Cytotoxic Diterpenoids from the Soft Coral *Sarcophyton crassocaule*

Cuixian Zhang,[†,‡] Jun Li,[†] Jingyu Su,[†] Yongju Liang,[§] Xiaoping Yang,[§] Kangcheng Zheng,[†] and Longmei Zeng*[,†]

School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou, 510275, People's Republic of China, School of Chinese Medicine, Guangzhou University of Traditional Chinese Medicine, Guangzhou, 510405, People's Republic of China, and Cancer Research Center, Sun Yat-Sen University, Guangzhou, 510060, People's Republic of China

Received November 30, 2005

Five new cembrane diterpenoids, sarcrassins A−E (**1**−**5**) along with a known compound, emblide (**6**), have been isolated from the soft coral *Sarcophyton crassocaule* collected from the Bay of Sanya, Hainan Island, China. The structures of **1**−**5** were determined by spectroscopic methods including 1D and 2D NMR techniques. Their relative configurations were determined by NMR data and NOESY experiments. Compounds **2**, **4**, and **6** exhibited significant cytotoxic activities against KB cell lines with IC_{50} values of 5.0, 4.0, and 5.0 μg/mL, respectively, while compounds **1** and **5** showed moderate cytotoxicity toward KB cell lines with IC_{50} values of 19.0 and 13.0 μg/mL, respectively.

Soft corals (phylum Coelenterata) are a rich source of structurally diverse terpenes. Cembranoid diterpenes are secondary metabolites characteristic of several genera of soft corals, in particular, *Lobophytum*, *Sinularia*, and *Sarcophyton*. Numerous cembranoids have been reported to have antitumor,[1−4] antimicrobial,[5] HIV inhibitory,[6,7] and antiinflammatory activities.[8] As a part of our search for bioactive substances and new compounds from marine organisms, the soft coral *Sarcophyton crassocaule* Mosre (Alcyoniidae) was investigated. The specimen was collected from the Bay of Sanya, Hainan Island, China. Five new cytotoxic cembrane diterpenes, sarcrassins A−E (**1**−**5**) together with a known diterpene, emblide (**6**),[9,10] were isolated. Their structures were determined mainly by spectroscopic methods.

Results and Discussion

The soft coral *S. crassocaule* was extracted with EtOH. The EtOH extract was concentrated, and the residue was partitioned between EtOAc and H_2O. The EtOAc fraction was subjected to CC on Si gel, using petroleum−ether (PE) and EtOAc mixtures of increasing polarity, and finally pure EtOAc, to yield 11 fractions. Fraction 3 eluted with PE−EtOAc (3:1) was further subjected to flash chromatography over Si gel H to give three fractions, 3A, 3B, and 3C. Fraction 3A was further purified on silica gel H eluted with PE−EtOAc (19:1) to afford compound **6**. Fraction 3B was separated by RP HPLC on C18 to yield compounds **4** and **5**. Fraction 3C was divided into two parts: 3C-a and 3C-b. Fraction 3C-a was further purified by RP HPLC on C18 to afford compounds **1** and **2**. Fraction 3C-b was further purified on Si gel H using *n*-hexane−EtOAc (19:1) to afford compound **3**.

Sarcrassin A (**1**) was isolated as a pale yellow, viscous oil. The molecular formula $C_{22}H_{32}O_5$ was determined by HRESIMS *m*/*z* 377.2333 $[M + H]^+$ (calcd 377.2327). Its IR [ν 3010 (m), 1714 (s), 1636 (m), 1263 (s) cm^{-1}], NMR signals [δ_C 168.5 (s), 167.8 (s), 51.7 (q), 51.6 (q) and δ_H 3.76 (s, 3H), 3.77 (s, 3H)], and UV [λ_{max} (log ϵ) 285.3 (4.19), 239.2 (3.89) nm] suggested the presence of two α,β-conjugated methyl esters. NMR signals revealed the presence of three trisubstituted double bonds [δ_C 158.6 (s), 118.9 (d), 136.6 (d), 127.7 (s), 144.4 (d), 130.1 (s) and δ_H 6.23 (1H, d, J = 12.0 Hz), 7.62 (1H, d, J = 12.0 Hz), 6.59 (1H, t, J = 9.0 Hz)]; an epoxymethine [δ_C 62.1 (d), δ_H 2.63 (1H, t, J = 7.5 Hz)]; an epoxydic quaternary carbon [δ_C 66.8 (s)]; an isopropyl group [δ_H 1.00 (3H, d, J = 7.0 Hz), 1.08 (3H, d, J = 7.0 Hz), 2.00 (1H, m)]; and a methyl group [δ_H 1.12 (s, 3H)]. According to the molecular formula and the above functionalities, **1** was suggested to be a cyclic diterpene. The gross structure of **1** was determined by a detailed analysis of 1D and 2D NMR spectra. The HMQC experiment led to the assignment of all the protons to the corresponding carbon atoms (Table 1). $^1H-^1H$ COSY revealed five sequences depicted by the bold lines in Figure 1. The location of the 1,2 double bond was disclosed by the $^1H/^{13}C$ long-range correlations observed in the HMBC spectrum between H-2 and both C-1 and C-15, between H-16 and both C-15 and C-1, and between H-17 and C-15 and C-1. The positions of the 3,4 and 11,12 double bonds as well as the epoxy ring were also established through HMBC correlations of H-3 to C-4 and C-18; H-5 to C-4 and C-18; H-7 to C-8 and C-9; H-9 to C-8 and C-19; H-19 to C-8 and C-7; H-13 to C-12 and C-20; H-11 to C-13 and C-20; and H-14 to C-1 and C-15. By coupling $^1H-^1H$ COSY and HMBC data the isolated spin systems were pieced together (Table 1, Figure 1).

