 dd (8.4, 12.4)							
5	2.49 m	2.89 dd (5.6, 8.4)	3.11 dd (5.5, 10.2)	2.55 t (6.5)			1.91 ddd (6.0, 6.0, 17.0)	1.97 ddd (7.8, 7.8, 15.5)
							2.32 ddd (6.0, 8.0, 17.0)	2.22 m
6	2.06 m	2.35 dd (8.4, 16.0)	2.49 dd (10.2, 15.5)	1.45 m	2.09 d (13.9)	2.21 s	2.49 ddd (6.0, 6.0, 18.0)	2.24 m
	2.29 dd (4.2, 13.4)	2.44 dd (5.6, 16.0)	3.08 dd (5.5, 15.5)	1.67 m	2.27 d (13.9)	2.21 s	2.78 ddd (6.0, 8.0, 18.0)	2.27 m
7				1.30 m				
				1.46 m				
8	5.12 t (7.2)	1.54 ddd (4.0, 11.0, 12.0)	1.79 ddd (4.7, 12.1, 13.5)	1.30 m	3.57 t (7.2)	3.56 dd (7.4, 7.4)	1.80 s	1.77 s
		1.58 ddd (5.5, 11.0, 12.0)	1.97 ddd (4.5, 12.1, 13.5)	1.32 m				
9	2.16 ddt (7.2, 7.4, 12.0)	0.53 m	1.10 m	0.88 t (7.2)	1.75 m	1.71 m	1.96 s	1.87 s
	2.19 ddt (7.2, 7.4, 12.0)	0.66 m	1.16 m		1.80 m	1.78 m		
10	2.44 dd (7.4, 7.4)	1.15 m	1.29 m	2.16 ddd (7.7, 10.6, 13.9)	1.27 m	1.30 m		
				2.23 ddd (6.2, 10.3, 13.9)	1.87 m	1.87 m		
11		1.18 m	1.29 m	2.72 ddd (7.7, 10.3, 18.4)	3.83 m	3.86 m		
				2.78 ddd (6.2, 10.6, 18.4)				
12	2.07 s	0.80 t (7.0)	0.86 t (7.1)		1.13 d (6.0)	1.14 d (6.0)		
13	1.01 s	1.62 s	1.67 s	1.65 s	1.15 s	1.15 s		
14	1.13 s	1.90 s	2.02 s	1.97 s	1.15 s	1.15 s		
15	1.61 s				0.87 s	0.84 s		
		4.10 q (7.1)					4.17 q (7.1)	3.13 dq (7.0, 12.0)
EtO		1.21 t (7.1)					1.27 t (7.1)	3.24 dq (7.0, 12.0)
								1.09 t (7.0)
								3.58 s
OH-4		5.28 s					5.26 s	
OH-7					4.16 s	4.16 s		

^a In DMSO- d_6 , ^b In CDCl₃.

Table 2. ^{13}C NMR data of sinulactones A–H (1–8) (125 MHz, δ_{C} , mult.).

