Cembrane Diterpenoids from the Taiwanese Soft Coral *Sarcophyton stolidotum*

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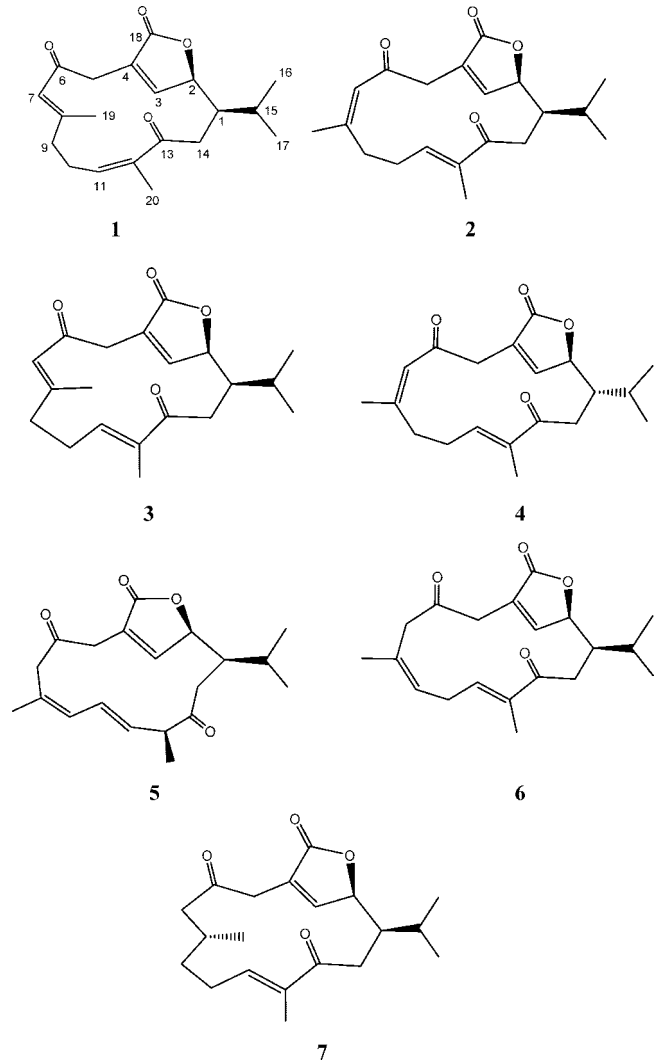
Investigation of an EtOAc-soluble extract of the soft coral *Sarcophyton stolidotum* resulted in the isolation of seven new 14-membered carbocyclic cembranes, sarcostolides A−G (**1**−**7**), together with two known cembrane diterpenes, isosarcophytoxide and isosarcophine. The structural elucidation of these metabolites was determined on the basis of spectroscopic analyses, particularly 2D NMR techniques. Sarcostolide E (**5**) exhibited weak to moderate cytotoxic activity against human WiDr and Daoy tumor cell lines. A biogenetic pathway and relationship for compounds **1**−**7** was also proposed.

Soft corals belonging to the genus *Sarcophyton* (family Alcyoniidae) are a rich source of cembrane diterpenoids with 14-membered rings.[1−5] It is widely believed that these diterpenes function as chemical defense substances against predator or competing reef organisms.[6] Cembranes have been reported to possess multiple biological activities such as cytotoxic,[1−9] neuroprotective,[3] anti-inflammatory,[8] antiarthritic,[8] calcium-antagonistic,[8] and antimicrobial[8] effects. The novelty of biscembrane structures[10] and the potential biological significance of these metabolites have provoked the present chemical investigation of *Sarcophyton stolidotum*, which led to the isolation of seven new oxygenated cembranes, sarcostolides A−G (**1**−**7**), together with two known cembrane diterpenes, isosarcophytoxide[11] and isosarcophine.[12] The structures of **1**−**7** were determined through detailed spectroscopic analyses, mainly 2D NMR techniques (HMQC, ^{1}H−^{1}H-COSY, and HMBC). The new cembranes all possess a γ-lactone and two ketone moieties (C-6 and C-13). The configuration at the stereogenic centers and the geometry of the double bonds were deduced from NOESY and CD spectra. The *in vitro* cytotoxic activity of the new metabolites was evaluated against human HeLa, WiDr, and Daoy tumor cell lines.