The chemical shift of H-11 at δ_H 6.59 was in favor of a *Z* configuration for the 11,12 double bond.[11,12] The NOE interaction between H-11/H-13b (δ_H 2.42) also supported this deduction. NOE correlations between H-3 (δ_H 7.62)/H_3-21 (δ_H 3.76) and H-2 (δ_H 6.23)/H-14a (δ_H 2.71) coupled with the chemical shift of H-3 at δ_H 7.62 and the *trans* orientation of H-2 and H-3 ($J_{2,3}$ = 12.0 Hz) indicated a *Z* configuration for the 1,2 double bond and an *E* configuration for the 3,4 double bond. In addition, the chemical shift δ_C 16.8 of the 19-CH_3 together with the lack of NOESY correlation between H-7 and 19-CH_3 indicated an *E* configuration[11,13,14] for the trisubstituted epoxide in **1**. That means H-7 and 19-CH_3 are located on opposite faces of the molecule. Thus, the structure of sarcrassin A was established as **1**.

Sarcrassin B (**2**) was isolated as a pale yellow, viscous oil. On the basis of its HRESIMS *m*/*z* 415.2091 $[M + Na]^+$ (calcd 415.2096), along with the ^{13}C NMR spectral data, the molecular formula was established as $C_{22}H_{32}O_6$. The ^1H and ^{13}C NMR spectral data of **2** were very similar to those of **1**. The main differences were the signals at δ_C 158.6 (s), 118.9 (d) and δ_H 6.23 (d, 1H, 12.0 Hz) for the 1,2 double bond in **1**, which were replaced by the signals at δ_C 69.7 (s), 57.5 (d) and δ_H 3.67 (d, 3H, J = 9.5 Hz) for epoxydic function in **2**. This implied that **2** might possess an additional epoxy ring. HMBC correlations between H-15 (δ_H 1.83)/C-1 and C-2 and between H-2 (δ_H 3.67)/C-4, C-3, C-1, C-15, C-16, and C-17 confirmed the location of the additional epoxy ring at C-1 (Table 1).

The stereochemistry of **2** was determined on the basis of the chemical shift and NOESY spectrum. The chemical shift of H-3 at

* Corresponding author. Tel: 86-20-84036447. Fax: 86-20-84112245. E-mail: ceszlm@zsu.edu.cn.

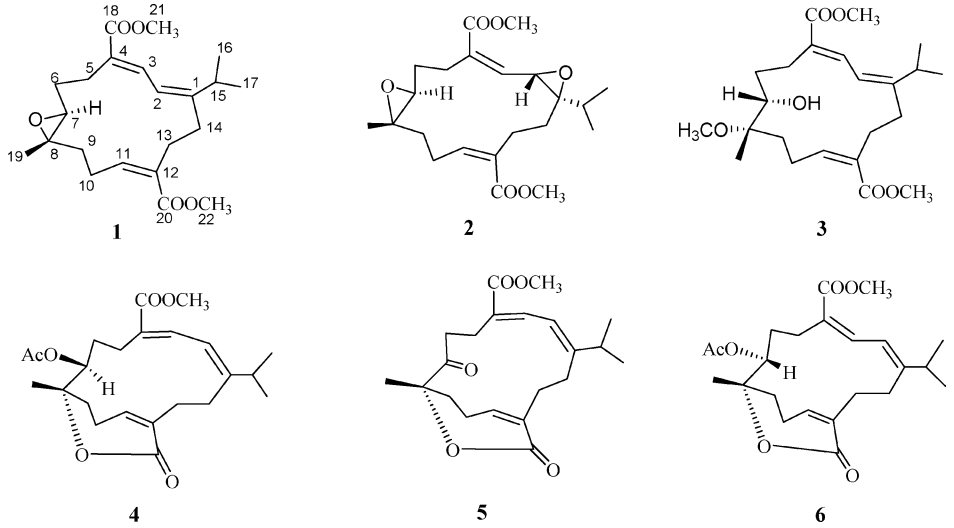
† School of Chemistry and Chemical Engineering, Sun Yat-Sen University.

