Structural and Stereochemical Studies of α-Methylene-γ-lactone-Bearing Cembrane Diterpenoids from a South China Sea Soft Coral *Lobophytum crassum*

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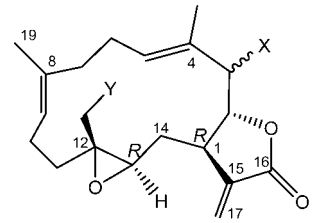
Four new α-methylene-γ-lactone-bearing cembranoids, 20-acetylsinularolide B (**6**), presinularolide B (**7**), 3-dehydroxylpresinularolide B (**8**), and 3-dehydroxyl-20-acetylpresinularolide B (**9**), together with five known analogues, sinularolides B−E (**1**−**4**) and 20-acetylsinularolide C (**5**), were isolated from a South China Sea soft coral *Lobophytum crassum*. Their structures and relative stereochemistry were established by a combination of detailed spectroscopic data analysis and chemical correlations. The structures of **1**−**9** were further confirmed by an X-ray diffraction study on a single crystal of sinularolide B (**1**). The absolute configurations of sinularolide B (**1**) and presinularolide B (**7**) were determined by a novel solid-state CD/TDDFT approach and by a modified Mosher's method, respectively. This study also revealed that the coupling constant between the lactonic methine protons ($^3J_{1,2}$) varies considerably with different functional groups on the cembrane ring and that the determination of the stereochemistry of lactone ring fusion based on this coupling constant is risky. In a bioassay, sinularolides B and C (**1** and **2**) and new cembranoids **7** and **8** showed *in vitro* cytotoxicity against the tumor cell lines A-549 and P-388.

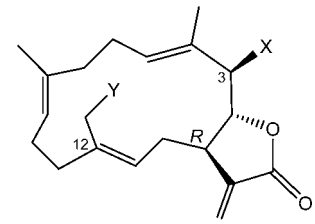
Cembrane diterpenoids are a large family of natural products having many structural variations, including further cyclizations and a multitude of functional groups, e.g., lactone, epoxide, furan, ester, ether, hydroxyl, aldehyde, and carboxylic moieties.[1] In particular, α-methylene-γ-lactone-bearing cembranoids represent an interesting group within this family, exhibiting mainly antitumor activity,[2] as well as other pharmacological effects such as anti-HIV[3] and antituberculosis.[2d] The occurrence of such lactonic cembranoids in gorgonians of the genus *Eunicea* and alcyonarians (soft corals) of the genera *Lobophytum*, *Sinularia*, *Sarcophyton*, and *Clavularia* was recently reported in an increasing number of examples.[2–4]

In the course of our ongoing project aimed at searching for biologically active secondary metabolites from South China Sea octocorals,[5] we had an opportunity to reinvestigate the soft coral *Lobophytum crassum* collected from the coast of Sanya, Hainan Island, China, which led to the isolation and structural elucidation of four new *trans*-fused α-methylene-γ-lactone cembranoids, namely, 20-acetylsinularolide B (**6**), presinularolide B (**7**), 3-dehydroxylpresinularolide B (**8**), and 3-dehydroxyl-20-acetylpresinularolide B (**9**), together with five analogues, sinularolides B−E (**1**−**4**) and 20-acetyl sinularolide C (**5**). We herein report the isolation, structural elucidation, and bioactivities of these compounds.

Results and Discussion

Freshly collected specimens of *L. crassum* were immediately frozen to −20 °C and stored at that temperature before extraction. The Et_2O-soluble portion of the acetone extract was repeatedly chromatographed over silica gel, Sephadex LH-20 columns, and RP-HPLC to afford nine diterpenoids (**1**−**9**). The structures and relative stereochemistry of the known compounds **1**−**5** were determined by extensive spectroscopic analysis combined with careful comparison with reported data and further confirmed by an X-ray diffraction study on a single crystal of sinularolide B (**1**) (Figure 1). Sinularolides B−E (**1**−**4**) were recently reported to be isolated from the Hainan soft coral *Sinularia gibberosa*.[2a] In fact, sinularolide B (**1**) was first isolated from a Red Sea soft coral *L. crassum*,[6] which also produced the 20-acetyl analogue (**5**).[7] A complete and rigorous ^{1}H and ^{13}C NMR assignment of **5** was then performed, since its relative stereochemistry had not been reported in the literature.[7] A preliminary ^{1}H NMR analysis of the newly isolated molecules revealed a structural similarity for all of them and indicated the presence of the common α-methylene-γ-lactone cembranoid frameworks in compounds **6**−**9**, according to previous chemical studies on *L. crassum*.[7] The chemical analysis of **6**−**9** was conducted starting from the main metabolite sinularolides B (**1**), followed by the remaining cembranoids **6**−**9**. Accordingly, the structure elucidation details of these new molecules are described in this order.