Results and Discussion

The HRESIMS (*m*/*z* 353.1727 [M + Na]$^+$) and ^{13}C NMR data of sarcostolide A (**1**) indicated the molecular formula $C_{20}H_{26}O_4$, implying a diterpene compound with eight degrees of unsaturation. The UV band (229 nm) and IR absorptions (1756 and 1684 cm^{-1}) indicated the presence of γ-lactone and conjugated ketone functionalities. The ^{1}H NMR data of **1** (Table 1) indicated the presence of one oxymethine (δ_H 4.97, H-2) and three olefinic methines of three trisubstituted double bonds at δ_H 6.93 (H-3), 6.04 (H-7), and 5.63 (H-11). The ^{13}C NMR data (Table 2) revealed two conjugated carbonyls (δ_C 195.6 and 203) and one lactone carbonyl (δ_C 172.4), suggesting a bicyclic structure. An isopropyl group was demonstrated by two methyl doublets (δ_H 1.02, 0.94) and a methine multiplet (δ_H 2.00) assigned to H-16, H-17, and H-15, respectively, with COSY correlations between H-15/H-1 (δ_H 2.50), H-16, and H-17. The HMBC correlations between H-2/C-1, C-3, and C-4 and H-3/C-2 and C-18, as well as COSY correlations between H-2/H-1 and H-3, proved the presence of an α,β-unsaturated γ-lactone moiety at C-4, C-3, C-2, and C-18 (Figure 1). The HMBC correlations between H_2-5/C-3, C-4, C-18, and C-6; H-7/C-6, C-9, and C-19; and H_3-19/C-7, C-8, and C-9 located an α,β-unsaturated carbonyl at C-6 and a vinylic C-19 methyl. Furthermore, HMBC correlations between H_3-20/C-11, C-12, and C-13; H-11/C-13; and H-14/C-1, C-2, C-13, and C-15, as well as COSY correlations of H_2-9/H_2-10/H-11 and H_2-14/H-1 indicated an α,β-unsaturated carbonyl at C-13 and a vinylic C-20 methyl. The NOESY correlations between H-3/H-7; H-7/H-9; H-11/H_3-19; and H-11/H_3-20 as well as the absence of NOE interaction between H-7/H_3-

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Table 1. ^{1}H NMR Data (300 MHz) for Compounds **1**−**7**[a]

position	**1**[b]	**2**[c]	**3**[c]	**4**[c]	**5**[b]	**6**[c]	**7**[c]
1	2.50 m	2.37 m	2.49 m	2.58 m	2.23 m	2.69 m	2.74 m
2	4.97 d (7.0)	5.04 d (4.4)	5.07 d (5.5)	5.21 br s	4.97 dd (1.0, 7.7)	5.14 d (4.5)	5.16 br s
3	6.93 br s	7.39 br s	7.47 br s	6.91 br s	7.25 d (1.0)	7.36 br s	7.35 br s
5	3.13 d (15.6)	2.96 d (11.8)	3.10 d (15.0)	3.09 d (15.3)	3.15 d (13.6)	3.04 d (11.7)	3.04 d (14.6)
	3.48 d (15.6)	3.37 d (11.8)	3.22 d (15.0)	3.46 d (15.3)	3.61 d (13.6)	3.70 d (11.7)	3.59 d (14.6)
7	6.04 s	6.21 s	5.98 s	5.59 s	2.99 d (17.0)	3.08 d (19.1)	2.42 dd (6.6, 13.0)
					3.84 d (17.0)	3.81 d (19.1)	2.50 dd (6.6, 13.0)
8							1.76 m
9	2.18 m	2.10 m	2.34 m	2.23 m	5.99 d (10.7)	5.68 t (7.4)	1.65 m
	2.45 m	3.68 dt (3.1, 12.0)		2.45 m			
10	2.47 m	2.23 m	2.34 m	2.42 m	6.11 dd (10.7,14.7)	2.66 m	2.22 m
	2.80 m	2.43 m	2.45 m	2.58 m		2.93 m	
11	5.63 dd (5.3, 10.7)	6.10 dd (4.2, 10.4)	6.26 dd (2.8, 8.9)	6.37 dd (4.4, 13.7)	5.37 dd (8.4, 14.7)	5.91 t (5.2)	6.15 dd (5.1, 7.8)
12					3.26 m		
14	2.24 m	1.87 m	2.01 m	2.04 m	2.02 dd (5.0, 15.9)	1.77 m	2.01 m
	2.33 m	2.62 dd (9.3, 16.1)	2.72 m	2.85 m	2.40 dd (5.0, 15.9)	2.45 dd (10.8, 14.8)	2.74 m
15	2.00 m	1.77 m	1.70 m	1.90 m	2.02 m	1.70 m	1.76 m
16	1.02 d (6.9)	0.95 d (6.8)	0.96 d (6.7)	1.10 d (6.7)	0.99 d (6.8)	1.06 d (6.6)	1.07 d (6.5)
17	0.94 d (6.9)	0.90 d (6.8)	0.90 d (6.7)	1.04d (6.7)	0.98 d (6.8)	1.02 d (6.8)	1.10 d (6.5)
19	2.13 s	1.77 s	1.97 s	2.11 s	1.92 s	1.81 s	1.09 d (6.5)
20	1.95 s	1.51 s	1.58 s	1.60 s	1.16 d (6.7)	1.73s	1.77 s

[a] Chemical shifts are in ppm; *J* values (Hz) are in parentheses. [b] Recorded in acetone-d_6. [c] Measured in $CDCl_3$.

