Cytotoxic Cembrenolides and Steroids from the Formosan Soft Coral *Sarcophyton crassocaule*

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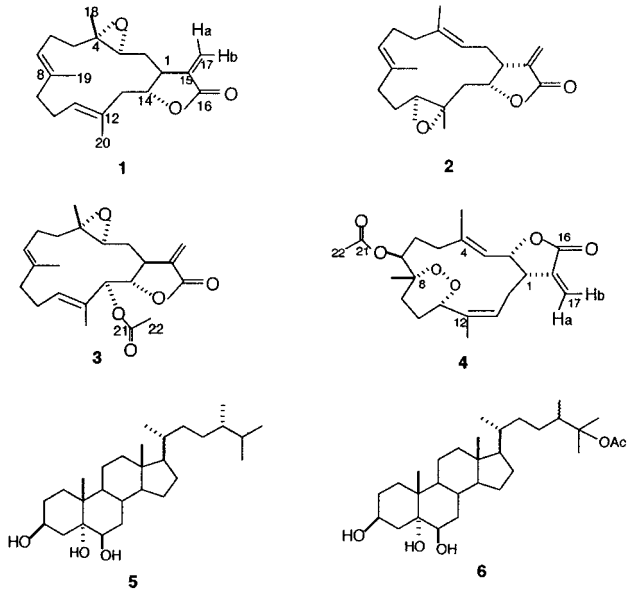
Received May 10, 2000

Three new cytotoxic cembrenolide diterpenes, sarcocrassolide (**1**), crassolide (**2**), and 13-acetoxysarcocrassolide (**3**); a known cytotoxic cembrenolide, denticulatolide (**4**); and two cytotoxic steroids, (24*S*)-24-methylcholestane-3β,5α,6β-triol (**5**) and 24ζ-methylcholestane-3β,5α,6β,25-tetraol-25-monoacetate (**6**), have been isolated from the Formosan soft coral *Sarcophyton crassocaule*. The structures of compounds **1**−**6** were determined by 1D and 2D spectral analysis.

As part of our search for bioactive substances from marine organisms, the soft coral *Sarcophyton crassocaule* Moser (Alcyoniidae) was studied based on the CH_2Cl_2 extracts showing significant cytotoxicity in A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[1,2] Bioassay-guided fractionations resulted in the isolation of three new cytotoxic cembrenolide diterpenes, sarcocrassolide (**1**), crassolide (**2**), and 13-acetoxysarcocrassolide (**3**); a known cytotoxic cembrenolide, denticulatolide (**4**); and two cytotoxic steroids, (24*S*)-24-methylcholestane-3β,5α,6β-triol (**5**) and 24ζ-methylcholestane-3β,5α,6β,25-tetraol-25-monoacetate (**6**).



Results and Discussion

Methylene chloride extraction of the freeze-dried animals followed by Si gel column chromatography and Sephadex LH-20 column chromatography yielded **1** as a colorless oil.

Table 1. NMR Data of **1**[a]

position	δ_H mult. (Hz)	δ_C mult.	COSY	NOESY
1	2.87 ddd (10.5, 3.6, 3.0)	41.2 d[c]	2α, 2β, 14, 17a, 17b	2α
2α	1.72 ddd (13.7. 7.2, 3.0)	33.4 t	1, 3	1
2β	1.87 ddd (13.0, 10.5, 3.9)		1, 3	14
3	2.68 dd (7.2, 3.9)[b]	60.5 d	2β	
4		60.5 s		
5	1.36 m	37.5 t	18	
	2.06 m			
6	2.26 m	23.7 t	7	
7	5.03 t (6.3)	123.3 d	6, 19	
8		134.9 s		
9	2.10 m	38.5 t	10	
10	2.08 m	24.9 t	9	
11	5.14 t (5.4)	130.0 d	20	10
12		129.3 s		
13α	2.12 m	44.7 t	13β, 14	
13β	2.56 br d (1.5, 13)		13α, 14	
14	4.53 ddd (9.6, 3.6, 3.5)	81.3 d	1, 13α	13β
15		138.9 s		
16		169.7 s		
17a	5.66 d (1.8)	122.9 t	1	1, 17b
17b	6.30 (1.8)		1	17a
18	1.29 s	17.2 q	5	3
19	1.61 s	16.3 q		
20	1.68 s	17.3 q	11	14

[a] Spectra recorded in $CDCl_3$. [b] *J* values (in Hz) in parentheses. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

HRFABMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **1** as $C_{20}H_{28}O_3$. Thus, seven degrees of unsaturation were determined for **1**. The IR spectrum of **1** indicated the presence of an α,β-unsaturated carbonyl group (ν_{max} 1752 cm^{-1}). A strong UV absorption at λ_{max} 235 nm suggested the presence of an α,β-unsaturated carbonyl. ^1H and ^{13}C NMR spectral data (Table 1) showed that **1** contained an α-methylene γ-lactone (C-1, -14, -15, -16, -17), a methyl-bearing trisubstituted epoxide (C-3, -4, -18), two isolated methyl-bearing trisubstituted double bonds (C-7, -8, -19, -11, -12, -20), and six methylene carbons (C-2, -5, -6, -9, -10, -13). These data suggested that **1** possessed a cembrane skeleton with functionalities of an α-methylene γ-lactone, a methyl-bearing trisubstituted epoxide, and two isolated methyl-bearing trisubstituted double bonds. From the HMBC experiment on **1**, the positioning of α-methylene γ-lactone at C-15 (α), C-1 (β), C-17 (β'), C-14 (γ), and C-16 (carbonyl carbon) was confirmed by long-range correlations between H-1 and C-2, C-3, C-15, C-16, and C-17; H-14 and C-2 and C-16; and H-17 and C-1, C-15, and C-16. The

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10.1021/np0002381 CCC: $19.00
Published on Web 11/03/2000

Table 2. NMR Data of **2**[a]

position	δ_H mult. (Hz)	δ_C mult.	COSY	NOESY
1	2.75 m	44.3 d[c]	2, 17a, 17b	13β, 14, 17a
2	2.29 m	28.2 t	1	
3	4.98 m	119.5 d	2, 18	17a
4		138.4 s		
5	2.05 m	36.4 t		
	2.22 m			
6	1.33 m	23.8 t	7	
	2.05 m			
7	4.98 m	125.5 d	6, 19	
8		133.2 s		
9	2.27 m	38.9 t	10	
10	2.11 m	24.2 t	11	
	2.39 m			
11	2.61 dd (9.9, 3.0)[b]	61.4 d	10	13β, 20
12		60.0 s		
13α	1.31 m	44.9 t	13β, 14	
13β	2.25 m		13α, 14	1, 11, 14, 20
14	4.25 ddd (8.7, 7.0, 1.8)	80.0 d	1, 13α	1, 13β, 20
			13β	
15		137.9 s		
16		170.2 s		
17a	5.59 d (3.0)	121.8 t	1	1, 3
17b	6.33 d (2.7)		1	
18	1.50 s	14.7 q	3	
19	1.65 s	14.7 q	7	
20	1.40 s	17.3 q		11, 14

[a] Spectra recorded in $CDCl_3$. [b] *J* values (in Hz) in parentheses. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

Table 3. NMR Data of **3**[a]

position	δ_H mult. (Hz)	δ_C mult.	COSY	NOESY
1	3.03 ddd (11.4, 2.5, 2.4)[b]	36.9 d[c]	2α, 2β, 14, 17b	2α
2α	1.67 m	34.6 t	2β	1, 3
2β	1.81 m		2α	
3	2.61 dd (6.9, 3.6)	60.0 d	2α, 2b	1, 2α, 5α, 18
4		60.4 s		
5α	1.30 m	37.3 t	5β	3, 18
5β	2.03 m		5α	7
6	2.05 m	23.4 t	7	
7	4.98 t (6.2)	122.9 d	6	
8		134.9 s		
9	2.17 m	37.8 t		
	2.33 m			
10	2.38 m	24.7 t	11	
11	5.29 m	128.9 d	10	
12		128.4 s		
13	5.35 d (2.3)	77.2 d	14	14, 20
14	4.57 dd (2.4, 2.3)	80.8 d	13	13
15		139.5 s		
16		169.0 s		
17a	5.60 d (1.5)	120.9 t	1	1, 17b
17b	6.21 (2.1)			17a
18	1.26 s	17.3 q		5α
19	1.60 s	16.7 q		
20	1.69 s	14.6 q		
OAc	1.98 s	20.6 q		
		169.2 s		