‡ Guangzhou University of Traditional Chinese Medicine.

§ Cancer Research Center, Sun Yat-Sen University.

10.1021/np050499g CCC: $33.50
Published on Web 09/27/2006

Scheme 1. Cembrane Diterpenoids from the Soft Coral *Sarcophyton crassocaule*



δ_H 6.74 and H-11 at δ_H 6.80 suggested a *Z* configuration for the 3,4 double bond and the 11,12 double bond, respectively.[11,12] The chemical shift at δ_C16.1 of the 19-CH_3 coupled with the lack of NOESY correlation between H-7 and H_3-19 indicated an *E* configuration for the methyl-bearing trisubstituted epoxide.[11,13,14] The NOESY correlation between H-2 and H-14b but lack of correlation between H-2 and H-15 indicated the H-2 and isopropyl group were situated on the opposite side of the second trisubstituted epoxide ring. The relative stereochemistry of four chiral carbons, C-1, C-2, C-7, and C-8, was defined by NOESY, which showed correlations between H-2 and H_3-19, H-15 and H-9b (δ_H 1.05), and H-9b and H-7. This observation implied H-2 and 19-CH_3 were on the same face of the molecule, while H-7 and isopropyl were on the other face. The relative stereochemistry determined by NOESY was supported by a Chem 3D molecular modeling study. There is only one energetically favorable conformation for the large ring, which revealed the distance of these corresponding protons are within 2.46−3.76 after the energy was minimized by MM2 (Figure 2). The structure of sarcrassin B was established as shown in **2**.

Sarcrassin C (**3**) was obtained as a colorless, viscous oil. HRESIMS (*m*/*z* 431.2401 $[M + Na]^+$ (calcd 431.2409)) established the molecular formula for **3** as $C_{23}H_{36}O_6$ and six degrees of unsaturation. UV and NMR spectral data suggested the presence of two α,*β*-conjugated methyl esters [δ_C 170.3 (s), 52.3 (q) and δ_H 3.82 (s); δ_C 167.9 (s), 51.6 (q) and δ_H 3.75 (s) and UV λ_{max} (log ϵ) 287.2 (4.29) nm]; three trisubstituted double bonds [δ_C 158.2 (s), 143.4 (d), 135.3 (d), 130.0 (s), 129.2 (s), 118.5 (d); δ_H 7.50 (d, 1H, J = 12.0 Hz), 6.60 (dd, 1H, J = 2.5, 10.5 Hz), 6.09 (d, 1H, J = 12.0 Hz)]; one secondary hydroxyl carbon [δ_C 73.0 (d), δ_H 3.14 (d, 1H, J =10.0, 1.0 Hz)]; one methoxy group [δ_C 78.3 (s), 50.0 (q) and δ_H 3.28 (s, 3H)]; and one isopropyl group [δ_H 0.98 (d, 3H, J = 7.0 Hz), 0.93 (d, 3H, J = 7.0 Hz), 1.98 (m, 1H)]. According to the molecular formula and the above functionalities, **3** is suggested to be a cyclic diterpene. Analysis of the ^1H and ^{13}C NMR spectral data (Table 1) of **3** clearly showed it to be structurally similar to **1**, the obvious differences being the absence of any epoxy group and the presence of an additional hydroxyl and one methoxyl group in **3**. The positions of the hydroxyl and methoxyl groups were determined through HMBC correlations of H-7 to C-8 and C-9; H-9 to C-8 and C-19; H_3-23 to C-8 and C-7; and H_3-19 to C-8 and C-7.

The chemical shift of H-11 at δ_H 6.60 was in favor of a *Z* configuration for the 11,12 double bond,[11,12] and the NOE interaction between H-11 and H-13 (δ_H 2.54) supported this deduction. In addition, a NOESY correlation was observed between H-3 and H_3-21 (δ_H 3.82) and between H-2 and H-14, which when coupled with the *trans* orientation of H-2 and H-3 ($J_{2,3}$ = 12.0 Hz) deduced an *E* configuration for the 3,4 double bond and a *Z* configuration for the 1,2 double bond. NOE correlations between H-7/H-9a; H-7/19-CH_3; H-9a/19-CH_3 H-11/H-7; H-11/H-9; and H-11/19-CH_3 were observed, implying H-7 and 19-CH_3 were located on the same face of the molecule. This orientation was supported by the very similar $J_{H-7,6}$ coupling constants (10.0, 1.0 Hz, Table 1) of **3** compared with the model compound **7** ($J_{H-7,6}$ = 10.8, 3.7 Hz).[15] It was further confirmed by Chem 3D molecular modeling study. The minimum energy conformation of **3** was calculated, and the dihedral angles between the coupled nuclei H-7 and H_2-6 were acquired from this model. The $J_{H-7,6}$ coupling constants (8.2, 2.4 Hz) calculated from the dihedral angle (H-7−C-7−C-6−H-6a = 5.7°; H-7−C-7−C-6−H-6b = 121.9°, Figure 3) of the model structure of **3** through the Karplus equation fit best with that of the $J_{H-7,6}$ value[16] of **3** (Table 1). Thus, the structure of sarcrassin C was established as **3**.