Table 2. ^{13}C NMR Data (75 MHz) for Compounds **1**−**7**[a]

	1[b]	**2**[c]	**3**[c]	**4**[c]	**5**[b]	**6**[c]	**7**[c]
1	43.7 CH	46.1 CH	46.1 CH	41.4 CH	44.7 CH	46.4 CH	46.5 CH
2	82.2 CH	84.3 CH	84.4 CH	82.5 CH	82.5 CH	83.6 CH	83.8 CH
3	148.5 CH	150.7 CH	150.9 CH	152.7 CH	150.5 CH	149.1 CH	148.6 CH
4	129.7 C	130.5 C	130.9 C	128.0 C	128.5 C	129.1 C	129.2 C
5	41.4 CH_2	42.3 CH_2	42.7 CH_2	41.2 CH_2	38.8 CH_2	38.5 CH_2	39.1 CH_2
6	195.6 C	196.8 C	195.8 C	194.5 C	204.5 C	205.0 C	205.0 C
7	121.8 CH	126.8 CH	124.4 CH	123.2 CH	46.7 CH_2	47.9 CH_2	49.8 CH_2
8	160.5 C	159.0 C	157.9 C	158.5 C	132.8 C	133.5 C	29.5 CH
9	39.1 CH_2	31.1 CH_2	40.0 CH_2	40.0 CH_2	126.7 CH	124.7 CH	34.0 CH_2
10	28.4 CH_2	28.0 CH_2	25.8 CH_2	26.1 CH_2	129.7 CH	27.3 CH_2	27.3 CH_2
11	138.8 CH	141.0 CH	141.0 CH	141.6 CH	131.5 CH	141.5 CH	143.6 CH
12	135.8 C	139.2 C	139.4 C	138.4 C	49.1 CH	139.6 C	138.9 C
13	203.0 C	200.2 C	200.8 C	199.3 C	210.2 C	199.9 C	200.3 C
14	38.1 CH_2	34.2 CH_2	34.5 CH_2	31.5 CH_2	36.9 CH_2	31.9 CH_2	33.2 CH_2
15	28.9 CH	30.3 CH	30.0 CH	30.9 CH	29.1 CH	29.3 CH	29.6 CH
16	20.4 CH_3	21.4 CH_3	21.3 CH_3	20.7 CH_3	20.2 CH_3	21.0 CH_3	21.0 CH_3
17	18.9 CH_3	21.1 CH_3	21.4 CH_3	20.9 CH_3	18.8 CH_3	21.3 CH_3	21.1 CH_3
18	172.4 C	172.5 C	173.0 C	174.0 C	172.4 C	171.9 C	171.9 C
19	21.9 CH_3	24.7 CH_3	19.0 CH_3	19.4 CH_3	25.3 CH_3	24.3 CH_3	21.7 CH_3
20	21.2 CH_3	11.8 CH_3	11.7 CH_3	11.5 CH_3	15.3 CH_3	11.6 CH_3	11.6 CH_3

[a] Assignments assisted by HMQC and HMBC techniques. [b] Recorded in acetone-d_6. [c] Recorded in $CDCl_3$.

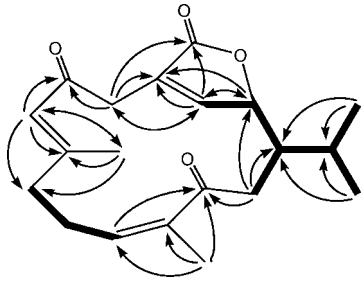

Figure 1. Selected COSY (bold lines) and HMBC (arrows) correlations of **1**.

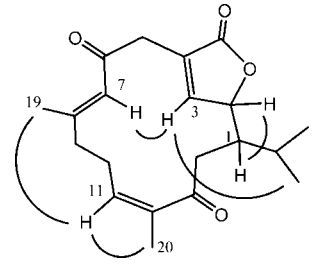

Figure 2. Selected NOESY correlations (curves) for **1**.

19 was consistent with *E*- and *Z*-geometry of the double bonds at C-7 and C-11, respectively. Assuming the β-orientation of the isopropyl group at C-1,[13–15] the NOESY interaction between H-1/H-2 and lack of correlation of C-2/H-15 indicated that both are situated on the same face of the molecule and favor the α-orientation of H-2 (Figure 2).[16] The 14-membered ring is conformationally so flexible that the NOE results need further confirmation. The relative stereochemistry of brassicolide was determined by X-ray analysis.[15] The CD spectrum of **1** exhibited a positive Cotton effect at 250 nm and a negative Cotton effect at 223.8 nm, suggesting the *S*-configuration at C-2. Thus, the structure of sarcostolide A was tentatively assigned as compound **1**.

