Diterpenoids, Norditerpenoids, and Secosteroids from the Formosan Soft Coral *Cespitularia hypotentaculata*

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Four new cespitularane diterpenes, cesputularins I−L (**1**−**4**), two new norverticillane norditerpenes, cespitularins M and N (**5** and **6**), two new verticillane diterpenes, cespitularins O and P (**7** and **8**), a new norditerpene, cespitularin Q (**9**) (having a novel carbon skeleton), a new xenicane diterpene, cespitolide (**10**), and two new secosteroids, 3β,11-dihydroxy-5β,6β-epoxy-9,11-secocholestan-9-one (**11**) and 3β,11-dihydroxy-5β,6β-epoxy-9,11-secogorgostan-9-one (**12**), were isolated from the methylene chloride solubles of the Formosan soft coral *Cespitularia hypotentaculata* Roxas. The structures were elucidated by extensive spectroscopic analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

Previous study of the soft coral *Cespitularia hypotentaculata* Roxas (Xeniidae) led to the isolation of diterpenoids with verticillane, norverticillane, and cespitularane skeletons.[1] In our continued study of the bioactive metabolites from the Formosan soft coral *C. hypotentaculata*, four new cespitularane diterpenes, cesputularins I−L (**1**−**4**), two new norverticillane norditerpenes, cespitularins M and N (**5** and **6**), two new verticillane diterpenes, cespitularins O and P (**7** and **8**), a new norditerpene, cespitularin Q (**9**) (having a novel carbon skeleton), a new xenicane diterpene, cespitolide (**10**), and two new secosteroids, 3β,11-dihydroxy-5β,6β-epoxy-9,11-secocholestan-9-one (**11**) and 3β,11-hydroxy-5β,6β-epoxy-9,11-secogorgostan-9-one (**12**), were isolated from the methylene chloride solubles.

Results and Discussion

Cespitularin I (**1**) was isolated as a colorless, amorphous solid. HREIMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **1** as $C_{20}H_{26}O_3$, indicating eight degrees of unsaturation. The ^{13}C NMR singlet at δ 133.2 and a doublet at δ 128.8 that was correlated in the HMBC experiment (Table S1) with the ^{1}H NMR (Table 1) signal at δ 4.86 (br d, J = 9.3 Hz, 1H) together with the vinylic methyl signals at δ 1.80 (s, 3H) in the ^{1}H NMR spectrum and at δ 15.7 (CH_3) in the ^{13}C NMR spectrum (Table 2) were assigned to an *E*-trisubstituted double bond bearing a methyl group. HMQC correlation of δ_H 4.92 (s, 2H) with δ_C 112.6 (CH_2) as well as HMBC correlation of δ_H 4.92 (s, 2H) with δ_C 144.8 (qC), 30.9 (CH_2), and 45.6 (CH_2) indicated that **1** contained an exocyclic methylene. The geminal methyls at δ_H 1.42 (s, 3H) and 1.26 (s, 3H) showed HMBC correlations with δ_C 35.1 (qC), 42.2 (CH), and 165.9 (qC), indicating **1** contained a *gem*-dimethyl-bearing quaternary carbon that was adjacent to a methine carbon and a quaternary olefinic carbon. HMBC correlations (Table S1) between H-9 and C-10, C-11, C-12, and C-20 indicated the presence of an α,β-unsaturated cyclopentadione (δ_H 3.25 s, δ_C 200.9 qC, 196.6 qC, 165.9 qC, 155.8 qC, 63.0 CH). The NMR features (Tables 1 and 2; Tables S2 and S3) of **1** were similar to those of cespitularin F (**13**),[1] except that the resonances for the secondary hydroxyl at C-20 were replaced by those of a keto group. HMBC correlations (Table S1) from H-9 to C-7, C-8, C-10, C-11, C-12, and C-20 further supported these assignments. Therefore, the structure of cespitularin I was defined as **1**. The relative stereochemistry of **1**