1 X = β-OH, Y = OH
2 X = α-OH, Y = OH
3 X = H, Y = OH
4 X = β-OH, Y = H
5 X = α-OH, Y = OAc
6 X = β-OH, Y = OAc
10 X = β-OAc, Y = OAc

7 X = Y = OH
8 X = H, Y = OH
9 X = H, Y = OAc

On the basis of the knowledge of the relative relationships of the chiral centers around the 14-membered cembrane ring of sinularolide B (**1**), advanced stereochemical studies were performed. A novel solid-state CD/TDDFT (time-dependent density functional theory) approach[8] was applied to determine the absolute stereochemistry of **1**. The CD spectra of (−)-**1** were recorded in both acetonitrile solution and the solid state as a KCl disk and showed

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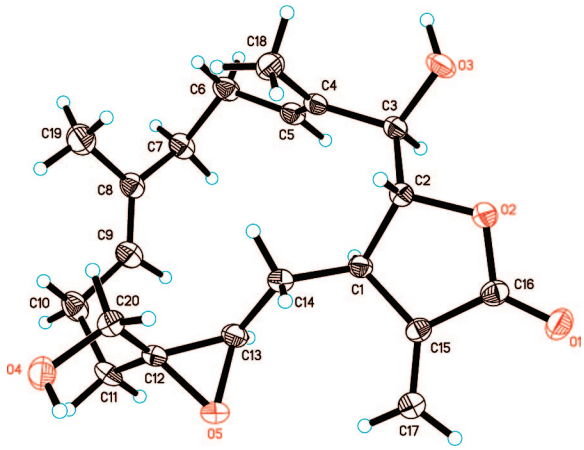

Figure 1. Molecular structure of sinularolide B (**1**) from the X-ray diffraction experiment.

similar profiles in the two cases (Figure 2a), which is in agreement with a pronounced rigidity of the macrocycle due to its many unsaturated bonds. The CD spectrum calculated with the TDDFT method[9] at the BH&HLYP/TZVP level, using as input geometry the X-ray coordinates for **1** with (1*R*,2*R*,3*R*,12*S*,13*R*) configurations, is shown in Figure 2b. It clearly reproduces the shapes of the main experimental bands very well, except for a 20 nm wavelength shift and some intensity overestimation. The smaller positive band at 224 nm (experimental spectrum) and the stronger negative one at 197 nm are assigned to the $\pi-\pi^*$ transitions of the enone and the isolated alkenes, respectively. The absolute configurations of the five stereogenic centers in **1** are thus determined to be 1*R*, 2*R*, 3*R*, 12*S*, 13*R*.

Compound **6**, 20-acetylsinularolide B, was isolated as an optically active {$[\alpha]^{20}_D$ −85.0 (*c* 0.25, $CHCl_3$)} colorless oil. The molecular formula $C_{22}H_{30}O_6$ was established by HRESIMS, ^{13}C NMR, and DEPT spectra. The IR spectrum of **6** indicated the presence of a hydroxyl group (3446 cm^{-1}), an ester group (1743 cm^{-1}), and an α-methylene-γ-lactone moiety (1766, 1660 cm^{-1}) in the molecule. The presence of an α-methylene-γ-lactone moiety was also supported by the observation of a strong UV absorption at 207 nm and a pair of characteristic exocyclic olefinic protons (δ 6.32, 6.03) in the ^1H NMR spectrum. The ^1H NMR data of **6** were very similar to those of **1**, except for the presence of an additional signal at δ 2.13 (s) (Table 1), which suggested the presence of an acetyl moiety in **6**, consistent with two carbon resonances at δ 170.7 (qC) and 20.7 (qC) in its ^{13}C NMR spectrum (Table 2). The acetylation was determined to occurr at C-20, since the AB-type proton signals of H_2-20 were significantly downfield shifted from δ 3.82 and 3.58 to δ 4.38 and 3.87 compared to **1**, respectively. Acetylation of **1** and **6** yielded the same diacetate **10**, giving further support to this conclusion. Thus, compound **6** was established as the 20-acetyl derivative of **1**.

A search of the literature revealed that compound **6** is identical in all aspects, except for optical rotation sign and magnitude, to a "known" cembranoid (namely, 13-hydroxylobolide[7]), previously isolated from a Red Sea soft coral of *L. crassum*. Unfortunately, the authors of the paper gave only a planar structure of 13-hydroxylobolide without stereochemical comment. Since the $[\alpha]_D$ sign of compound **6** is opposite that of 13-hydroxylobolide {$[\alpha]^{24}_D$ +16.0 (*c* 0.9, $CHCl_3$)}, it is obvious that **6** should be an enantiomer of 13-hydroxylobolide. However, because the 13-epimer (3-epimer in our atom-numbering system) of **6** and that of 13-hydroxylobolide were also isolated from both Chinese and Israeli samples and they are identical including the $[\alpha]_D$ values and signs, it is necessary to remeasure the optical rotation value of 13-hydroxylobolide. In fact, we twice recorded the $[\alpha]_D$ value of compound **6** in order to confirm the correctness of our measurement.

Presinularolide B (**7**) was obtained as an optically active colorless oil. HRESIMS, ^{13}C NMR, and DEPT spectra established the molecular formula as $C_{20}H_{28}O_4$, indicating seven double-bond equivalents. IR bands at 1736, 1728, and 1660 cm^{-1} and the strong UV absorption at 210 nm suggested the presence of an α-methylene-γ-lactone moiety. This conclusion was supported by the observation of the signals for a lactone group (δ_H 4.23; δ_C 169.8, qC and 83.7, CH) and an exocyclic double bond (δ_H 6.30 and 5.71; δ_C 138.5, qC and 123.4, CH_2) in the ^1H and ^{13}C NMR spectra, which accounted for three degrees of unsaturation. The remaining degrees of unsaturation were assigned to three double bonds and an additional ring by 1D and 2D NMR (HSQC and HMBC) experiments (Tables 1 and 2).

A comparison of the ^1H and ^{13}C NMR data of **7** with those of **1** revealed a close similarity with the exception that the epoxy group (δ_H 2.88; δ_C 63.4, CH and 62.8, qC) in **1** was replaced by a trisubstituted double bond (δ_H 5.20; δ_C 140.3, qC and 124.6, CH_2) in **7**. This fact suggested the gross structure of **7** to be a 12,13-double bond precursor of **1**, which was strongly supported by the $^1H-^1H$ COSY and HMBC experiments, as shown in Figure 3a. The *trans* ring fusion and α-orientation of H-3 were deduced from the significant NOE effect between H-1 and H-3 and the lack of NOE effect between H-2 and both H-1 and H-3 in NOE difference spectra (Figure 3b).