The HRESIMS spectrum indicated a molecular weight for sarcostolide B (**2**) identical to that of **1**. The UV, IR, and CD (negative Cotton effect at 219.7 nm and positive Cotton effect at 248 nm) spectra of **2** are similar to those of **1**, suggesting close structural analogues. The ^{1}H and ^{13}C NMR data showed that the numbers of methyls, methylenes, methines, and quaternary carbons were the same as those of **1** (Tables 1 and 2). The olefinic proton resonating at δ_H 6.21 was assigned to H-7 by virtue of its HMBC correlations to C-6, C-8, C-9, and C-19, indicating a double bond

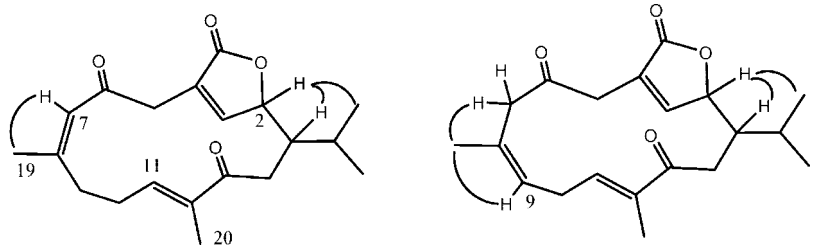

Figure 3. Selected NOESY correlations for **2** and **6**.

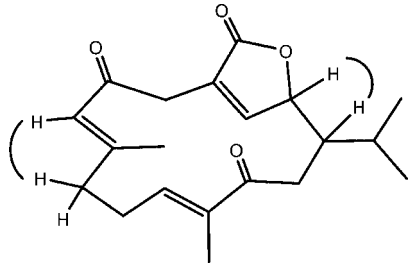

Figure 4. Selected NOESY correlations for **3**.

at C-7. The downfield shift of C-8 pointed to its β-position in the α,β-unsaturated ketone moiety. The chemical shift of the olefinic H-7 and H-11 (δ_H 6.21 and 6.10, respectively) at positions 7 and 11 showed some differences in comparison with those of **1**. The HMBC correlations between δ H-7/C-6, C-8, C-9, and C-19 and H-11/C-10, C-12, and C-20 unveiled unsaturation at C-11, which was further proved by COSY correlations between H_2-9/H_2-10/H-11. The *Z*-geometry of the double bond at C-7 was indicated by NOESY correlation between H-7 and H_3-19, while the *E*-form of the double bond at C-11 was deduced from absence of NOE interaction between H-11 and H-20 (Figure 3). On the basis of the above discussion, it was concluded that **2** was a geometric isomer of **1**, with reversal of the geometry of the double bonds at C-7 and C-11.

The molecular formula of sarcostolide C (**3**) was determined as $C_{20}H_{26}O_4$ with eight degrees of unsaturation, as inferred from HRESIMS and NMR data. The CD spectrum of **3** exhibited a strong negative Cotton effect at 215.4 nm and a strong positive Cotton effect at 245.6 nm, similar to those of **1** and **2**. A detailed comparison of the ^{1}H and ^{13}C NMR, COSY, and HMBC spectra of **3** with those of **1** revealed that they were similar. Significant differences between **3** and **1** were the proton and carbon chemical shifts at position 11. The *E*-geometry of the double bonds at C-7 and C-11 were both proven through absence of NOESY cross-peaks between H-7/H-19 or H-11/H-20 (Figure 4). The NOESY correlation between H-1 and H-2 was consistent with the β-orientation of the isopropyl group. Consequently, the structure of sarcostolide C (**3**) was tentatively determined to be the 7*E* and 11*E* geometric isomer of compounds **2** and **1**, respectively.

Sarcostolide D (**4**) had a molecular formula of $C_{20}H_{26}O_4$ and eight degrees of unsaturation, i.e., the same as **2**. However, the CD spectrum of **4** exhibited a negative Cotton effect at 228.4 nm. The ^{1}H and ^{13}C NMR spectroscopic data were quite similar to those of **2**, showing the same number of methyls, methylenes, methines, and quaternary carbons (Tables 1 and 2). The HMBC correlations between H-7/C-6, C-8, C-9, and C-19 and between H-11/C-10, C-12, and C-20 verified the position of the double bonds at C-7 and C-11. A NOESY correlation was observed between H-7/H-19 but not between H-11/H-20, implying 7*Z*- and 11*E*-configuration of the double bonds, i.e., similar to those of **2** (Figure 5). The absence of NOE correlation between H-2 and H-1 as well as the presence of correlation between H-2/H-15 indicated that H-2 and the isopropyl group were tentatively assigned on the same face of the molecule (α), which is opposite the case in the previously discussed compounds. It was suggested that sarcostolide D (**4**) was a C-1 epimer of **2**.