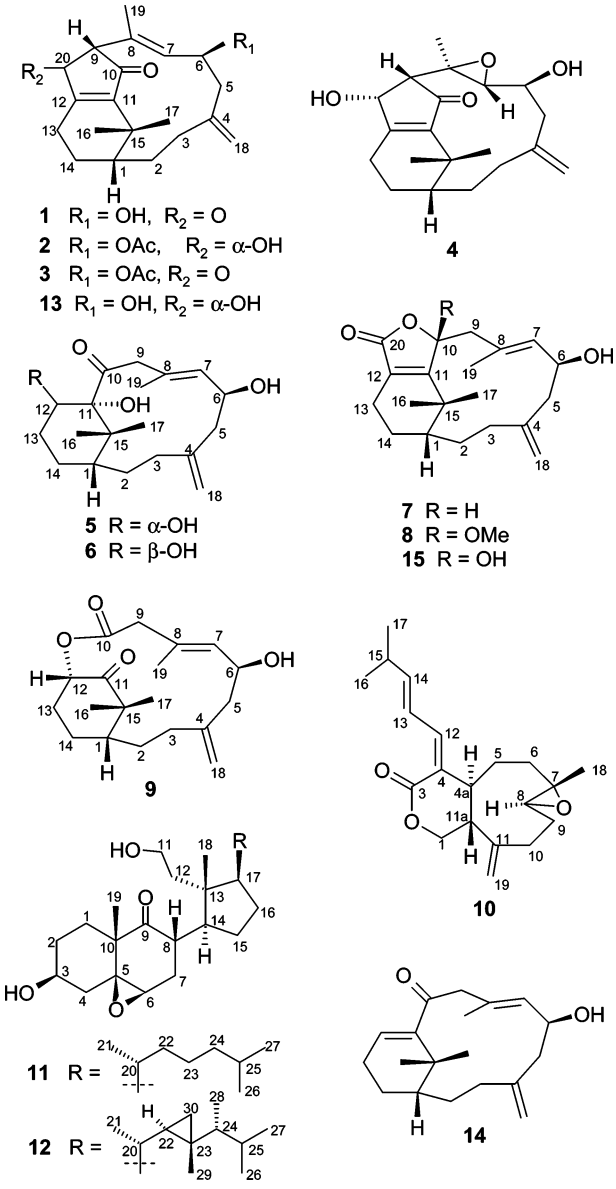

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Table 1. ^{1}H NMR Spectral Data[a] (300 MHz) of **1**−**4** in $CDCl_3$

	1	2	3	4
1	1.84 m	1.68 m	1.84 m	1.69 m
2	1.68 m, 2.08 m	1.98 m, 2.41 m	1.66 m, 2.16 m	1.78 m, 2.00 m
3	1.87 m, 2.10 m	1.89 m, 2.29 m	1.90 m, 2.08 m	2.20 m
5	2.53 m, 1.95 m	2.62 m, 1.94 m	1.95 m, 2.60 m	2.51 m, 2.06 m
6	5.53 m	5.54 m	5.55 m	3.63 m
7	4.86 d (9.3)	5.01 d (9.8)[b]	4.93 d (9.9)	2.59 d (9.5)
9	3.25 s	3.28 d (5.7)	3.29 s	3.08 d (5.3)
13	2.32 m, 2.57 m	2.31 m, 2.62 m	2.32 m, 2.58 m	1.95 m, 2.31 m
14α	2.33 m	2.10 m	2.34 m	2.76 m
14*β*	1.88 m	1.69 m	1.90 m	2.31 m
16	1.26 s	1.14 s	1.28 s	1.12 s
17	1.42 s	1.38 s	1.46 s	1.29 s
18	4.92 s	4.82 s, 4.87 s	4.85 s, 4.87 s	4.97 s, 5.00 s
19	1.80 s	1.87 s	1.86 s	1.58 s
20		4.90 d (6.0)		4.97 m
OAc		1.99 s	2.02 s	

[a] Recorded in $CDCl_3$ (assigned by COSY, HSQC, NOESY, and HMBC experiments). [b] *J* values (in Hz) in parentheses.