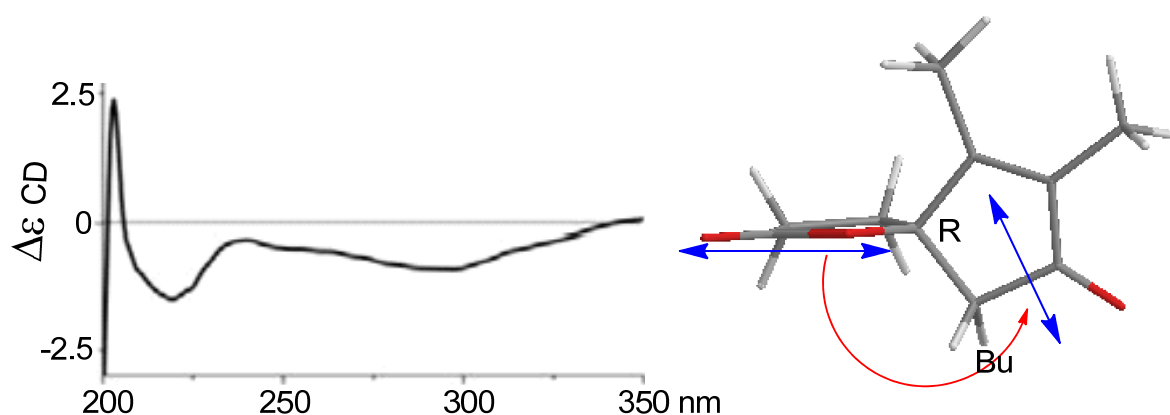
No.	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	7 ^a	8 ^b
1	220.0 C	203.8 C	204.9 C	203.4 C	209.4 C	209.4 C	171.8 C	171.1 C
2	52.8 CH ₂	134.3 C	138.7 C	136.4 C	49.8 CH ₂	49.8 CH ₂	124.7 C	127.1 C
3	34.0 C	170.0 C	166.7 C	164.9 C	38.9 C	38.9 C	158.2 C	156.7 C
4	43.5 CH ₂	80.1 C	92.4 C	91.5 C	170.8 C	170.7 C	105.5 C	109.1 C
5	46.7 CH	55.3 CH	46.4 CH	54.5 CH	138.8 C	138.9 C	31.3 CH ₂	31.3 CH ₂
6	32.3 CH ₂	29.6 CH ₂	32.3 CH ₂	24.8 CH ₂	33.5 CH ₂	32.2 CH ₂	28.9 CH ₂	28.0 CH ₂
7	133.9 C	172.5 C	174.9 C	29.3 CH ₂	72.5 C	72.3 C	174.8 C	172.9 C
8	125.7 CH	35.9 CH ₂	34.0 CH ₂	22.2 CH ₂	85.2 CH	85.2 CH	8.4 CH ₃	8.6 CH ₃
9	22.3 CH ₂	24.6 CH ₂	23.0 CH ₂	13.7 CH ₃	26.2 CH ₂	26.1 CH ₂	10.6 CH ₃	11.0 CH ₃
10	43.4 CH ₂	31.9 CH ₂	31.7 CH ₂	25.3 CH ₂	33.3 CH ₂	33.2 CH ₂		
11	208.5 C	22.3 CH ₂	22.3 CH ₂	28.7 CH ₂	75.3 CH	75.2 CH		
12	30.2 CH ₃	14.3 CH ₃	14.3 CH ₃	176.1 C	21.3 CH ₃	21.4 CH ₃		
13	28.1 CH ₃	8.0 CH ₃	8.3 CH ₃	7.8 CH ₃	28.6 CH ₃	28.5 CH ₃		
14	29.9 CH ₃	11.6 CH ₃	12.3 CH ₃	10.6 CH ₃	28.4 CH ₃	28.5 CH ₃		
15	23.5 CH ₃				21.9 CH ₃	23.4 CH ₃		
16								
17								
18								
EtO		14.5 CH ₃					14.1 CH ₃	15.4 CH ₃
		60.5 CH ₂					61.5 CH ₂	58.4 CH ₂
MeO								51.9 CH ₃

^a In DMSO-*d*₆, ^b In CDCl₃.**Figure 4.** CD and ECD spectra of (2).

The molecular formula ($\text{C}_{14}\text{H}_{20}\text{O}_3$) of sinularone D (4) was determined to be the same as that of 3 on the basis of the HRESIMS data (m/z 259.1313 [$\text{M} + \text{Na}]^+$, calcd. 259.1310) and NMR data. The IR absorptions at 1778, 1710 and 1657 cm^{-1} were characteristic of lactone, ketone, and olefinic groups. Comparison of the NMR data revealed the resonances of 4 in respect to those of a 2,3-dimethylcyclopent-2-enone nucleus (Tables 1 and 2) compatible to the data of 3. However, the COSY and HMBC relationships revealed a *n*-butyl group to replace a *n*-pentyl group of 3. This unit was determined to be linked to C-5 (δ_{C} 54.5) as evident from the COSY correlation between H₂-6 (δ_{H} 1.45, 1.67) and H-5 (δ_{H} 2.55, t, $J = 6.5\text{ Hz}$) in association with the HMBC interactions from H₂-6 to