Sarcostolide E (**5**) had a molecular formula of $C_{20}H_{26}O_4$, the same as that of **1**. The close similarity of IR, UV, and ^{1}H and ^{13}C NMR data of **1** and **5** suggested that **5** was an isomer of **1**. The CD spectrum of **5** showed a positive Cotton effect at 245.7 nm and a negative Cotton effect at 301.9 nm, suggesting that **5** contains a diene system in addition to an α,β-unsaturated γ-lactone moiety. Significant differences in NMR data were observed at C-7 to C-12. In the ^{1}H NMR spectrum, compound **5** showed four olefinic protons (δ_H 5.99, 6.11, 5.37, and 7.25) instead of three in **1**, the most downfield proton apparently belonging to the α,β-unsaturated γ-lactone as in **1**. Compared to **1**, compound **5** lacked one methylene and contained one additional methine (δ_H 3.26, H-12) and a methyl doublet (δ_H 1.16, H-12). The ^{13}C NMR data showed that the carbonyl groups (δ_C 210.2 and 204.5) in **5** were relatively downfield-shifted compared to the corresponding conjugated carbonyls (δ_C 195.6 and 203) in **1**, while the lactone carbonyl (δ_C 172.4) was identical to that of **1**. The HMBC spectrum of **5** showed correlations between H-10/C-8; H_3-19/C-7, C-8, and C-9; and H_2-7/C-6, C-8, C-9, and C-19, indicating 8,9-unsaturation. The HMBC correlation of the methyl doublet H_3-20 to C-11, C-12, and C-13 suggested unsaturation at H-10 and not at C-11 as in the case of **1**. This was confirmed by COSY connectivities between H-12/H-11/H-10/H-9. The large coupling constant, $J_{10,11}$ 14.7 Hz, implied *E*-geometry of the C-10/C-11 double bond, while the *Z*-configuration of the double bond at C-8 was proved by NOESY correlation between H-9 and H-19. The NOESY correlations between H-1/H-2 and H-12 agreed with the β-orientation of the isopropyl group as well as the methyl at C-12 (Figure 6). The collective 2D NMR data confirmed that the only difference between **1** and **5** was the arrangement of double bonds that were positioned at C-8 and C-10 in **5** instead of C-7 and C-11 in **1**.

Sarcostolide F (**6**) was assigned the molecular formula $C_{20}H_{26}O_4$, as derived from HRESIMS, identical to those of **5** and **2**. The CD spectrum of **6** had a negative Cotton effect at 214.7 nm and a positive Cotton effect at 237.7 nm, similar to those of **2**. The ^{1}H and ^{13}C NMR spectra of **6** were similar to those of **5**, suggesting it was closely related to **2** and **5**. Comparison of the NMR spectrum of **6** with those of **5** revealed differences at positions 10, 11, and 12. The ^{1}H NMR spectrum of **6** did not show signals of *trans*-coupled protons (H-10 and H-11), but, instead, an additional methylene appeared at δ_H 2.66, 2.93 (H_2-10), which was coupled to an olefinic proton at δ_H 5.91 (H-11). HMBC correlations were detected between H-20/C-11, C-12, and C-13; H-11/C-9, C-13, and C-20; and H_3-19/C-7 and C-8, thereby locating two double bonds at C-8 and C-11. The COSY correlations between H-9/H_2-10/H-11 confirmed the aforementioned double-bond arrangement. The *Z*-geometry of the double bond at C-8 was proved from the NOESY correlation between H-9 and H-19 (Figure 3). In turn, the double bond at C-11 was assigned an *E*-configuration on the basis of the absence of NOE correlation between H-11 and H-20 as well as the coupling pattern of H-11, which was different from that of **1** (Table 1). The NOE interaction between H-1 and H-2 indicated the same disposition of the isopropyl group as in the case of **2** and **5**.

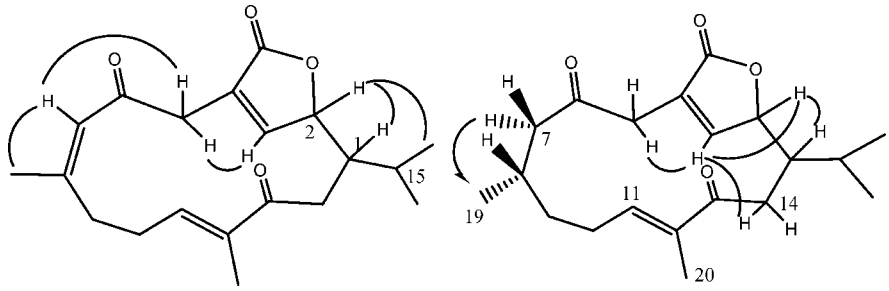

Figure 5. Selected NOESY correlations for **4** and **7**.