Table 2. ^{13}C NMR Spectral Data[a] (75 MHz) of **1**−**9** in $CDCl_3$

	1	2	3	4	5	6	7	8	9
1	42.2	42.0	42.3	41.7	45.5	46.9	42.7	44.1	43.1
2	28.2	31.1	28.2	28.8	38.9	39.3	18.4	17.8	29.1
3	30.9	22.8	30.9	31.3	31.6	33.0	31.9	29.8	33.6
4	144.8	144.9	143.8	145.1	145.0	145.0	146.3	146.0	146.3
5	45.6	42.6	42.4	41.9	47.0	46.9	43.7	43.8	44.4
6	66.9	70.0	69.8	68.2	70.0	70.3	68.3	68.5	68.8
7	133.2	127.8	128.3	62.9	131.9	132.9	134.6	135.9	134.4
8	128.8	136.2	131.4	60.0	133.7	133.4	133.1	131.4	130.5
9	63.0	62.9	63.1	58.7	47.8	49.5	42.4	46.7	46.4
10	200.9	203.8	200.9	201.5	212.2	208.2	82.1	110.1	169.7
11	165.9	146.8	166.0	147.0	89.1	92.3	170.1	168.4	211.2
12	155.8	168.9	155.8	169.2	77.5	74.9	127.1	130.2	72.3
13	19.4	22.2	19.4	22.0	34.5	24.4	31.5	32.2	26.4
14	22.2	28.6	22.3	22.4	24.8	24.6	24.0	24.6	20.5
15	35.1	33.8	35.2	34.0	45.6	46.9	36.8	37.6	47.7
16	30.2	30.8	30.2	30.1	27.3	25.9	34.3	33.8	27.8
17	23.8	23.9	23.8	23.8	27.0	26.6	25.0	24.5	23.2
18	112.6	112.7	113.0	112.8	115.6	115.5	113.5	114.7	112.3
19	15.7	19.8	15.9	21.4	18.7	17.6	18.3	17.2	17.0
20	196.6	73.9	196.4	71.2			172.5	170.5	
OMe								50.9	
OAc		21.3	21.3						
		170.7	170.7						

[a] Assigned by DEPT, COSY, HSQC, and HMBC experiments.

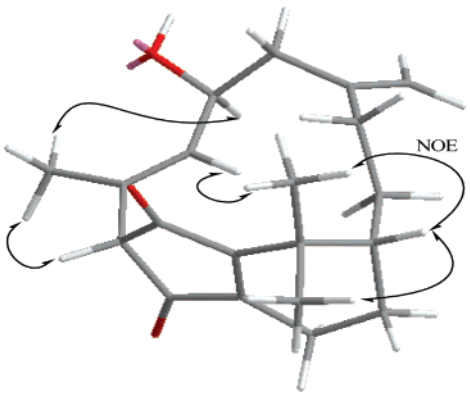

Figure 1. Computer-generated perspective model for **1** using MM2 force field calculations.

was deduced from a 2D NOESY experiment (Table 4), which indicated that Me-16, Me-17, H-7, and H-1 are on one side of the molecule, while Me-19 and H-6 are on the opposite side of the molecule. The computer-modeled structure of **1** was generated by CS Chem 3D version 9.0 using MM2 force field calculations for energy minimization, as shown in Figure 1. The result was consistent with the stereochemistry of **1** as established by NOESY experiments.

Cespitularin J (**2**) gave a formula of $C_{22}H_{30}O_4$, from the interpretation of its HRESIMS and ^{13}C NMR spectroscopic data. The NMR features (Tables 1 and 2; Tables S2 and S3) of **2** were analogous to those of **1** with the exception that the resonances for the secondary hydroxyl at C-6 were replaced by those of an acetoxy group and the resonances for the keto at C-20 were replaced by a secondary hydroxyl. COSY correlations from H-6 to H-5 and H-7 and HMBC correlations (Table S1) from H-6 to C-5, C-7, C-8, and the carbonyl carbon of OAc-6 as well as HMBC correlations (Table S1) from H-9 to C-7, C-8, C-10, C-11, C-12, and C-20 suggested these assignments. The structure of cespitularin J was thus determined as **2**.