C-1 (δ_C 203.4). The remaining resonances included two methylenes and a carbonyl carbon (δ_C 176.1, C-12). The COSY correlation between H₂-10 (δ_H 2.16, 2.23) and H₂-11 (δ_H 2.72, 2.78) and the HMBC interactions from H₂-10 to C-12 and from H₂-11 to C-4 (δ_C 91.5, C), in association with the molecular formula requiring 5° unsaturation, conducted the quaternary carbon C-4 to be located by a γ -lactone. The NOE interaction between H₂-6 and H₂-10 reflected the same orientation of H-5 and the heterocyclic atom of lactone. In regard to the absolute configuration of the *spiro* carbon C-4, we extended the CD method originally used for the chiral center of allylic amines [14]. The negative Cotton effect at 222 nm belonging to the π - π^* charge-transfer transition polarized by the lactone carbonyl group and the enone unit, correlated with anti-clockwise screw (Figure 5). Therefore, the sign of the Cotton effect reflected a 4*R* configuration. In combination with NOE data, C-5 was thus assigned to *S* configuration.

Figure 5. Cotton effect of (4).



The NMR spectroscopic data of **5** and **6** are very similar, while HRESIMS data revealed both compounds shared the same molecular formula (C₁₅H₂₄O₃), indicating 4° unsaturation. APT spectrum of **5** displayed 15 carbon resonances, involving one ketone (δ_C 209.4, C-1), two olefinic carbons (δ_C 138.8, C-5; 170.8, C-4), two oxymethines at δ_C 85.2 (C-8) and 75.3 (C-11) and four methyls (δ_C 21.3, 21.9, 28.4, 28.6). The observation of HMBC interactions from the isolated methylene protons at δ_H 2.18 (2H, s, H₂-2) to C-1, C-3 (δ_C 38.9, C), C-4, and C-5 and from H-4 (δ_H 7.35, s) to C-1, C-2, and C-3, in addition to the methyl singlets at δ_H 1.15 (6H, s, H₃-13 and H₃-14) to C-2, C-3, and C-4, ascertained the presence of 5-substituted 3,3-dimethylcyclopent-4-enone. In regard to the remaining resonances, the COSY correlations extended a moiety from C-8 to C-12, in which C-8 and C-11 were oxygenated. Additionally, the HMBC interactions from H₃-15 (δ_H 0.87, s) to the methylene carbon C-6 (δ_C 33.5), quaternary carbon C-7 (δ_C 72.5) and C-8 and from H-8 (δ_H 3.57, t, J = 7.2 Hz) to C-11 indicated the formation of an ether bridge across C-8 and C-11, while C-7 was co-positioned by a methyl and a hydroxy groups. The side chain was linked to C-5 as evident from the HMBC relationships of H₂-6 (δ_H 2.09, 2.27) with C-1, C-5, and C-4. Based on the NOE interaction between H-8 and H-11, a *cis*-geometry of the 8,11-epoxy bonding was assigned. Thus, the relative configurations were suggested to be 8*S** and 11*S**.

Analysis of 2D NMR data revealed sinularone F (**6**) to be a stereoisomer of **5**. Both compounds showed the NOE interaction between H-8 and H-11, indicating them to be oriented in the same face

toward tetrahydrofuran ring. The major difference was found by the chemical shifts at C-6 and C-15 (Table 2), implying **6** to be a C-7 epimer of **5** rather than an enantiomer. Since the calculated ECD data could not provide confidential evidence to judge the configurations, the calculation for specific rotation and ^{13}C NMR data was performed. The conformations with relative energies from 0 to 2.5 kcal/mol were used in optical rotation computations at B3LYP/6-311+G(2d,p) level, while Boltzmann statistics were used for rotation computations of all conformations. The computed specific rotation [15] for 7*S*,8*R*,11*R*-isomer is -16.9 and -27.5 for 7*R*,8*R*,11*R*-isomer. These data are in opposite sign to the experimental data that of **5** ($[\alpha]_{\text{D}}^{23} +18.0$) and **6** ($[\alpha]_{\text{D}}^{23} +22.6$), indicating **5** and **6** to be the enantiomers of the calculated isomers. These assignments were also supported by the relative shift errors of the experimental ^{13}C NMR data of **6** and **5** that were in accordance with the error distribution calculated at B3LYP/6-311+G(2d,p) level (Table 3) [16]. Thus, the absolute configurations of **5** were in agreement with 7*R*,8*S*,11*S*, whereas those of **6** were assigned to 7*S*,8*S*,11*S*.