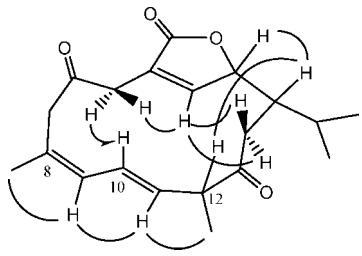

Figure 6. Selected NOESY correlations (curves) for **5**.

Table 3. Results of Cytotoxic Activities (inhibition %) of Compounds **1**−**7** at 10 μg/mL[a]

compound	HeLa[b]	Widr[c]
sarcostolide A (**1**)	22.26 ± 6.84	19.97 ± 5.88
sarcostolide B (**2**)	5.88 ± 2.54	8.31 ± 2.31
sarcostolide C (**3**)	1.65 ± 2.13	19.35 ± 1.99
sarcostolide D (**4**)	11.05 ± 0.50	29.09 ± 3.43
sarcostolide E (**5**)	16.75 ± 0.69	27.48 ± 4.08
sarcostolide F (**6**)	7.32 ± 2.68	29.84 ± 4.18
sarcostolide G (**7**)	18.45 ± 4.14	20.06 ± 4.82

[a] Results of standard mitomycin C: HeLa, 0.4 μg/mL 53.87%; WiDr, 0.4 μg/mL 64.16%. [b] HeLa: human cervical epitheloid carcinoma. [c] WiDr: human colon adenocarcinoma.

Sarcostolide G (**7**) had a molecular formula of $C_{20}H_{28}O_4$ with seven degrees of unsaturation, as inferred from HRESIMS. The ^{1}H NMR spectrum disclosed two olefinic protons (δ_H 6.15 and 7.35); the latter was assigned to H-3 as deduced from its downfield shift as well as its HMBC correlations to the lactone carbonyl (δ_C 171.9). The ^{13}C NMR data revealed the presence of five methylenes, two olefinic methines (δ_C 148.6, 143.6; C-3, C-11), and three aliphatic methines (δ_C 46.5, 29.5, 29.6) in addition to one oxymethine (δ_C 83.8, C-2). Both the olefinic proton (δ_H 6.15) and H-1 (δ_H 2.74) correlated to the carbonyl at δ_C 200.3 (C-13), suggesting a double bond at C-11, which was confirmed by HMBC correlations between H-11/C-20 and H-20/C-11, C-12, and C-13. One of the upfield-shifted methines (δ_C 29.6) was assigned to C-15 on the basis of COSY correlation between H-1 and H-15 and HMBC correlation of H-15/C-1. The other methine (δ_C 29.5) was assigned to C-8 as a result of COSY correlation between H-8/H_2-7, H_2-9, and H_3-19. The COSY connectivities between H-11/H_2-10/H_2-9/H-8/H_2-7, as well as HMBC correlations of H_3-19/C-7, and C-9 and H_2-5, H_2-7/unconjugated carbonyl at δ_C 205.0 (C-6), were consistent with the aliphatic nature of C-8 and carbon sequence from C-11 to C-5. The geometry of the double bond at C-11 was proved to be *E*-form from the absence of NOESY correlation between H-11 and H-20. The NOESY correlations between H-1/H-2 and H_β-14 and between H_3-19/H_β-7 suggested the β-orientation of the isopropyl group as well as the α-orientation of CH_3-19 (Figure 5).

The *in vitro* cytotoxic activity of the new metabolites was tested against human HeLa (cervical epitheloid carcinoma) and WiDr (colon adenocarcinoma) tumor cell lines. As illustrated in Table 3, sarcostolides D (**4**), E (**5**), and F (**6**) exhibited very weak cytotoxic activity against HeLa and WiDr cell lines. Compounds **1**, **2**, and **4**−**7** were further tested against human Daoy (medulloblastoma) tumor cells. Sarcostolide E (**5**) showed moderate cytotoxicity against Daoy with ED_{50} at 5.5 μg/mL, while others were inactive.