Sarcrassin D (**4**) was obtained as a yellow, viscous oil. HRESIMS (*m*/*z* 405.2270 $[M + H]^+$ (calcd 405.2277)) established the molecular formula for **4** as $C_{23}H_{32}O_6$. UV and NMR spectral data suggested the presence of an α,*β*-unsaturated methyl ester [δ_C 168.6(s), 51.7 (q) and δ_H 3.71 (3H, s); UV λ_{max} (log ϵ) 282.8 (4.10) nm]; an acetoxy [δ_C 169.8 (s), 21.0 (q) and δ_H 2.03 (3H, s)]; and an α,*β*-unsaturated lactone function [δ_C 141.8 (d), 132.3 (s), 166.5 (s), 82.1 (s); UV λ_{max} (log ϵ) 239.2 (3.87) nm]. Moreover, the NMR data showed signals at δ_C 155.3 (s), 141.8 (d), 137.1 (d), 132.3 (s), 129.0 (s), 119.2 (d) and δ_H 5.92 (1H, d, 9.0 Hz), 7.50 (1H, d, 9.0 Hz), 6.29 (1H, br s), indicating the presence of three trisubstituted double bonds. On the basis of the above finding, **4** was suggested to be a bicyclic diterpenoid. The downfield chemical shift of H-15 (δ_H 2.63) suggested the presence of a double bond at C-1. The connectivities H-2/H-3, H-5/H-6/H-7, H-9/H-10/H-11, H-13/H-14, and H-16/H-15/H-17 were provided by $^1H-^1H$ COSY. Connections of these five isolated spin systems were provided by HMBC experiment.

The *E* geometry of the 1,2 double bond was assigned since NOE interactions between H-2 and both H-15 and H-16 were observed. Furthermore, a NOESY interactions observed between H-3 and H_3-21 (δ_H 3.71), coupled with the *cis* orientation of H-2 and H-3 ($J_{2,3}$ = 9.0 Hz), implied an *E* configuration for the 3,4 double bond. The chemical shift of H-11 at δ_H 6.29 favored a *Z* configuration for the 11,12 double bond,[11,12] and the NOE interaction observed between H-11 and H-13 confirmed this deduction. H_3-19 showed a strong NOE interaction with H_3-23 (δ_H 2.03), revealing that 19-CH_3 and the acetoxy group were located on the same face of the molecule, assigned arbitrarily as a *β*-orientation (Supporting Information). Thus, the structure of sarcrassin D was determined as **4**.

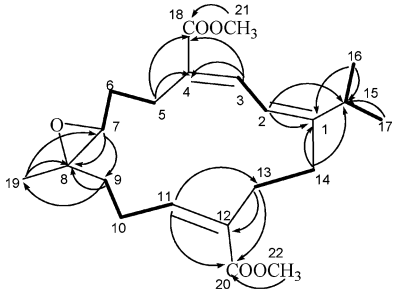
Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds **1**−**6** in $CDCl_3$