The presence of a secondary alcohol at C-3 of **7** allowed us to determine its absolute stereochemistry by utilizing the modified Mosher's method.[10] (*S*)- and (*R*)-MTPA esters of presinularolide B (**7**) were prepared by treatment with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride in dry pyridine at room temperature, respectively. Compound **7** was converted to the corresponding MTPA esters **7S** and **7R**, respectively. Significant $\Delta\delta$ values ($\Delta\delta = \delta_{S\text{-MTPA-ester}} - \delta_{R\text{-MTPA-ester}}$) for the protons near the chiral center C-3 were observed as shown in Figure 4. According to the Mosher's rule, the absolute configuration at C-3 was determined as *R*. As a consequence, the absolute configurations of the other two stereogenic centers in **7** were determined to be 1*R*, 2*R*, on the basis of the established relative stereochemistry, which is in agreement with the configuration of the related compound **1** determined above.

Although compound **1** was the 12,13-epoxide of **7**, the marked difference between the coupling constants of the protons attached to the bridgehead carbons in **1** ($^3J_{1,2}$ = 6.6 Hz) and **7** ($^3J_{1,2}$ = 2.3 Hz) was puzzling. To further confirm the established structure of **7**, a Sharpless epoxidation[11] was performed to produce **1** from **7** by using (*R*,*R*)-diethyl tartrate. The structure and stereochemistry of **7** were thus unambiguously determined.

Compound **8**, 3-dehydroxylpresinularolide B, was an optically active colorless oil. It had the molecular formula $C_{20}H_{28}O_3$ as deduced from HRESIMS, ^{13}C NMR, and DEPT spectra and thus one oxygen atom less than **7**. The IR and UV spectra of **8** closely resembled those of **7**. The ^1H and ^{13}C NMR data of **8** (Tables 1 and 2) were also closely related to those of **7**, with only a difference at C-3 [the former possessed a methylene (δ_H 2.44, 2.02; δ_C 44.9, CH_2) and the latter had a hydroxyl-bearing methine (δ_H 3.75; δ_C 78.9, CH)]. Due to the loss of the 3-OH, ^{13}C NMR chemical shifts of C-2−C-5 were all upfield shifted while that of C-1 was downfield shifted with respect to those of **7**. This evidence suggested that **8** was a 3-deoxy derivative of **7**. A series of obvious proton connectivities of H-1/H-2/H_2-3 as established by a $^1H-^1H$ COSY experiment and the diagnostic long-range correlations of H_2-3 with C-1, C-2, C-4, C-5, and C-18 and of H_3-18 with C-3, C-4, and C-5 observed in the HMBC spectrum confirmed this conclusion about the structure **8**.

Compound **9**, 3-dehydroxyl-20-acetylpresinularolide B, had a molecular formula of $C_{22}H_{30}O_4$ as determined by HRESIMS {*m*/*z* 381.2016 $[M + Na]^+$, corresponding to $C_{22}H_{30}O_4Na$ (calculated for 381.2042)}. Its UV, IR, and ^1H and ^{13}C NMR

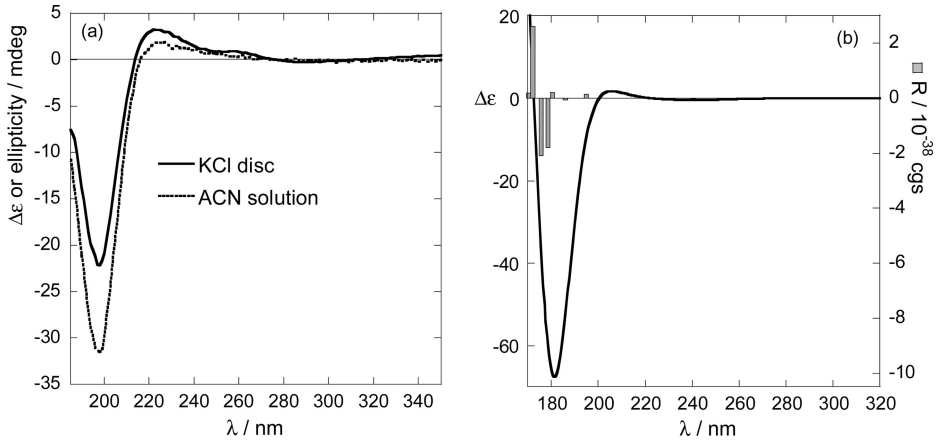

Figure 2. (a) Experimental CD spectra of (−)-**1** in acetonitrile and in the solid state as a KCl disk. (b) TDBH&HLYP/TZVP-calculated CD spectrum of (1*R*,2*R*,3*R*,12*S*,13*R*)-**1** using X-ray coordinates; vertical bars represent rotational strengths.