A plausible biogenetic pathway and relationship for **1**−**7** was proposed as illustrated in Scheme 1 (Supporting Information). The usual cyclization of GPP coupled with oxidation and lactonization may produce compound **3**. Sarcostolides A, B, and D−G (**1**, **2**, **4**−**7**) may be further transformed from sacostolide C (**3**) via isomerization and migration of double bonds.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. UV and IR spectra were taken with a Hitachi U-3210 and a Horiba FT-720 spectrophotometer, respectively. CD spectra were measured on a JASCO J-720 spectrophotometer. The ^{1}H and ^{13}C NMR spectra as well as 2D NMR spectra (COSY, HMQC, HMBC, and NOESY) were recorded in $CDCl_3$ using Bruker DRX NMR spectrometers operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C using the solvent peak as internal standard. Low-resolution EIMS spectra were recorded on a VG Quattro 5022 mass spectrometer. High-resolution ESIMS spectra were measured on a JEOL HX 110 mass spectrometer. LiChrospher Si 60 (5 μm, 250−10, Merck) and LiChrospher 100 RP-18e (5 μm, 250−10, Merck) were used for NP-HPLC and RP-HPLC (Hitachi), respectively.

Animal Material. *Sarcophyton stolidotum* Quoy and Gaimard was collected near Kenting, off the southern coast of Taiwan, in March 2005. The soft coral was identified by Prof. Keryea Soong (Institute of Marine Biology, National Sun Yat-sen University). A voucher specimen (LG-14) was deposited at the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The animal material (wet, 0.6 kg) was chopped and extracted with CH_2Cl_2/MeOH (1:1, 5 L) at room temperature and then was concentrated under reduced pressure to afford a crude extract (6.0 g). The latter was partitioned between H_2O/EtOAc (1:1) to yield an EtOAc-soluble portion (3.6 g), which was chromatographed on a Si gel column (70 g) and eluted gradiently with *n*-hexane/EtOAc to give 21 fractions. Fraction 2 (180 mg) was further chromatographed on a Si gel column (10 g) using *n*-hexane/EtOAc (10:1 to 1:1) to yield isosarcophytoxide and isosarcophine. Fraction 14 (67 mg) was further purified with an RP-HPLC column (MeOH/H_2O, 65:35) to give **1** (11.7 mg) and **5** (4.5 mg). Separation of fraction 15 (180 mg) by HPLC used the latter solvent system, yielding **6** (36.4 mg). Fraction 16 was separated by NP-HPLC using *n*-hexane/EtOAc (2:1) to afford **2** (30 mg). Fraction 17 was also chromatographed by RP-HPLC (MeOH/H_2O, 65:35) to give **7** (3.4 mg) and **4** (4.3 mg). Compound **3** (45 mg) was obtained by precipitation from fraction 18.

Sarcostolide A (1): colorless oil; $[\alpha]_D$ +60 (*c* 1.17, CH_2Cl_2); IR (neat) ν_{max} 1757, 1684, 1615, 1372, 1069, 735, 734 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 229 (3.44) nm; CD (*c* 0.1, MeOH) (mdeg) 223.8 (−55.3), 232.7 (+59.1), 250 (+129) nm; ^{1}H NMR (300 Mz), Table 1; ^{13}C NMR (75 MHz), Table 2; HRESIMS *m*/*z* 353.1727 [M + Na]$^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1729).

Sarcostolide B (2): colorless oil; $[\alpha]_D$ +364 (*c* 2.44, CH_2Cl_2); IR (neat) ν_{max} 1756, 1715, 1666, 1371, 1264, 1094, 1032, 735 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 228 (3.41) nm; CD (*c* 0.1, MeOH) (mdeg) 219.7 (−109.4), 248 (+196.7) nm; ^{1}H NMR (300 Mz), Table 1; ^{13}C NMR (75 MHz), Table 2; HRESIMS *m*/*z* 353.1727 [M + Na]$^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1729).

Figure 7. Computer-generated perspective models for **5** and **7** using MM2 force field calculation.

Sarcostolide C (3): colorless oil; $[\alpha]_D$ +173 (*c* 1.46, CH_2Cl_2); IR (neat) ν_{max} 1755, 1675, 1658, 1620, 1424, 1362, 1329, 1090, 1033, 644 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 228 (3.38) nm; CD (*c* 0.1, MeOH) (mdeg) 215.4 (−169.6), 245.6 (+273) nm; ^1H NMR (300 Mz), Table 1; ^{13}C NMR (75 MHz), Table 2; HRESIMS *m/z* 353.1727 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1729).

Sarcostolide D (4): colorless oil; $[\alpha]_D$ +4.7 (*c* 0.43, CH_2Cl_2); IR (neat) ν_{max} 1755, 1679, 1664, 1613, 1450, 1370, 1220, 1071, 734 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 229 (3.39) nm; CD (*c* 0.1, MeOH) (mdeg) 220.8 (−31.7), 228.4 (−33.3) nm; ^1H NMR (300 Mz), Table 1; ^{13}C NMR (75 MHz), Table 2; HRESIMS *m/z* 331.1895 $[M + H]^+$ (calcd for $C_{20}H_{27}O_4$, 331.1381).