Table 3. The error patents of ^{13}C NMR data of **6** and **5** in experiments and computations.

Position	6	5	$\Delta\delta_{6-5}$	6 *	5 *	$\Delta\delta_{6-5}$
1	209.4	209.4	0.0	206.0	205.8	0.2
2	49.8	49.8	0.0	49.2	49.2	0.0
3	38.9	38.9	0.0	41.9	42.0	-0.1
4	170.7	170.8	-0.1	171.2	171.1	0.1
5	138.9	138.8	0.1	142.8	142.5	0.3
6	32.2	33.5	-1.3	32.7	39.8	-7.1
7	72.3	72.5	-0.2	72.4	72.7	-0.3
8	85.2	85.2	0.0	84.8	84.1	0.7
9	26.1	26.2	-0.1	27.2	27.4	-0.2
10	33.2	33.3	-0.1	34.3	34.7	-0.4
11	75.2	75.3	-0.1	75.7	75.8	-0.1
12	21.4	21.3	0.1	18.5	18.6	-0.1
13	28.5	28.6	-0.1	26.2	26.4	-0.2
14	28.5	28.4	0.1	26.2	26.2	0.0
15	23.4	21.9	1.5	24.4	17.4	7.0

6*: calculated for 7*S*,8*S*,11*S*-isomer; **5***: calculated for 7*R*,8*S*,11*S*-isomer.

Sinularone G (**7**) has a molecular formula of $\text{C}_{11}\text{H}_{16}\text{O}_5$ as determined by HRESIMS data (m/z 251.0886 $[\text{M} + \text{Na}]^+$, calcd. 251.0895), requiring 4° unsaturation. The ^1H NMR exhibited two olefinic methyl singlets at δ_{H} 1.80 (3H, s, H₃-8) and 1.96 (3H, s, H₃-9) and a methyl triplet (δ_{H} 1.27, H₃-1'), while ^{13}C NMR involved two carbonyl carbons at δ_{C} 171.8 (C-1) and 174.8 (C-7), two olefinic carbons at δ_{C} 124.7 (C-2) and 158.2 (C-3), and an acetal carbon at δ_{C} 105.5 (C-4). The HMBC interactions of H₃-8 to C-1, C-2, and C-3 and from H₃-9 to C-2, C-3, and C-4 disclosed a α,β -unsaturated 2,3-dimethyl- γ -lactone, the same as that of a known butenolide [17]. In addition, an ethylpropanoate was recognized by the COSY relationships between two vicinal methylenes along with a methyl triplet H₃-1' coupled to the oxymethylene at δ_{H} 4.17 (H₂-2'), in combination with the HMBC interactions from C-7 to the protons of H₂-6 (δ_{H} 2.49, 2.78), H₂-5 (δ_{H} 1.91, 2.32), and the oxymethylene (δ_{C} 61.5). The positive specific rotation ($[\alpha]_{\text{D}} +4.03^\circ$) and the similar Cotton effect in comparison with those of a known butenolide (**11**) supposed C-4 to be 4*S* configuration [11,17].

The NMR data of sinularone H (**8**) were similar to those of **7** with the exception of the presence of an additional methoxy group. Examination of the HMBC cross-peaks afforded the interactions between the carbonyl carbon (δ_C 172.9, C-7) and the methoxy protons (δ_H 3.58, s) and between the acetal carbon (δ_C 109.1, C-4) and the oxymethylene (δ_H 3.13, 3.24), requiring the formation of a methyl ester, while the ethoxy group was substituted to C-4. The absolute configuration of C-4 was supposed to be the same as that of **7** on the basis of similar specific rotation and CD data.

The NMR data of sinularone I (**9**) were mostly identical to those of a known butenolide (**11**) [17]. The distinction was attributed to the presence of an ethyl ester to replace a methyl ester of the known analogue, as evident from the molecular weight of **9** (C₂₁H₃₆O₅) to be 14 amu more than that of the latter, and the presence of an ethoxy group in its NMR spectra. The absolute configuration was determined to be 4*S* on the basis of the similar specific rotation and Cotton effect as those of **8** and the known analogues [17].