Table 1. ^{1}H NMR Data[a,b] for Compounds **6**−**9**

	6	**7**	**8**	**9**
position	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)
1	2.74, m	2.73, dd (7.8, 2.3)	2.73, ddd (8.2, 3.6, 2.8)	2.72, ddd (8.3, 3.7, 2.7)
2	4.07, dd (9.1, 6.6)	4.23, dd (7.5, 2.3)	4.33, dt (9.6, 2.8)	4.31, dt (9.4, 2.7)
3	4.02, d (9.1)	3.75, d (7.5)	2.44, br d (14.1)	2.44, br d (14.1)
			2.02,dd (14.1, 9.6)	2.03, dd (14.1, 9.4)
5	5.46, m	5.34, dd (8.5, 3.5)	5.03, m	5.04, m
6	2.53, ddd (22.7, 9.1, 4.1)	2.33, m	2.30, m	2.29, m
	2.20, m	2.14, m	2.09, m	2.09, m
7	2.35, m	2.13, m	2.08, m	2.06, m
	2.11, m	2.08, dd (18.0, 9.8)	2.06, m	2.04, m
9	5.02, t, 7.1	4.89, br d (7.2)	4.93, m	4.88, m
10	2.26, m	2.35, m	2.30, m	2.28, m
	2.14, m	2.18, m	2.21, m	2.22, m
11	2.33, m	2.32, m	2.35, m	2.28, m
	1.24, dt (11.9, 1.5)	2.21, m	2.23, m	2.21, m
13	2.85, dd (7.3, 3.0)	5.20, dd (8.2, 7.3)	5.22, t (7.7)	5.29, t (7.7)
14	1.87, ddd (14.5, 3.4, 3.0)	2.36, m	2.33, m	2.32, m
	1.50, ddd (14.5, 9.7, 7.3)	2.29, m	2.29, m	2.28, m
17	6.32, d (2.3)	6.30, d (2.1)	6.27, d (1.6)	6.28, d (2.0)
	6.03, d (2.3)	5.71, d (2.1)	5.68, d (1.6)	5.69, d (2.0)
18	1.72, s	1.72, s	1.66, s	1.66, s
19	1.63, s	1.62, s	1.61, s	1.60, s
20	4.38, d (12.2)	4.10, s	4.12, s	4.56, s
	3.87, d (12.2)	4.10, s	4.12, s	4.56, s
OAc	2.13, s			2.06, s

[a] Bruker DPX-300 spectrometer, chemical shifts referenced to $CHCl_3$ (δ 7.26 ppm). [b] Assignments made by HSQC, ^{1}H−^{1}H COSY, and HMBC experiments.

data (Tables 1 and 2) were very similar to those of **8**, suggesting it shares the same framework as **8**. In fact, the presence of an acetyl group (δ_H 2.13, s; δ_C 170.9, qC and 20.9, CH_3) as compared to **8** was the only difference recognized in the spectroscopic data of **9**. Furthermore, the apparent downfield shifted ^{1}H NMR resonance of H_2-20 (from δ_H 4.12 to 4.56) indicated that the 20-hydroxyl group was acetylated. Compound **9** was therefore the 20-acetyl derivative of **8**.

It is worthy to point out that both compounds **8** and **9** are formally identical to "20-deacetyllobolide" and "3,4-deepoxylobolide", both of which were previously reported from the same species of Red Sea origin by Kashman et al.[6] However, neither relative stereochemistry nor $[\alpha]_D$ values of "20-deacetyllobolide" and "3,4-deepoxy lobolide" were disclosed.[6] In particular, to our surprise, the ^{13}C NMR data of "20-deacetyllobolide"[6] were found to be quite different from those of compound **8** (Table 2). Moreover, careful comparison of their ^{13}C NMR data with ours revealed that the significant differences between them were observed for three olefinic carbons (C-5, C-8, and C-12) and the methylene carbons (C-7, C-7, C-10, and C-14) that linked to these double bonds. Since the NMR data assignments of **8** were correct even after careful interpretation of its NMR spectra, we suspect that the above-mentioned differences between the ^{13}C NMR data may be caused by the different geometry of the double bonds in "20-deacetyllobolide". This observation creates a need to reanalyze the NMR spectra of "20-deacetyllobolide" so as to verify its correct structure.

"3,4-Deepoxylobolide" and compound **9** shared almost identical NMR data, indicating their structures might be the same if the former had the same optical rotation sign as **9**.

The close biosynthetic relationship between metabolites **1**−**9** is apparent. They differ from each other only in the oxidative pattern of the 14-membered ring. Compounds **7**−**9** could be the formal precursors of epoxides **1**−**6**, as suggested by the chemical conversion of **7** to **1**. Moreover, since the absolute configuration of **1** and **7** was determined, it is reasonable to assign the same configurations for the corresponding chiral centers within molecules **2**−**6**, **8**, and **9** based on biogenetic consideration. In addition, it is interesting to note that the coupling constant between the lactone methine protons ($^3J_{1,2}$) was significantly changed during the transformation of **7** to **1** (e.g., $^3J_{1,2}$ = 2.3 Hz in **7** and 6.6 Hz in **1**) in spite of the fact that the same ring junction is present in the two groups of metabolites. This fact supports Coll's suggestion that the coupling constant