	1		**2**		**3**	
position	δ_C mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)	δ_C mult	δ_H (J in Hz)
1	158.6 C		69.7 C		158.2 C	
2	118.9 CH	6.23 d (12.0)	57.5 CH	3.67 d (9.5)	118.5 CH	6.09 d (12.0)
3	136.6 CH	7.62 d (12.0)	136.5 CH	6.74 d (9.5)	135.3 CH	7.50 d (12.0)
4	127.7 C		136.9 C		129.2 C	
5	23.6 CH_2	2.67 t (7.5), Ha 2.55 t (7.5) Hb	23.1 CH_2	2.78 dt (5.5,7.5) Ha 2.46 t (13.0) Hb	21.8 CH_2	2.90 t (4.0) Ha 2.50 m Hb
6	26.8 CH_2	1.91 m Ha 1.70 m Hb	26.7 CH_2	1.55 m	36.1 CH_2	1.8 m Ha 1.31 m Hb
7	62.1 CH	2.63 t (7.5)	62.1 CH	2.70 dd (3.0,5.5)	73.0 CH	3.14 dd (10.0,1.0)
8	66.8 C		61.3 C		78.3 C	
9	36.3 CH_2	1.15 m	38.5 CH_2	2.20 m Ha 1.05 m Hb	29.7 CH_2	1.82 m Ha 1.65 m Hb
10	23.9 CH_2	2.11 m Ha 2.02 m Hb	26.1 CH_2	2.28 m Ha 2.00 m Hb	23.7 CH_2	1.91 m Ha 1.70 m Hb
11	144.4 CH	6.59 (9.0)	140.8 d	6.80 t (8.0)	143.4 CH	6.60 dd (2.5,10.5)
12	130.1 C		134.0 s		130.0 C	
13	26.9 CH_2	2.82 m Ha 2.42 m Hb	21.7 CH_2	2.33 m Ha 2.17 m Hb	27.9 CH_2	2.54 m (2H)
14	28.8 CH_2	2.71 m Ha 2.33 m Hb	29.6 CH_2	2.08 m Ha 1.65 m Hb	29.1 CH_2	2.78 td (3.5) Ha 2.45 m Hb
15	36.7 CH	2.00 m	31.7 CH	1.83 m	37.6 CH	1.98 m
16	22.2 CH_3	1.00 d (7.0)	17.6 CH_3	1.13 d (7.0)	20.4 CH_3	0.98 d (7.0)
17	20.7 CH_3	1.08 d (7.0)	19.0 CH_3	0.97 d (7.0)	21.9 CH_3	0.93 d (7.0)
18	168.5 C		166.8 C		170.3 C	
19	16.8 CH_3	1.12 s	16.1 CH_3	1.32 s	18.7 CH_3	1.28 s
20	167.8 C		167.7 C		167.9 C	
21	51.7 CH_3	3.76 s	52.2 CH_3	3.77 s	52.3 CH_3	3.82 s
22	51.6 CH_3	3.77 s	51.8 CH_3	3.75 s	51.6 CH_3	3.75 s
23					50.0 CH_3	3.28 s

	4		**5**		**6**	
position	δ_C mult	δ_H (J in Hz)	δ_C mult	δ_H (J in Hz)	δ_C mult	δ_H (J in Hz)
1	155.3 C		155.8 C		155.2 C	
2	119.2 CH	5.92 d (9.0)	118.9 CH	5.99 d (9.5)	120.8 CH	7.11 d (12.0)
3	137.1 CH	7.50 d (9.0)	136.3 CH	7.24 d (9.5)	135.4 CH	6.28 dd (12.0,1.0)
4	129.0 C		129.7 C		124.4 C	
5	20.7 CH_2	2.79 dt (5.5,14.5) Ha 2.47 dt (5.5,12.5) Hb	20.2 CH_2	3.02 m Ha 2.55 m Hb	26.2 CH_2	2.48 m
6	26.0 CH_2	2.18 m Ha 1.76 dt (4.0,12.5) Hb	33.9 CH_2	2.51 m Ha 2.32 m Hb	25.2 CH_2	2.18 m Ha 1.86 m Hb
7	68.3 CH	5.23 brd (11.5)	209.8 C		68.2 CH	5.40 dd (9.5,2.5)
8	82.1 C		87.0 C		82.4 C	
9	34.3[a] CH_2	2.21 m Ha 1.87 m Hb	33.8 CH_2	2.60 m Ha 1.90 m Hb	34.5 CH_2	2.08 m Ha 2.02 m Hb
10	27.3[a] CH_2	2.35 m Ha 2.28 m Hb	27.0 CH_2	2.30 m Ha 2.24 m Hb	27.1 CH_2	2.65 t (13.0) Ha 2.30 m Hb
11	141.8 CH	6.29 br s	143.7 CH	6.29 t (5.0)	142.2 CH	6.12 t (4.5)
12	132.3 C		131.8 C		131.9 C	
13	34.2[a] CH_2	3.21 dt (3.5,10.0) Ha 2.04 m Hb	32.3 CH_2	3.10 t (11.0), Ha 2.27 t (11.0) Hb	37.1 CH_2	3.17 t (8.5) Ha 1.89 m Hb
14	33.0[a] CH_2	2.61 m Ha 2.39 m Hb	31.8 CH_2	2.60 m Ha 1.90 m Hb	27.1 CH_2	2.38 m Ha 2.32 m Hb
15	31.1 CH	2.63 m	22.6 CH	2.62 m	35.9 CH	2.38 m
16	19.8 CH_3	1.07 d (7.0)	22.7 CH_3	1.07 d (6.5)	21.9 CH_3	1.13 d (7.0)
17	22.8 CH_3	0.99 d (7.0)	20.5 CH_3	1.12 d (6.5)	22.6 CH_3	1.07 d (7.0)
18	168.6 C		168.8 C		168.2 C	
19	24.0 CH_3	1.36 s	28.8 CH_3	1.44s	23.7 CH_3	1.47 s
20	166.5 C		167.1 C		166.3 C	
21	51.7 CH_3	3.71 s	51.9 CH_3	3.80 s	51.2 CH_3	3.75 s
22	169.8 C				169.6 C	
23	21.0 CH_3	2.03 s			20.9 CH_3	2.03 s