[a] Spectra recorded in $CDCl_3$. [b] *J* values (in Hz) in parentheses. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

methyl-bearing trisubstituted epoxide at C-3 (methine), C-4 (quaternary carbon) was deduced from HMBC correlations between H-2 and C-1, C-3, C-4, C-14, and C-15; H-3 and C-2 and C-4; H-5 and C-3, C-4, C-6, C-7, and C-18; H-18 and C-3, C-4, and C-5; and H-6 and C-4 and C-8. The vinyl methyl group attached at C-8 was confirmed by HMBC correlations between H-19 and C-7, C-8, and C-9; H-7 and C-6, C-9, and C-19; and H-9 and C-7, C-8, C-10, and C-19. The other vinyl methyl group attached at C-12 was revealed by the HMBC correlations between H-20 and C-11, C-12, and C-13; H-11and C-20; and H-13 and C-1, C-12, C-14, and C-20. The relative stereochemistry at C-1, C-3, C-4, and C-14 was deduced from NOESY correlations between H-1 and H-2α, H-2α and H-3, and H-3 and H-18. The $J_{1,14}$ coupling constant (3.6 Hz) confirmed the relative stereochemistry at H-1/H-14 as trans.[3] The *E*-type stereochemistry of two trisubstituted carbon−carbon double bonds and the trisubstituted epoxide in **1** was indicated by the chemical shift observed for the methyl signals in the ^{13}C NMR spectrum (δ 17.2, 16.3, 17.3). The methyl group of the cis double bond resonated downfield from 20 ppm.

Compound **2** was isolated as a colorless oil, whose molecular formula, $C_{20}H_{28}O_3$, was revealed by HRFABMS. The IR spectrum of **2** exhibited the presence of an α,β-unsaturated carbonyl group (ν_{max} 1755 cm^{-1}). A strong UV absorption at λ_{max} 233 nm suggested the presence of an α,β-unsaturated carbonyl functionality. ^1H and ^{13}C NMR spectral data (Table 2) showed that **2** contained an α-methylene γ-lactone (C-1, -14, -15, -16, -17), a methyl-bearing trisubstituted epoxide (C-11, -12, -20), two isolated methyl-bearing trisubstituted double bonds (C-3, -4, -18, -7, -8, -19), and six methylene carbons (C-2, -5, -6, -9, -10, -13). These data suggested that **2** possessed a cembrane skeleton with functionalities of an α-methylene γ-lactone, a methyl-bearing trisubstituted epoxide, and two isolated methyl-bearing trisubstituted double bonds. From the HMBC experiment on **2**, the positioning of α-methylene γ-lactone at C-15 (α), C-1 (β), C-17 (β'), C-14 (γ), and C-16 (carbonyl carbon) was confirmed by long-range correlations between H-14 and C-1, C-2, C-12 and C-16 and between H-17 and C-1, C-15, and C-16. The methyl-bearing trisubstituted epoxide at C-11 (methine), C-12 (quaternary carbon) was deduced from HMBC correlations between H-11 and C-10, C-12, and C-13; H-13 and C-11, C-12, C-14, and C-20; H-20 and C-11, C-12 and C-13; and H-10 and C-12. The vinyl methyl group attached at C-8 was confirmed by HMBC correlations between H-19 and C-8, H-7 and C-6 and C-19; and H-9 and C-10 and C-11. The other vinyl methyl group attached at C-4 was revealed by the HMBC correlations between H-18 and C-4, H-3 and C-1 and C-2, and H-5 and C-18. The relative stereochemistry at C-11, C-12, C-14, and C-1 was deduced from NOESY correlations between H-1 and H-14, H-14 and H-13β, H-13β and H-20, and H-11 and H-20. Irradiation of the proton at δ 2.75 (H-1) simplified the signal at δ 4.25 (H-14) to a doublet of doublets (J = 8.7, 1.8 Hz). These findings revealed that the coupling constant between H-1 and H-14 was 7.0 Hz, which was consistent with their being cisoid.[4] The *E*-type stereochemistry of two trisubstituted carbon−carbon double bonds and a trisubstituted epoxide in **2** was decided on the basis of ^{13}C NMR chemical shifts of the methyl carbons (14−17 ppm).

Compound **3** was isolated as a colorless oil. HRFABMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **3** as $C_{22}H_{30}O_5$. The IR spectrum of **3** exhibited absortions consistent with the presence of an α,β-unsaturated γ-lactone carbonyl group (ν_{max} 1752 cm^{-1}) and an ester carbonyl (ν_{max} 1726 cm^{-1}). A strong UV absorption at λ_{max} 234 nm suggested the presence of an α,β-unsaturated carbonyl. ^1H and ^{13}C NMR spectral data (Table 3) showed that **1** contained an α-methylene γ-lactone (C-1, -14, -15, -16, -17), a methyl-bearing trisubstituted epoxide (C-3, -4, -18), two isolated methyl-bearing trisubstituted double bonds (C-7, -8, -19, -11, -12, -20), a secondary acetoxy at C-13, and five methylene carbons (C-2, -5, -6, -9, -10). These data suggested that **3** possessed a cembrane skeleton with functionalities of an α-methylene γ-lactone, a methyl-