In addition, the spectroscopic data and the specific rotation indicated the butenolide **10** to be identical to (*S*)-4-hydroxy-2,3-dimethyl-4-pentyl- γ -lactone, isolated from the fruiting bodies of a fungus [11].

All compounds were tested for their cytotoxicity against a panel of tumor cell lines including human ovarian carcinoma A2780, human lung adenocarcinoma A549, human gastric carcinoma BGC823, human hepatoma Bel7402, and human colonic carcinoma HCT-8. However, they showed weak inhibitory activity with IC₅₀ > 10 μ g/mL. In order to detect whether these compounds play a role for ecological functions, the test for antifouling activity against the larvae of the barnacle *Balanus amphitrite* was performed [18,19]. The bioassay results revealed compounds **1–2**, **7–10** showed potent inhibition with the EC₅₀ values (Table 4) lower than the standard requirement (EC₅₀ < 25 μ g/mL) in regard to the efficacy level of natural antifouling agents as established by the US navy program [20]. However, compounds **3–6** showed weak inhibition with EC₅₀ > 50 μ g/mL. In addition, the bioactive compounds (**1–2**, **7–10**) showed weak toxicity against the barnacle with LC₅₀ > 50 μ g/mL. A primary discussion of structure-activity relationship implied that α,β -unsaturated 2,3-dimethyl- γ -lactone is a functional unit for anti-barnacle. Among the active compounds, **10** is the most active, suggesting it to be a promising candidate as a nontoxic natural antifouling agent.

Table 4. Antifouling activity of compounds against the larvae of barnacle *B. amphitrite* *.

Compounds	<i>Balanus amphitrite</i> Larvae		
	EC ₅₀ (μ g/mL)	LC ₅₀ (μ g/mL)	LC ₅₀ /EC ₅₀
1	13.86	>50	>3.61
2	23.50	>50	>2.13
7	18.65	>50	>2.69
8	21.39	>50	>2.34
9	12.58	>50	>3.97
10	3.84	>50	>13.02

* EC₅₀ represents the concentration of a compound where 50% of larval population was inhibited to settle compared to control, while LC₅₀ represents the concentration of a compound required to kill 50% of larvae of a tested population.

3. Experimental Section

3.1. General

Optical rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were determined on a Thermo Nicolet Nexus 470 FTIR spectrometer. CD spectra were measured using J-810-150s spectropolarimeter (Jasco, Darmstadt, Germany). ^1H and ^{13}C NMR and 2D NMR spectra were recorded on a Bruker Avance 600 MHz and Bruker Avance 500 MHz using TMS as an internal standard. HRESIMS data were obtained on a LTQ Orbitrap XL instrument. HPLC was performed with a C_{18} packed column (250×10 nm) and using a DAD detector.

3.2. Animal Material

The soft coral *Sinularia* sp. 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 Leen van Ofwegen (National Museum of Natural History Naturalis). The voucher specimens (HSF-15) are deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China.

3.3. Extraction and Isolation

The soft coral *Sinularia* sp. (3.7 kg) was homogenized, and then extracted with 95% EtOH ($5 \text{ L} \times 3$). The EtOH extract (102.5 g) was partitioned between H_2O (400 mL) and EtOAc (200 mL). The EtOAc fraction (10.0 g) was subjected to column chromatography (3.5×25 cm) using 160–200 mesh Si gel (120 g) and was eluted with a gradient of petroleum ether (PE)/acetone (20:1, 10:1, 1:1) to obtain nine fractions (F1–F9). F3 (260 mg) was fractionated on an ODS column (C_{18} , 2.0×25 cm) and eluted with a gradient of MeOH/ H_2O (75%–100%) to collect three portions PA–PC. Portion PB (85% MeOH, 180 mg) were then subjected to a Sephadex LH-20 column (1.5×35 cm) eluting with acetone to give **2** (7.0 mg), **3** (11.7 mg), and **4** (2.6 mg). Portion PA (75% MeOH, 70 mg) was subjected to semi-preparative HPLC (ODS, $5 \mu\text{m}$, 2×25 cm) with 50% MeOH as a mobile phase to obtain **5** (1.5 mg), **6** (1.4 mg), **7** (2.4 mg), and **10** (3.2 mg). Compounds **1** (14.9 mg), **8** (3.5 mg) and **9** (3.4 mg) were isolated from F4 (300 mg) using Sephadex LH-20 column (1.5×35 cm) and semi-preparative RP-18 HPLC ($5 \mu\text{m}$, 2×25 cm) with 45% MeOH as a mobile phase.