Cespitularin K (**3**) was assigned a molecular formula of $C_{22}H_{28}O_4$, as indicated by HRESIMS and ^{13}C NMR spectroscopic data. The NMR features (Tables 1 and 2; Tables S2 and S3) of **3** closely resembled those of **1**, except that the resonances for the secondary hydroxyl at C-6 were replaced by those of a secondary acetoxyl. COSY correlations from H-6 to H-5 and H-7 and HMBC correlations (Table S1) from H-6 to C-4, C-5, C-7, and the carbonyl carbon of OAc-6 revealed the location of a ketone at C-6. The relative stereochemistry of **1** was deduced from a 2D NOESY experiment (Table 4), which indicated that Me-16, Me-17, H-7, and H-1 are on one side of the molecule, while Me-19 and H-6 are on the opposite side of the molecule. The structure of cespitularin K was thus formulated as **3**.

Cespitularin L (**4**) was shown to have a molecular formula of $C_{20}H_{28}O_4$ from its HRESIMS and ^{13}C NMR spectroscopic data. The NMR features (Tables 1 and 2; Tables S2 and S3) of **4** exhibited some similarity to those of cespitularin F[1] expect that the resonances for a trisubstituted olefin of cespitularin F (**13**) were replaced by those of a trisubstituted epoxy in **4**. COSY correlation from H-7 to H-6 and HMBC correlations (Table S1) from H_3-19 to C-7, C-8, and C-9 and from H-6 to C-4, C-7, and C-8 clearly positioned the trisubstituted epoxy. NOESY correlation (Table 4) from H-7 to H_3-17 indicated that Me-17 and H-7 are on the same side of the molecule, while Me-19 and H-6 are on the opposite side of the molecule. The computer-modeled structure of **4** was generated by CS Chem 3D version 9.0 using MM2 force field calculations for energy minimization, as shown in Figure 2. The result was consistent with the stereochemistry of **4** as established by NOESY experiments. Therefore, the structure of cespitularin L was characterized as **4**.

Cespitularin M (**5**) analyzed for $C_{19}H_{30}O_4$ from HRESIMS and ^{13}C NMR spectroscopic data, indicating five degrees of unsaturation. The IR spectrum of **1** indicated the presence of hydroxyl group(s) (ν_{max} 3558 cm^{-1}) and a carbonyl group (ν_{max} 1742 cm^{-1}). The ^{13}C NMR signals at δ 133.7 (qC), 131.9 (CH), and 18.7 (CH_3) (Table 2) together with the ^{1}H NMR signals at δ 5.58 (br d, J = 8.7 Hz, 1H) and 1.89 (s, 3H) (Table 3) were assigned to an *E*-trisubstituted