Table 4. NMR Data of **4**[a]

position	δ_H mult. (Hz)	δ_C mult.	HMBC	COSY	NOESY
1	2.84 m	44.5 d[c]	2, 3	2	2, 14β, 18
2	5.20 dd (7.0, 6.5)[b]	79.3 d	1, 3	3	1, 3, 18
3	5.50 br d (7.0)	123.3 d	5, 18		2, 5α, 17
4		138.4 s			
5α	2.00 m	35.5 t	6, 7, 18	5β, 6β	3, 7
5β	2.05 m		6, 7	5α, 6α	14β
6α	1.72 m	26.0 t	5, 7	5β, 6β, 7	
6β	1.96 m		5, 7	5α, 6α,	19
7	5.78 d (9.5)	70.1 d	5, 6, 19, 21	6β	3, 5α, 6α, 10α, 14β
8		80.0 s			
9α	1.76 m	31.0 t	10	9β, 10β	
9β	1.57 m		7, 8	9β, 10α	11
10α	2.38 m	24.7 t	11	10β, 11	7
10β			8, 9	10α	11
11	4.33 dd (12.5, 2.5)	84.2 d	12, 13, 20	10α	9β, 10β, 20
12		135.5 s			
13	5.36 dd (9.6,1.8)	125.4 d	11, 20	14β, 20	14α, 20
14α	1.85 br d (14.0)	28.0 t	1, 12, 13	14β	13, 20
14β	3.09 ddd (14.0, 11.1, 2.5)		1, 2, 12, 13, 15	13, 14α	1, 5β, 7, 18
15		140.2 s			
16		170.3 s			
17a	5.63 s	121.1 t	1, 16	17b	13, 17b
17b	6.23 s		1, 15, 16	17a	17a
18	1.68 s	16.4 q	3, 4	3	1, 2, 14β
19	1.09 s	19.5 q	7, 8, 9		6β
20	1.77 br s	24.3 q	11, 12, 13	13	11, 13
21		170.5 s			
22	2.11 s	21.0 q	21		

[a] Spectra recorded in $CDCl_3$. [b] *J* values (in Hz) in parentheses. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

bearing trisubstituted epoxide, a secondary acetoxy, and two isolated methyl-bearing trisubstituted double bonds. From the HMBC experiment on **3**, the positioning of α-methylene γ-lactone at C-15 (α), C-1 (β), C-17 (β'), C-14 (γ), and C-16 (carbonyl carbon) was confirmed by long-range correlations between H-1 and C-2, C-3, C-14, C-15, C-16, and C-17; H-14 and C-1, C-2, C-13, C-15, and C-16; and H-17 and C-1, C-15, and C-16. The methyl-bearing trisubstituted epoxide at C-3 (methine), C-4 (quaternary carbon) was deduced from HMBC correlations between H-3 and C-2, H-5 and C-3 and C-4, and H-18 and C-3 and C-4. The secondary acetoxy at C-13 was deduced from HMBC corelations between H-13 and C-1, C-11, C-12, and C-14; H-14 and C-1, C-2, C-13, C-15, and C-16; and H-1 and C-2, C-3, C-14, C-15, C-16, and C-17.The vinyl methyl group attached at C-8 was confirmed by HMBC correlations between H-19 and C-7, C-8, and C-9; H-7 and C-5, C-6, and C-9; and H-9 and C-8, C-10, and C-11. The other vinyl methyl group attached at C-12 was revealed by the HMBC correlations between H-20 and C-11 and C-12; H-11and C-9, C-12, C-13, and C-20; and H-13 and C-1, C-11, C-12, and C-14. The relative stereochemistry at C-1, C-3, C-4, C-13, and C-14 was deduced from NOESY correlations between H-1 and H-2α, H-2α and H-3, H-3 and H-18, and H-14 and H-13. The $J_{1,14}$ coupling constant (2.4 Hz) confirmed the relative stereochemistry at H-1/H-14 as trans.[3] Finally, in the ^{13}C NMR spectrum of **3** the methyl resonances occur upfield of 20 ppm, indicating the *E*-configuration of the trisubstituted double bonds and trisubstituted epoxide.

The identity of compounds **4**−**6** was established by direct comparison of the $[\alpha]^{20}_D$, IR, EIMS, ^1H NMR, and ^{13}C NMR data with literature data.[5−7] The H and ^{13}C NMR chemical shifts of **4**, which were not thoroughly assigned in previous literature,[5] were assigned by COSY, NOESY, HMQC, and HMBC experiments (Table 4) in this investigation.