3.4. Computational Calculation

The computational ECD, specific rotation, and ^{13}C NMR calculations were performed by the B3LYP functional and a generic basis set, employ the 6-311+G(*d,p*) basis set [21,22]. This generic basis set has been shown to be effective, both efficient and reliable, in predicting structural and reactivity properties for homogeneous systems. All calculations are performed with the Gaussian 03 package with tight self-consistent field convergence and ultrafine integration grids.

3.5. Larval Settlement Bioassays

Adults of the barnacle *Balanus 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.* [23]. 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.

3.6. Cytotoxic Assay

The cytotoxic properties of the isolated compounds were tested in vitro using human tumor cell lines including human ovarian carcinoma cell line A2780, human lung adenocarcinoma epithelial cell line A549, human gastric carcinoma cell line BGC823, human hepatoma cell line Bel7402, and human colonic carcinoma cell line HCT-8 by MTT method.

Sinularone A (**1**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} +7.26$ ($c = 0.27$, MeOH); IR (KBr) ν_{max} cm^{-1} : 2956, 2868, 1738, 1717, 1457, 1407, 1368, 1262, 1158, 1079; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 259.1674 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{15}\text{H}_{24}\text{O}_2\text{Na}$, 259.1674); CD λ_{max} nm ($\Delta\epsilon$): 212 (−2.8).

Sinularone B (**2**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} +0.60$ ($c = 0.43$, MeOH); IR (KBr) ν_{max} cm^{-1} : 3489, 2957, 2868, 1737, 1707, 1653, 1377, 1326, 1046; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 305.1732 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{16}\text{H}_{26}\text{O}_4\text{Na}$, 305.1729); CD λ_{max} nm ($\Delta\epsilon$): 210 (+3.6).

Sinularone C (**3**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} +2.20$ ($c = 0.14$, MeOH); IR (KBr) ν_{max} cm^{-1} : 2954, 2920, 2863, 1730, 1710, 1654, 1458, 1377, 1164, 1062; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 259.1315 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{16}\text{H}_{26}\text{O}_4\text{Na}$, 259.1310); CD λ_{max} nm ($\Delta\epsilon$): 203 (+5.0).

Sinularone D (**4**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} -3.22$ ($c = 0.09$, MeOH); IR (KBr) ν_{max} cm^{-1} : 2958, 2926, 2872, 1778, 1710, 1657, 1458, 1378, 1165; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 259.1313 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{16}\text{H}_{26}\text{O}_4\text{Na}$, 259.1310); CD λ_{max} nm ($\Delta\epsilon$): 222 (−1.3).

Sinularone E (**5**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} +18.0$ ($c = 0.04$, MeOH); IR (KBr) ν_{max} cm^{-1} : 3422, 2959, 2854, 1735, 1629, 1458, 1377, 1165; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 275.1612 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{15}\text{H}_{24}\text{O}_3\text{Na}$, 275.1623); CD λ_{max} nm ($\Delta\epsilon$): 221 (+5.59).

Sinularone F (**6**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} +22.6$ ($c = 0.03$, MeOH); IR (KBr) ν_{max} cm^{-1} : 3420, 2958, 2856, 1730, 1612, 1459, 1377, 1120; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 275.1610 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{15}\text{H}_{24}\text{O}_3\text{Na}$, 275.1623); CD λ_{max} nm ($\Delta\epsilon$): 219 (−2.56).