Table 2. ^{13}C NMR Data[a,b] for Compounds **6**−**9**

	6	7	8	9	8[c]
1	42.2, CH	42.2, CH	44.7, CH	44.5, CH	44.8, CH
2	82.1, CH	83.7, CH	81.1, CH	81.1, CH	81.2, CH
3	81.0, CH	78.9, CH	44.9, CH_2	45.0, CH_2	38.0, CH_2
4	131.9, qC	131.9, qC	129.4, qC	129.3, qC	128.5, qC
5	132.1, CH	131.2, CH	128.4, CH	128.5, CH	124.7, CH
6	24.8, CH_2	24.2, CH_2	24.5, CH_2	24.6, CH_2	24.5, CH_2
7	38.5, CH_2	37.6, CH_2	37.9, CH_2	37.9, CH_2	34.6, CH_2
8	135.1, qC	134.0, qC	134.0, qC	134.0, qC	129.5, qC
9	124.2, CH	123.6, CH	124.0, CH	123.8, CH	124.2, CH
10	23.6, CH_2	24.4, CH_2	24.4, CH_2	24.4, CH_2	29.6, CH_2
11	32.6, CH_2	34.7, CH_2	34.6, CH_2	34.6, CH_2	33.7, CH_2
12	60.4, qC	140.3, qC	140.1, qC	135.5, qC	134.1, qC
13	62.4, CH	124.6, CH	124.7, CH	126.9, CH	125.6, CH
14	31.5, CH_2	33.8, CH_2	33.5, CH_2	33.8, CH_2	30.9, CH_2
15	138.5, qC	138.5, qC	139.3, qC	139.2, qC	142.0, qC
16	168.8, qC	169.8, qC	170.2, qC	170.0, qC	169.2, qC
17	124.2, CH_2	123.4, CH_2	122.8, CH_2	122.8, CH_2	122.8, CH_2
18	12.4, CH_3	13.2, CH_3	17.5, CH_3	17.4, CH_3	17.5, CH_3
19	15.8, CH_3	17.0, CH_3	16.8, CH_3	16.7, CH_3	16.8, CH_3
20	64.0, CH_2	60.0, CH_2	60.1, CH_2	61.7, CH_2	60.0, CH_2
OAc	20.7, CH_3			20.9, CH_3	
	170.7, qC			170.9, qC	

[a] Varian INOVA-600 NMR spectrometer; chemical shifts referenced to $CDCl_3$ (δ_C 77.0). [b] Assignments made by DEPT, HSQC, and HMBC experiments. [c] Data reported for 20-deacetyllobolide in ref 6.

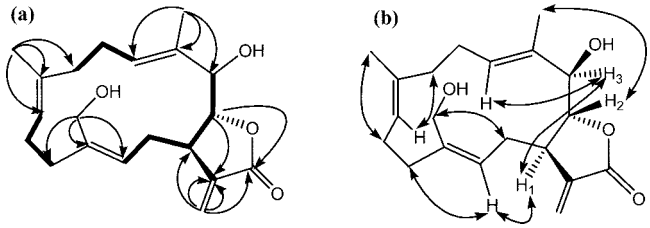

Figure 3. (a) $^1H-^1H$ COSY (bold bonds) and selected HMBC correlations (curved arrows) for **7**. (b) Key NOESY correlations for **7**.

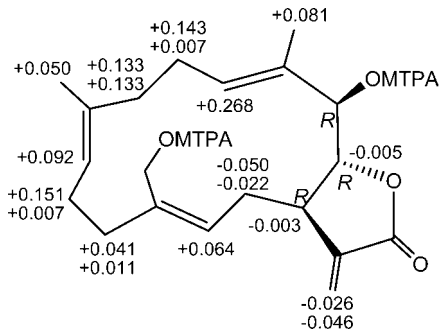

Figure 4. $\Delta\delta$ ($\delta_S - \delta_R$) values (in ppm) for the MTPA ester of **7**.

around the five-membered lactone ring does not permit unequivocal stereochemical assignments due to the flexibility of the cembrane ring.[12] Unfortunately, Coll's suggestion has not been widely appreciated and the $^3J_{1,2}$ value is still widely used to deduce relative stereochemistry (e.g., a small coupling constant indicates a *transoid* ring junction, whereas a large coupling constant suggests a *cisoid* ring linkage). This general trend was proposed mainly on the basis of several early X-ray studies[6,13] and played a crucial role in the assignment of the bridgehead fusion pattern of lactonic cembranoids. A literature survey revealed that the coupling constants of protons ($^3J_{1,2}$) for the *trans* ring junction ranged from 2.9 to 7.5 Hz, while those for the corresponding *cis* linkage extended over the range 6.5 to 9.3 Hz.[2–4,6,7,12,13] It seems to be appropriate to employ coupling constants in stereochemical assignments of the bicyclic fusion pattern when these values lie at either of the two extremes, while those lying in the middle (i.e., 6−8 Hz) should be used with much greater caution. Our observation of the dependence of the $^3J_{1,2}$ coupling constant on the transformation of a $\Delta^{12(13)}$ double bond (**7**−**9**) into an epoxy group (**1**−**6**) demonstrates the sensitivity of such parameters to the nature of different functional groups attached to the cembrane ring scaffold. Similar phenomena were also observed upon oxidation of 20-acetylsinularolide C (**5**, $^3J_{1,2}$ = 6.5) to its 3-ketone analogue ($^3J_{1,2}$ = 3.3) and conversion of *ent*-lobophilide A ($^3J_{1,2}$ = 6.8) into the corresponding 4,5-epoxy-3-ketone derivative ($^3J_{1,2}$ = 3.3).[7] These observations indicate that the conformational flexibility of the macrocycle renders somewhat risky the stereochemical determination of the ring fusion based on the proton coupling constants. To obtain an unambiguous stereochemical assignment, other techniques, for example, more advanced NMR methods and X-ray diffraction analysis, are necessary.