Sarcrassin E (**5**) was obtained as a colorless oil. The molecular formula for **5** was established as $C_{21}H_{28}O_5$ on the basis of FABMS m/z 361 $[M + H]^+$ and the data of DEPT. The UV and NMR spectral data suggested the presence of an α,β-unsaturated methyl ester [δ_C 168.8(s), 51.9 (q) and δ_H 3.80 (3H, s); UV λ_{max} (log ϵ) 284.2 (4.08) nm]; an α,β-unsaturated lactone function [δ_C 143.7-(d), 131.8(s), 167.1(s), 87.0 (s); UV (log ϵ) 237.2 (3.88) nm]; a ketone group [δ_C 209.8 (s)]; and three trisubstituted bonds [δ_C 155.8 (s), 118.9 (d), 136.3 (d), 129.7 (s), 143.7 (d), 131.8 (s); δ_H 5.99 (1H, d, J = 9.5 Hz), 7.24 (1H, d, J = 9.5 Hz), 6.29 (1H, t, J = 5.0 Hz)]. According to the molecular formula and the functionalities mentioned above and in comparison of the ^{13}C NMR data of **5** with those of ketoemblide,[10] the planar structure of **5** was identical with ketoemblide. Examination of their ^{1}H NMR spectral data revealed a crucial difference. H-3 in **5** resonated at δ_H 7.24 as opposed to δ_H 6.97 in ketoemblide, indicating that the 3,4 double bond is *trans* rather than *cis*.[11,12] In addition, the chemical shift of H-15 (δ_H 2.62) in **5** is very close to that of **4** (δ_H 2.63) but quite different from those of **1** (δ_H 2.00) and **3** (δ_H 1.98), implying that the 1,2 and 3,4 double bonds in **5** have the same geometry as that of **4**. Coupled with the *cis* orientation of H-2 and H-3 ($J_{2,3}$ = 9.5 Hz), the *E* geometry of the 1,2 and 3,4 double bonds was assigned for **5**. Thus, the structure of sarcrassin E was determined as **5**.

Compound **6** was obtained as colorless crystals, mp 119−120 °C; $[\alpha]^{20}_D$ +114.7 (c 0.10, $CHCl_3$). By a careful comparison of physical and spectral data, **6** was identified to be emblide, which was previously isolated from the soft coral *Sarcophyton elegans*[9] and *Sarcophyton glaucum*,[10] respectively. In the articles cited, the



1

Figure 1. ^{1}H−^{1}H COSY correlations (bold lines) and key HMBC correlations (arrows) of compound **1**.

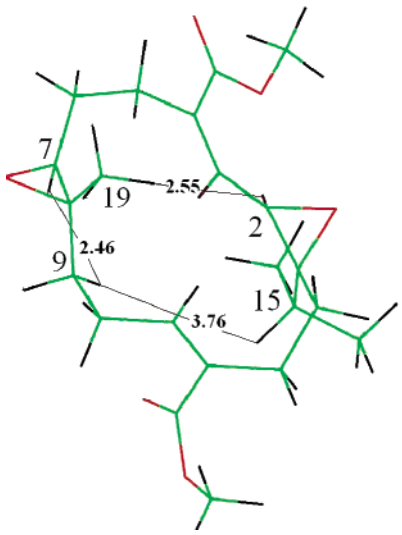


Figure 2. Energy-optimized model of compound **2** (lines: important NOEs).

structure of emblide was solved by X-ray analysis. We report detailed C-H assignments by a combination of DEPT, HMQC, ^{1}H−^{1}H COSY, and HMBC. The ^{1}H and ^{13}C NMR data are included in Table 1.

Compounds **1**−**6** were evaluated for cytotoxic activities against human tumor cell lines KB and MCF. Compounds **2**, **4**, and **6** exhibited significant cytotoxic activity against the KB cell lines with IC_{50} values of 5.0, 4.0, and 5.0 μg/mL, respectively, while compounds **1** and **5** showed moderate cytotoxicity toward the KB cell line with IC_{50} values of 19.0 and 13.0 μg/mL. Compounds **2**, **4**, and **5** showed moderate activities against human MCF tumor cell lines, but compound **1** was weakly active in the MCF cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined using a X-6 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Schmidt + Haensch polaptronic hnqw5 polarimeter. UV spectra were recorded on a Shimadzu UV-2501PC. IR spectra were recorded with an EQUINOX55 (Bruker) spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity INOVA spectrometer at 500 and 125 MHz, respectively, with TMS as internal standard. HRESIMS spectra were obtained with an API QSTAR Pulsari System mass spectrometer. HPLC was conducted on a Perkin-Elmer series 200 using a diode array detector 235C and a reversed-phase Symmetry Prep C18 column (7 μm, 7.8 × 300 mm). Preparative TLC was performed with Si gel H F_{254}. Si gel H (200−300 mesh) was used for flash chromatography. All calculations of the configuration in the energy minima were performed with the Gaussian 98 quantum chemistry program package (revision A.11.4)