Sarcostolide E (5): colorless oil; $[\alpha]_D$ −20 (*c* 0.45, CH_2Cl_2); IR (neat) ν_{max} 1758, 1709, 1420, 1372, 1324, 1201, 1050, 974, 735 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 228 (3.41) nm; CD (*c* 0.1, MeOH) (mdeg) 211.7 (−29.1), 245.7 (+87.1), 301.9 (−44.7) nm; ^1H NMR (300 Mz), Table 1; ^{13}C NMR (75 MHz), Table 2; HRESIMS *m/z* 353.1718 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1729).

Sarcostolide F (6): colorless oil; $[\alpha]_D$ +157 (*c* 3.67, CH_2Cl_2); IR (neat) ν_{max} 1756, 1716, 1661, 1427, 1370, 1315, 1205, 1126, 1095, 1063, 950, 850, 735 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 225 (3.55) nm; CD (*c* 0.1, MeOH) (mdeg) 214.7 (−80.4), 237.7 (+100.1) nm; ^1H NMR (300 Mz), Table 1; ^{13}C NMR (75 MHz), Table 2; HRESIMS *m/z* 353.1727 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1729).

Sarcostolide G (7): colorless oil; $[\alpha]_D$ +274 (*c* 0.34, CH_2Cl_2); IR (neat) ν_{max} 1758, 1667, 1616, 1449, 1372, 1330, 1271, 1237, 1204, 1113, 799, 734 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 228 (3.41) nm; CD (*c* 0.1, MeOH) (mdeg) 214.7 (−5.7), 254.9 (−15.1) nm; ^1H NMR (300 Mz), Table 1; ^{13}C NMR (75 MHz), Table 2; HRESIMS *m/z* 333.2066 $[M + H]^+$ (calcd for $C_{20}H_{29}O_4$, 333.2067).

Cytotoxicity Assay. Cytotoxicity was determined against HeLa (human cervical epitheloid carcinoma) and WiDr (human colon adenocarcinoma) tumor cells and was based on the MTT assay method. The assay procedure was carried out as previously described.[17] ED_{50} values were defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance.

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Supporting Information Available: Plausible biogenetic pathway and relationship for **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) König, G. M.; Wright, A. D. *J. Nat. Prod.* **1998**, *61*, 494–496.
(2) Duh, C.-Y.; Wang, S.-K.; Chung, S.-G.; Chou, G.-C.; Dai, C.-F. *J. Nat. Prod.* **2000**, *63*, 1634–1637.
(3) Pham, N. B.; Butler, M. S.; Quinn, R. J. *J. Nat. Prod.* **2002**, *65*, 1147–1150, and references therein.
(4) Gross, H.; Kehraus, S.; Nett, M.; König, G. M.; Beil, W.; Wright, A. D. *Org. Biomol. Chem.* **2003**, *1*, 944–949.
(5) Gross, H.; Wright, A. D.; Beil, W.; König, G. M. *Org. Biomol. Chem.* **2004**, *2*, 1133–1138.
(6) Coll, J. C.; Price, I. R.; Konig, G. M.; Bowden, B. F. *Mar. Biol.* **1987**, *96*, 129–135.
(7) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259–302.
(8) Jia, R.; Guo, Y.-W.; Mollo, E.; Gavagnin, M.; Cimino, G. *J. Nat. Prod.* **2006**, *69*, 819–822.
(9) Badria, F. A.; Guirguis, A. N.; Perovic, S.; Steffen, R.; Müller, W. E. G.; Schröder, H. C. *Toxicology* **1998**, *131*, 133–143.
(10) Yan, X.-H.; Gavagnin, M.; Cimino, G.; Guo, Y.-W. *Tetrahedron Lett.* **2007**, *48*, 5313–5316.
(11) Bowden, B. F.; Coll, J. C. *Heterocycles.* **1989**, *28*, 669–672.
(12) Kusumi, T.; Yamada, K.; Ishitsuka, M. O.; Fujita, Y.; Kakisawa, H. *Chem. Lett.* **1990**, *8*, 1315–1318.
(13) Williams, D.; Andersen, R. J.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1987**, *52*, 332–335.
(14) Marrero, J.; Rodriguez, A. D.; Baran, P.; Raptis, R. G. *J. Org. Chem.* **2003**, *68*, 4977–4979.
(15) Duh, C.-Y.; Wang, S.-K.; Weng, Y.-L.; Chiang, M. Y.; Dai, C.-F. *J. Nat. Prod.* **1999**, *62*, 1518–1521.
(16) Jia, R.; Guo, Y.-W.; Mollo, E.; Cimino, G. *Helv. Chim. Acta* **2005**, *88*, 1028–1033.
(17) Shen, Y. C.; Lin, Y. S.; Cheng, Y. B.; Cheng, K. C.; Khalil, A. T.; Kuo, Y. H.; Chien, C. T.; Lin, Y. C. *Tetrahedron* **2005**, *61*, 1345–1352.

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