All the compounds were examined for growth-inhibition activities *in vitro* toward human lung adenocarcinoma A-549 cells and murine leukemia P-388 cells. 3-Dehydroxylpresinularolide B (**8**) showed significant cytotoxicity against both A-549 and P-388 cell lines with 95.7% and 100% inhibition at 10^{-5} mol/L, while sinularolide B (**1**) demonstrated cytotoxic activity against P-388 with an inhibition of 100% at 10^{-5} mol/L. Moderate antitumor activities were observed for presinularolide B (**7**) against A-549 cells and for sinularolide C (**2**) against P-388 cells with 64.0% and 63.8% inhibition at a concentration of 10^{-5} mol/L, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured on an X-4 digital micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer polarimeter 341 at the sodium D-line, cell length 100 mm. CD spectra were measured on a Jasco J-810 spectropolarimeter (for the solid-state CD protocol, see refs 8b,e). UV spectra were recorded on a 756 CRT spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrophotometer; peaks are reported in cm^{-1}. The NMR spectra were recorded at 293 K on Bruker DPX-300, Bruker Avance-500, and Varian INOVA-600 NMR spectrometers, using the residual $CHCl_3$ signal (δ_H 7.26 ppm) as an internal standard for ^1H NMR and $CDCl_3$ (δ_C 77.0 ppm) for ^{13}C NMR; coupling constants (*J*) are given in Hz. ^1H and ^{13}C NMR assignments were supported by $^1H-^1H$ COSY, HSQC, HMBC, ROESY, and NOEDIFF experiments. EI mass spectra were obtained on a MAT 8200 mass spectrometer, and ESI mass spectra were performed on a Q-TOF Micro LC-MS-MS mass spectrometer. The X-ray diffraction study was carried out on a Bruker SMART APEX CCD diffractometer with Mo Kα radiation (λ = 0.71073 Å). Commercial silica gel (Qing Dao Hai Yang Chemical Group Co., 200−300

and 400−600 mesh) was used for column chromatography. Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical thin-layer chromatography (TLC).

Animal Material. The soft coral *L. crassum* was collected off the coast of Sanya, Hainan Province, China, in December 2004, at a depth of 20 m and identified by Professor R.-L. Zhou of South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (LS-167) is available for inspection at Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The frozen animals (630 g, dry weight) were cut into pieces and exhaustively extracted with acetone at room temperature (1.5 L × 3). The organic extract was evaporated to give a residue, which was partitioned between ether and H_2O. The ether solution was concentrated under reduced pressure to give a dark green residue (3.8 g), which was fractionated by gradient silica gel column chromatography (0−100% acetone in petroleum ether), yielding 16 fractions. Fractions 6−11 showed interesting red TLC spots after spraying with H_2SO_4. Fraction 6 gave compound **9** (9.0 mg) after a column chromatography (CC) on Sephadex LH-20 ($CHCl_3$). Fraction 8 was subjected to silica gel CC (400−600 mesh, petroleum ether/acetone, 85:15), followed by CC on Sephadex LH-20 ($CHCl_3$) to yield **8** (136.2 mg) and crude **5** (9.4 mg), which afforded pure **5** (6.0 mg) after purification by RP-HPLC (semipreparative ZORBAX ODS (5 μm, 250 × 9.4 mm), MeO/H_2O (7:3), 2.5 mL/min, 8.4 min). Fraction 9 was first split by Sephadex LH-20 CC ($CHCl_3$/MeOH/petroleum ether, 2:1:1) and then chromatographed on a silica gel column (400−600 mesh, petroleum ether/acetone, 85:15) to afford **3** (39.0 mg) and **6** (18.3 mg), respectively. Compounds **1** (35.0 mg) and **2** (77.6 mg) were isolated from fraction 10 by a CC sequence on Sephadex LH-20 ($CHCl_3$/MeOH/petroleum ether, 2:1:1) and silica gel (400−600 mesh, petroleum ether/acetone, 4:1) as well. Last, CC of fraction 11 on Sephadex LH-20 CC ($CHCl_3$/MeOH/petroleum ether, 2:1:1) yielded **4** (50.0 mg) and **7** (227.0 mg).

Sinularolide B (1): colorless crystals, mp 128−130 °C; $[\alpha]^{20}_D$ −123.6 (*c* 0.47, $CHCl_3$); CD (MeCN, λ [nm] ($\Delta\epsilon$), $c = 7.4 \times 10^{-4}$) 225 (2.09), 198 (−31.58), CD (KCl, λ [nm] ϕ [mdeg], 47 μg of **1** and 250 mg of KCl, 224 (3.18), 197 (− 21.28); UV (MeOH) λ_{max} (log ε) 208 (3.83) nm; IR (KBr) ν_{max} 3610, 3460, 2949, 2866, 1751, 1659, 1261, 1149, 1043 cm^{-1}; HRESIMS *m/z* 371.1833 (calcd for $C_{20}H_{28}O_5Na$, 371.1834).

20-Acetylsinularolide C (5): colorless oil; $[\alpha]^{20}_D$ −89.0 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (3.84) nm; IR (KBr) ν_{max} 3466, 2924, 2870, 1765, 1743, 1660, 1267, 1145, 1037 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.27 (1H, d, J = 3.1 Hz, H-16a), 5.98 (1H, d, J = 3.1 Hz, H-16b), 5.62 (1H, m, H-5), 5.03 (1H, t, J = 7.8 Hz, H-9), 4.46 (1H, br s, H-3), 4.35 (1H, d, J = 12.1 Hz, H-20a), 4.22 (1H, dd, J = 6.8, 1.7 Hz, H-2), 3.87 (1H, d, J = 12.1 Hz, H-20b), 3.29 (1H, m, H-1), 2.90 (1H, dd, J = 7.7, 2.8 Hz, H-13), 2.54 (1H, m, H-6a), 2.35 (1H, m, H-7a), 2.30 (1H, m, H-11a), 2.22 (1H, m, H-10a), 2.19 (1H, m, H-7b), 2.13 (1H, m, H-6b), 2.13 (3H, s, H_3-OAc), 2.11 (1H, m, H-10b), 1.79 (1H, ddd, J = 14.5, 3.6, 2.8 Hz, H-14a), 1.71 (3H, s, H_3-18), 1.61 (3H, s, H_3-19), 1.49 (1H, ddd, J = 14.5, 8.7, 7.7 Hz, H-14b), 1.25 (1H, dt, J = 12.4, 2.9 Hz, H-11b) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9 (qC, *C*=O-OAc), 170.1 (qC, C-17), 139.8 (qC, C-15), 135.2 (qC, C-8), 131.5 (qC, C-4), 127.8 (CH, C-5), 124.3 (CH, C-9), 123.6 (CH_2, C-16), 81.9 (CH, C-2), 73.7 (CH, C-3), 64.3 (CH_2, C-20), 62.6 (CH, C-13), 60.5 (qC, C-12), 38.8 (CH_2, C-7), 37.4 (CH, C-1), 33.1 (CH_2, C-14), 32.8 (CH_2, C-11), 24.8 (CH_2, C-6), 23.7 (CH_2, C-10), 21.0 (CH_3, *Me*-OAc), 16.1 (CH_3, C-19), 15.7 (CH_3, C-18) ppm; HRESIMS *m/z* 413.1970 (calcd for $C_{22}H_{30}O_6Na$, 413.1940).