The cytotoxicity of compounds **1**−**6** is shown in Table 5. Compounds **1**−**6** exhibited cytotoxicity against the P-388 cell line with ED_{50} values of 0.16, 0.14, 0.38, 0.15, 0.14, and 3.96 μgmL^{-1}, respectively.

Table 5. Cytotoxicitiy[a] of **1**−**6** ($n = 8$)

	ED_{50} (μg /mL)			
compd	A549	HT-29	KB	P-388
1	8.31	7.55	9.15	0.16
2	4.29	4.97	8.35	0.14
3	4.66	5.67	7.39	0.38
4	6.46	5.78	6.29	0.15
5	6.26	8.35	5.38	0.14
6	22.43	4.32	26.84	3.96

[a] For significant activity of pure compounds, an ED_{50} of $\leq$ 4.0 μg/mL is required.[1]

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are reported uncorrected. Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26-30 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Bruker AMX 400 NMR spectrometer at 400 and 100.6 MHz, respectively, in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 ev. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *S. crassocaule* (Alcyoniidae) was collected at Green Island, off Taiwan, in September 1998, at a depth of 10 m and was stored for 3 days in a freezer until extraction. A voucher specimen, NSUGN-1026, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *S. crassocaule* were freeze-dried to give 980 g of a solid, which was extracted with CH_2Cl_2 (2.0 L × 3). After removal of solvent in vacuo, the residue (70 g) was chromatographed over Si gel 60 using CH_2Cl_2 and CH_2Cl_2−MeOH mixtures of increasing

polarity. Elution by CH_2Cl_2 afforded fractions containing compounds **1** and **2**. Elution by CH_2Cl_2−MeOH (49:1) afforded fractions containing compounds **3** and **4**. Elution by CH_2Cl_2−MeOH (17:3) afforded fractions containing steroids **5** and **6**. Compounds **1** and **2** were obtained by Sephadex LH-20 column chromatography, by eluting with *n*-hexane and *n*-hexane−CH_2Cl_2 (2:1), respectively. Compounds **3** and **4** were obtained by Sephadex LH-20 column chromatography, by eluting with *n*-hexane and *n*-hexane−CH_2Cl_2 (1:1), respectively. Compounds **5** and **6** were obtained by Si gel column chromatography, by eluting with *n*-hexane−EtOAc (3:17) and *n*-hexane−EtOAc (1:4), respectively.

Sarcocrassolide (1): colorless oil (27 mg) $[\alpha]^{25}_D$ +7.8° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max}(log ϵ) 235 (4.2) nm; IR (KBr) ν_{max} 1763, 1668 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 316 $[M]^+$ (0.7), 271 (0.7), 255 (1), 245 (1), 233 (2), 219 (2), 201 (2), 189 (3), 68 (100); HRFABMS *m*/*z* 316.2031 (calcd for $C_{20}H_{28}O_3$, 316.2039).

Crassolide (2): colorless oil (54 mg); $[\alpha]^{25}_D$ +127.1° (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max}(log ϵ) 233 (4.3) nm; IR (KBr) ν_{max} 1755 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 316 $[M]^+$ (0.3), 301 (0.4), 283 (0.5), 264 (3), 203 (2), 189 (4); 177 (5), 149 (26), 121 (22), 43 (100); HRFABMS *m*/*z* 316.2035 (calcd for $C_{20}H_{28}O_3$, 316.2039).

13-Acetoxysarcocrassolide (3): colorless oil (200 mg); $[\alpha]^{25}_D$ +56.6° (*c* 0.19, MeOH); UV (MeOH) λ_{max}(log ϵ) 232 (4.2) nm; IR (KBr) ν_{max} 1752, 1726 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; EIMS *m*/*z* 374 $[M]^+$ (0.5), 332 (1), 315 (5), 297 (6), 231 (8), 187 (12), 181 (24), 175 (17), 162 (38), 147 (27), 135 (27), 119 (27), 108 (42), 93 (63), 55 (100); HREIMS $[M]^+$ *m*/*z* 374.2120 (calcd for $C_{22}H_{30}O_5$, 374.2124).

Cytotoxicity Testing. KB and P-388 cells were kindly supplied by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[8]

Acknowledgment. We thank Prof. J. M. Pezzuto, Program for Collaborative Research in Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, for the provision of P-388 and KB cell lines. This work was supported by a grant from the National Science Council of Taiwan awarded to C.-Y.D.

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NP0002381