Collection. *Sarcophyton crassocaule* Mosre was collected from the Bay of Sanya, Hainan Island, China. A voucher specimen (No. 98-SY-12) is preserved in the Research Centre of Organic Natural Products, Sun Yat-Sen University.

Extraction and Isolation. The soft coral *S. crassocaule* (215 g dried wt) was extracted with EtOH at RT. The EtOH extract was concentrated under vacuum to give a light brown gum (60 g). The residue was partitioned between EtOAc and H_2O. The EtOAc fraction (48 g) was subjected to CC on Si gel, using petroleum ether (PE) and EtOAc mixtures of increasing polarity, and finally pure EtOAc, to yield 11 fractions. Fraction 3 eluted with PE−EtOAc (3:1) and was further subjected to flash chromatography over Si gel H using PE−EtOAc (19:1) to give three fractions, 3A, 3B, and 3C. Fraction 3A was further purified on silica gel H using PE−EtOAc (19:1) to afford compound **6** (600 mg). Fraction 3B containing compounds **4** and **5** was separated by RP HPLC on C18 Si gel and using MeOH−H_2O (17:3) as eluant to yield pure **4** (15 mg) and **5** (6 mg), respectively. Fraction 3C was divided into two parts: fractions 3C-a and 3C-b. Fraction 3C-a was further purified by RP HPLC on C18 Si gel using MeOH−H_2O (17:3) as eluate to afford pure **1** (30 mg) and **2** (10 mg), respectively. Fraction 3C-b was further purified on Si gel H using *n*-hexane−EtOAc (19:1) to afford compound **3** (20 mg).

Sarcrassin A (1): yellow, viscous oil, $[\alpha]^{20}_D$ −6.3 (*c* 0.208, $CHCl_3$); UV($CHCl_3$) λ_{max} (log ϵ) 285.3 (4.19), 239.2 (3.89) nm; IR (KBr) ν_{max}, 3010, 2957, 1714, 1636, 1439, 1384, 1263, 1202, 1125, 1076, 766 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 377.2333 $[M + H]^+$ (calcd for $C_{22}H_{33}O_5$, 377.2327).

Sarcrassin B (2): yellow, viscous oil, $[\alpha]^{20}_D$ +6.9 (*c* 0.13, $CHCl_3$); UV($CHCl_3$) λ_{max} (log ϵ) 238.0 (3.88) nm; IR (KBr) ν_{max}, 2958, 2930, 1717, 1641, 1462, 1439, 1383, 1262, 1199, 1120, 1074, 767 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 415.2091 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_6Na$, 415.2096); FABMS *m*/*z* 393 $[M + H]^+$.

Sarcrassin C (3): colorless oil, $[\alpha]^{20}_D$ −6.5 (*c* 0.280, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 287.2 (4.29) nm; IR (KBr) ν_{max}, 3484, 2956, 2878, 1713, 1637, 1439, 1375, 1259, 1203, 1116, 1069, 763 cm^{-1}; ^{1}H

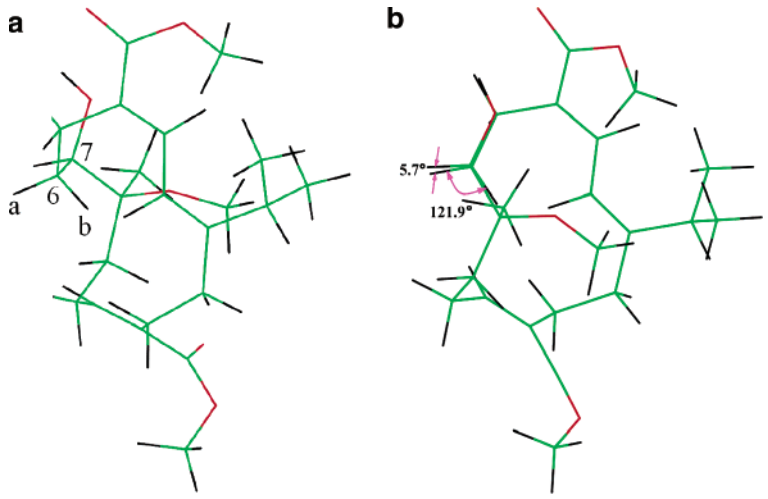


Figure 3. Minimum energy conformation (a) and dihedral angles (H-7−C-7−C-6−H-6a; H-7−C-7−C-6−H-6b) (b) of **3**.

and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 431.2401 $[M + Na]^+$ (calcd for $C_{23}H_{36}O_6Na$, 431.2409); FABMS *m*/*z* 409 $[M + H]^+$.