20-acetylsinularolide B (6): colorless oil; $[\alpha]^{20}_D$ −85.0 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (3.80) nm; IR (KBr) ν_{max} 3446, 2923, 2850, 1766, 1743, 1660, 1263, 1140, 1040 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 413.1939 (calcd for $C_{22}H_{30}O_6Na$, 413.1940).

Presinularolide B (7): colorless oil; $[\alpha]^{20}_D$ +33.8 (*c* 0.54, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.06) nm; IR (KBr) ν_{max} 3411, 3323, 2910, 2850, 1736, 1728, 1660, 1271, 1138, 1020 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 355.1883 (calcd for $C_{20}H_{28}O_4Na$, 355.1885).

3-Dehydroxylpresinularolide B (8): colorless oil; $[\alpha]^{20}_D$ +66.1 (*c* 0.32, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (3.96) nm; IR (KBr) ν_{max} 3479, 2908, 2846, 1732, 1664, 1271, 1140, 1028 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 339.1955 (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

3-Dehydroxyl 20-acetylpresinularolide B (9): colorless oil: $[\alpha]^{20}_D$ +81.0 (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (3.97) nm; IR (KBr) ν_{max} 2920, 2852, 1766, 1738, 1664, 1232, 1118, 1026 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 381.2016 (calcd for $C_{22}H_{30}O_4Na$, 381.2042).

Acetylation of 1 and 6. To **1** (2.0 mg) and **6** (2.0 mg) in dry pyridine (0.5 mL) was added 1 drop of Ac_2O, respectively. Both mixtures were stirred at room temperature overnight, and the reactions were stopped by adding 1 drop of H_2O. The crude acetylated products, after evaporating the solvent *in vacuo*, were purified on silica gel column chromatography (petroleum ether/EtOAc in gradient), respectively, to afford the expected diacetate **10**: ^1H NMR (500 MHz, $CDCl_3$) δ 6.32 (1H, d, J = 2.9 Hz, H-16a), 6.00 (1H, d, J = 2.9 Hz, H-16b), 5.53 (1H, dd, J = 8.2, 5.2 Hz, H-5), 5.21 (1H, d, J = 8.8 Hz, H-3), 5.04 (1H, t, J = 7.0 Hz, H-9), 4.36 (1H, d, J = 12.2 Hz, H-20a), 4.19 (1H, dd, J = 8.8, 6.7 Hz, H-2), 3.89 (1H, d, J = 12.2 Hz, H-20b), 2.88 (1H, dd, J = 6.8, 3.6 Hz, H-13), 2.86 (1H, m, H-1), 2.44 (1H, m, H-6a), 2.34 (2H, m, H-7a, H-11a), 2.24 (1H, m, H-10a), 2.17 (1H, m, H-6b), 2.14 (1H, m, H-10b), 2.14 (3H, s, H_3-OAc-20), 2.10 (3H, s, H_3-OAc-3), 2.10 (1H, m, H-7b), 1.85 (1H, dt, J = 14.6, 3.7 Hz, H-14a), 1.70 (3H, s, H_3-18), 1.63 (s, 3H, H_3-19), 1.58 (1H, ddd, J = 14.6, 9.3, 7.0 Hz, H-14b), 1.28 (1H, dt, J = 11.2, 2.9 Hz, H-11b) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (qC, C=O-OAc-20), 169.6 (qC, C=O-OAc-3), 168.9 (qC, C-17), 138.2 (qC, C-15), 135.1 (qC, C-8), 133.5(CH, C-5), 129.2 (qC, C-4), 124.3 (CH, C-9), 124.0 (CH_2, C-16), 80.1 (CH, C-3), 79.7 (CH, C-2), 64.1 (CH_2, C-20), 62.0 (CH, C-13), 60.4 (qC, C-12), 42.1 (CH, C-1), 38.3 (CH_2, C-7), 32.4 (CH_2, C-11), 31.7 (CH_2, C-14), 24.8 (CH_2, C-6), 23.5 (CH_2, C-10), 21.0 (CH_3, *Me*-OAc-3), 20.8 (CH_3, *Me*-OAc-20), 15.9 (CH_3, C-19), 13.3 (CH_3, C-18) ppm; HREIMS *m/z* 432.21508 (calcd for $C_{24}H_{32}O_7$, 432.21481) {$[\alpha]^{20}_D$ −27 (*c* 0.10 $CHCl_3$)}.