Sinularone G (**7**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} +4.03$ ($c = 0.10$, MeOH); IR (KBr) ν_{max} cm^{-1} : 3446, 2958, 2921, 1730, 1633, 1457, 1376, 1163; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 251.0886 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{11}\text{H}_{16}\text{O}_5\text{Na}$, 251.0895); CD λ_{max} nm ($\Delta\epsilon$): 213(+1.21), 245 (+0.31).

Sinularone H (**8**): obtained as colorless oil; $[\alpha]_{\text{D}}^{23} +3.70$ ($c = 0.12$, MeOH); IR (KBr) ν_{max} cm^{-1} : 3445, 2958, 2924, 1725, 1630, 1456, 1376, 1165; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS (m/z): 265.1041 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{12}\text{H}_{18}\text{O}_5\text{Na}$, 265.1052); CD λ_{max} nm ($\Delta\epsilon$): 213 (+0.25), 230 (−0.01).

Sinularone I (**9**): obtained as colorless oil; $[\alpha]_D^{23} +5.44$ (c 0.18, MeOH); IR (KBr) ν_{\max} 3432, 2959, 2923, 1734, 1633, 1458, 1376, 1162 cm^{-1} ; ^1H NMR (600 MHz, DMSO- d_6) δ : 1.67 (1H, m, H-5a), 1.86 (1H, m, H-5b), 1.03 (1H, m, H-6a), 1.12 (1H, m, H-6b), 1.22 (14H, H₂-7-H₂-14), 1.50 (2H, m, H₂-15), 2.25 (2H, t, $J = 7.4$ Hz, H₂-16), 1.70 (3H, s, H₃-18), 1.85 (3H, s, H₃-19), 4.04 (2H, q, $J = 7.1$ Hz, Me-CH₂), 1.17 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (150 MHz, DMSO- d_6) δ : 172.1 (C, C-1), 123.9 (C, C-2), 158.9 (C, C-3), 107.7 (C, C-4), 36.1 (CH₂, C-5), 23.0 (CH₂, C-6), 29.3 (CH₂, C-7), 29.2 (CH₂, C-8), 29.1 (CH₂, C-9), 28.8 (CH₂, C-10), 29.1 (CH₂, C-11), 29.2 (CH₂, C-12), 29.2 (CH₂, C-13), 29.1 (CH₂, C-14), 24.9 (CH₂, C-15), 33.9 (CH₂, C-16), 173.2 (C, C-17), 8.5 (CH₃, C-18), 11.0 (CH₃, C-19), 14.1 (CH₃), 60.0 (CH₂); HRESIMS (m/z): 391.2456 [$\text{M} + \text{Na}$]⁺ (calcd. for C₂₁H₃₆O₅Na, 391.2460); CD λ_{\max} nm ($\Delta\epsilon$): 213 (+1.25), 221 (−0.02), 244 (−1.10).

4. Conclusions

The *Sinularia* genus commonly produces cembranoids, which are considered to be the biomarkers for chemotaxonomy. The structural patterns such as cyclopentenone/cyclopentanone and unsaturated γ -lactone are unusual natural products found from the soft coral genus *Sinularia*. Present work provided a group of new cyclopentanone/cyclopentanone and butenolide-type analogues to enrich the chemical diversity of *Sinularia* corals. The unprecedented 2,3-dimethylbutenolide based natural products were originally found from marine red algae [24] and brittle star [17], implying that dimethylbutenolides may be the signal molecules of the soft coral specimen to maintain coexistence with other benthos. This is the first report to indicate derivatives of unsaturated γ -lactone possessing potent antifouling activity. The metabolites containing a cyclopentenone or cyclopentanone ring are rarely discovered in marine soft corals, but they are often obtained from marine microorganisms. In addition, dimethylbutenolides showed potent antifouling activities, indicating that they contribute to a chemical ecological function. The literature survey revealed that the cyclopentenone group is relevant for cytotoxicity [25], thus cyclopentenone containing metabolites are regarded as the cytotoxins involved in chemical defense against predators. Whether the butenolides or cyclopentenones originated from a coral host or derived from other benthos serving as a food chain are uncertain.

The ethoxylated compounds such as **2**, **7–9** are probably artifacts derived during the extraction process, whereas the naturally occurring forms are suggested to be free of the ethyl group.

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