Sarcrassin D (4): yellow, viscous oil, $[\alpha]^{20}_D$ +187 (*c* 0.108, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 282.8 (4.10), 239.2 (3.87) nm; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 405.2270 $[M + H]^+$ (calcd for $C_{23}H_{33}O_6$, 405.2277).

Sarcrassin E (5): colorless oil, $[\alpha]^{20}_D$ +51.5 (*c* 1.7, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 284.2 (4.08), 241.6 (3.88), 237.2 (3.88) nm; ^1H NMR and ^{13}C NMR, see Table 1; FABMS *m*/*z* 361 $[M + H]^+$.

Emblide (6): colorless crystals, mp 119−120 °C; $[\alpha]^{20}_D$ +114.7 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 287.4 (4.19), 238.0 (3.89) nm; ^1H and ^{13}C NMR, see Table 1; FABMS *m*/*z* 405 $[M + H]^+$.

Cytotoxicity Bioassays. The tetrazolium-based colorimetric assay (MTT assay) was used for the in vitro assay of cytotoxicity to KB and MCF tumor cell lines.

Acknowledgment. This work was supported by a grant from National High Technology Development Project of China (863 Project, No. 2001AA620403) and a grant from the National Natural Science Foundation of China (No. 29932030). We acknowledge Z. C. Tang of Qingdao Institute of Oceanology, Academia Sinica, for identification of the soft coral species.

Supporting Information Available: $^1H-^1H$ COSY correlations and key HMBC correlations of compound **3**; $J_{H\text{-}7,6}$ value of the model compound **7**; $^1H-^1H$ COSY correlations, key HMBC correlations, and NOE interactions of compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Mathee, G. F.; Konig, G. M.; Wright, A. D. *J. Nat. Prod.* **1998**, *61*, 237−240.
(2) Yamda, K.; Ryu, K.; Miyamoto, K.; Komori, T. *Toxicon* **1997**, *60*, 798−801.
(3) Su, J. Y.; Yang, R. L.; Zeng, L. M. *J. Nat. Prod.* **2000**, *63*, 1543−1545.
(4) Wang, S. K.; Duh, C. Y.; Wu, Y. C.; Wang, Y.; Cheng, M. C.; Soong, K.; Fang, L.S. *J. Nat. Prod.* **1992**, *55*, 1430−1435.
(5) Duh, C. Y.; Wang, S. K.; Tseng, H. K.; Sheu, J. Y.; Chiang, M. Y. *J. Nat. Prod.* **1998**, *61*, 844−847.
(6) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 577−586.
(7) Mohammad, A. R.; Kirk, R. G.; Michael, R. B. *J. Nat. Prod.* **2000**, *63*, 531−533.
(8) Norton, R. S.; Kazlauskas, R. *Experientia* **1980**, *36*, 276−278.
(9) Uchio, Y.; Nitya, M.; Twaoawa, T. *Chem. Lett.* **1983**, 613−616.
(10) Toth, J. A.; Burreson, P. J.; Scheuer, P. J.; Moore, J. A.; Clardy, J. *Tetrahedron* **1980**, *36*, 1307−1308.
(11) Kagan, H. B. *Stereochemistry, Foundamentals and Methods*, Vol. 1; Academic Press: New York, 1977; pp 56−57.
(12) Longeon, A.; Lise, M.; Kondracki, B.; Guyot, M. *Tetrahedron Lett.* **2002**, *43*, 5937−5939.
(13) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: Braunschweig, 1969; pp 184−185.
(14) Duh, C. Y.; Wang, S. K.; Chung, S. G.; Chou, G. C.; Dai, C. F. *J. Nat. Prod.* **2000**, *63*, 1634−1637.
(15) Duan, Z. Y.; Zhang, J. Z.; Xu, X. X. *Acta Chem. Sin.* **2004**, *62*, 811−817.
(16) The minimum energy conformation of the C-7 epimer of **3** was also calculated. Its dihedral angles (H-7−C-7−C-6−H-6a = 128.3°; H-7−C-7−C-6−H-6b = −115°) between the coupled nuclei H-7 and H_2-6 were acquired from this model. The coupling constants $J_{H-7,6}$ = 3.4, 1.4 Hz calculated from the dihedral angle mentioned above through the Karplus equation was obviously different from the $J_{H-7,6}$ value of **3** (Table 1).

NP050499G