Esterification of 7 with MTPA Chlorides. Both (*S*)- and (*R*)-MTPA esters of **7** (**7S**, **7R**) were obtained by treatment of **7** (3.0 mg, respectively) with (*R*)- and (*S*)-MTPA chlorides (10 μL) in dry pyridine (0.5 mL) catalyzed with dimethylaminopyridine and stirred at room temperature overnight. The MTPA esters (6.0 mg, 87% yield) were purified by minicolumn chromatography on silica gel (300 mesh, petroleum ether/EtOAc, 7:1).

^1H NMR data of **7S** (500 MHz, $CDCl_3$): δ 6.29 (1H, s, H-16a), 5.67 (1H, d, J = 1.6 Hz, H-16b), 5.46 (1H, t, J = 6.9 Hz, H-5), 5.29 (1H, t, J = 7.3 Hz, H-13), 5.13 (1H, d, J = 7.6 Hz, H-3), 4.82 (1H, d, J = 12.1 Hz, H-20a), 4.81 (1H, m, H-9), 4.70 (1H, d, J = 12.1 Hz, H-20b), 4.21 (1H, d, J = 7.6 Hz, H-2), 2.69 (1H, br d, J = 10.9 Hz, H-1), 2.34 (1H, m, H-14a), 2.33 (1H, m, H-10a), 2.28 (1H, m, H-6a), 2.15 (2H, m, H_2-11), 2.14 (1H, m, H-10b), 2.13 (1H, m, H-14b), 2.10 (3H, m, H-6b, H_2-7), 1.69 (3H, s, H_3-18), 1.59 (3H, s, H_3-19) ppm.

^1H NMR data of **7R** (300 MHz, $CDCl_3$): δ 6.31 (1H, s, H-16a), 5.71 (1H, s, H-16b), 5.23 (1H, t, J = 6.8 Hz, H-13), 5.19 (1H, t, J = 7.0 Hz, H-5), 4.84 (1H, d, J = 8.6 Hz, H-3), 4.74 (2H, s, H_2-20), 4.72 (1H, m, H-9), 4.21 (1H, dd, J = 8.6, 1.6 Hz, H-2), 2.69 (1H, br d, J = 10.0 Hz, H-1), 2.36 (1H, m, H-14a), 2.18 (2H, m, H-10a, H-14b), 2.14 (2H, m, H-6a, H-11a), 2.13 (1H, m, H-10b), 2.11 (1H, m, H-11b), 2.10 (1H, m, H-6b), 1.97 (2H, m, H_2-7), 1.61 (3H, s, H_3-18), 1.54 (3H, s, H_3-19) ppm.

Sharpless Epoxidation of 7. To a mixture of Ti(O*i*Pr)$_4$ (0.0041 mL, 0.01 mmol) and 4 Å molecular sieves (2 mg) in CH_2Cl_2 (0.5 mL) were added L-(+)-DET (0.0034 mL, 0.02 mmol) and *t*-BuOOH (0.018 mmol, 0.0057 mL, 3.16 M in toluene) sequentially under an Ar atmosphere at −20 °C. The reaction mixture was stirred for an additional 30 min, and **7** (3 mg, 0.009 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise. The mixture was stirred for an additional 2 h at −20 to −15 °C until TLC showed disappearance of **7**. Then 10% aqueous tartaric acid solution (0.5 mL) was added and the mixture stirred for 1 h. The mixture was evaporated to remove the solvent and was then extracted with Et_2O (3 mL × 3). The ethereal solution was washed with saturated $NaHCO_3$ solution (1.5 mL × 2) and brine (1.5 mL × 2), evaporated, and then subjected to a minicolumn chromatography (petroleum ether/EtOAc, 1:1) to afford the pure product **1** (36%) { $[\alpha]^{20}_D$ −125.7 (*c* 0.11, $CHCl_3$)}.

X-ray Crystallographic Studies of Sinularolide B (1). Colorless block crystals of **1** were obtained by recrystallization in CH_3OH/H_2O (100:1). The crystal (0.475 × 0.466 × 0.345 mm) belongs to the orthorhombic system, $C_{20}H_{28}O_5 \cdot H_2O$ (M_r = 366.44), space group $P2(1)2(1)2(1)$ with a = 9.2900(14) Å, b = 9.3200(15) Å, c = 21.944(4) Å, $\alpha = \beta = \gamma = 90.0°$, V = 1899.9(5) Å^3, Z = 4, D_{calcd} = 1.281

mg/m^3, $\lambda = 0.71073$ Å. Intensity data were measured on a Bruker SMART APEX CCD diffractometer. A total of 11 090 reflections were collected to a maximum 2θ value of 54.60° by using the ω/ω scan technique at 293(2) K. The structure was solved by the direct method and was refined by the full matrix least-squares procedure. The collection data were reduced using the Saint program, and the empirical absorption correction was performed using the SADABS program. All non-hydrogen atoms were given anisotropic thermal parameters, whereas the hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. The refinement converged to the final $R = 0.0434$, $R_w = 0.0949$ for 2473 observed reflections ($I > 2\sigma(I)$, $2\theta \leq 51.28°$) and 277 variable parameters. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Center with the deposition no. CCDC 653250, which contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Computational Section. TDDFT calculations were run with the Gaussian'03 program (Gaussian, Inc., Pittsburgh, PA, 2003). The input geometry was obtained starting from the solid-state structure of **1**, where only the C−H bond distances were reoptimized with the DFT method at the B3LYP/6-31G(d) level.[14] The native hybrid functional BH&HLYP and Ahlrich's TZVP basis set were employed for the TDDFT calculations. CD spectra were generated using dipole length-computed rotational strengths to which a Gaussian band-shape was applied with 5800 cm^{-1} half-height width (corresponding to 23 at 200 nm). Dipole velocity-computed rotational strengths differed from dipole length values by less than 5% for the main bands.

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