Table 3. ^{1}H NMR Spectral Data[a] (300 MHz) of **5**−**9** in $CDCl_3$

	5	6	7	8	9
1	1.58 m	1.49 m	1.78 m	1.63 m	1.42 m
2	2.06 m, 2.23 m	1.95 m, 2.18 m	2.31 m	2.33 m	1.65 m
3	1.09 m, 1.51 m	1.15 m, 1.83 m	2.06 m, 2.38 m	1.51 m	1.88 m, 2.00 m
5	2.67 m, 2.15 m	2.14 m 2.73 d (12.6)	2.36 m	2.43 m	2.29 m, 2.49 m
6	4.52 m	4.55 m	4.38 m	4.37 m	4.48 m
7	5.58 d (8.7)[b]	5.56 d (9.6)	5.40 d (7.5)	5.50 d (7.5)	5.47 d (8.4)
9	2.60 d (14.1) 4.01 d (14.1)	2.84 d (13.5) 3.88 d (13.5)	2.94 br d (14.7) 2.72 dd (14.7, 3.3)	3.02 d (14.4) 2.84 d (14.4)	3.23 d (13.5) 2.86 d (13.5)
10			5.24 br s		
12	4.10 m	4.39 m			5.07 m
13	2.51 m, 2.75 m	1.92 m, 2.08 m	1.65 m	2.16 m, 2.29 m	1.92 m, 2.41 m
14α	1.64 m	1.44 m	1.79 m	1.74 m	1.68 m
14*β*	2.22 m	2.18 m	2.25 m	2.26 m	1.98 m
16	1.32 s	0.78 s	1.21 s	1.26 s	1.17 s
17	1.55 s	1.47 s	1.39 s	1.44 s	1.23 s
18	4.87 s, 4.92 s	4.92 s, 4.95 s	4.81 s, 4.85 s	4.84 s, 4.85 s	4.86 s, 4.88 s
19	1.89 s	1.87 s	1.60 s	1.58 s	1.74 s
OH-12		4.39 s			
OMe				3.28 s	

[a] Assigned by COSY, HSQC, NOESY, and HMBC experiments. [b] *J* values (in Hz) in parenthese.

Table 4. Selected NOE Correlations of **1**−**9**

H	1	2	3	4	5	6	7	8	9
1	H-16, 17	H-16, 17	H-16, 17	H-16, 17	H-16, 17	H-16, 17	H-16, 17	H-16, 17	H-16, 17
2	H-3	H-3, 18	H-3	H-3	H-3, 18	H-3, 18	H-3, 17, 18	H-3, 17, 18	H-3, 18
3	H-2, 18	H-2, 7	H-2, 18	H-2, 17	H-1, 18	H-1, 18	H-18	H-18	H-18
5	H-6, 18	H-6, 18	H-6, 18	H-6, 18	H-6, 18	H-6, 18	H-6, 18	H-6, 18	H-6, 18
6	H-5, 7, 19	H-5, 7, 19	H-5, 7, 19	H-5, 19	H-5, 7, 19	H-5, 7, 19	H-5, 7, 19	H-5, 7, 19	H-5, 7, 19
7	H-5, 6, 17	H-5, 6, 17	H-5, 6, 17	H-5, 17	H-6, 9*β*, 17	H-6, 9*β*, 17	H-6, 9*β*, 17	H-6, 9*β*, 17	H-6, 9α
9α	H-19, 20	H-19	H-19	H-19, 20	H-19	H-19	H-19	H-19	H-7
9*β*					H-7, 17	H-7, 17	H-10, 7, 17	H-10, 7, 17	H-19
10							H-9, 16, 17		
12					H-13*β*, 16	H-13*β*			H-13*β*, 16
13	H-14	H-14	H-14	H-14	H-12, 14	H-12, 14	H-14	H-14	H-12, 14
14α	H-13	H-13	H-13	H-13	H-13	H-13	H-13	H-13	H-13
14*β*	H-16	H-16	H-16	H-16	H-16	H-16	H-16	H-16	H-16
16	H-1, 14*β*	H-1, 14*β*	H-1, 14*β*	H-1, 14*β*	H-1, 12, 14*β*	H-1, 14*β* OH-12	H-1, 10, 14*β*	H-1, 14*β* OMe-10	H-1, 12, 13*β*, 14*β*
17	H-1, 7	H-1, 7	H-1, 7	H-1, 7	H-1, 7, 9*β*	H-1, 7, 9*β*	H-1, 7, 10, 9*β*	H-1, 7, 9*β*	H-1
18	H-2, 5	H-2, 5	H-2, 5	H-2, 5	H-3, 5	H-3, 5	H-3, 5	H-3, 5	H-2, 3, 5
19	H-6, 9	H-6, 9	H-6, 9	H-6, 9	H-6, 9α	H-6, 9α	H-6, 9α	H-6, 9α	H-6, 9*β*
20	H-9			H-9					
OH-12						H-16			
OMe								H-16	

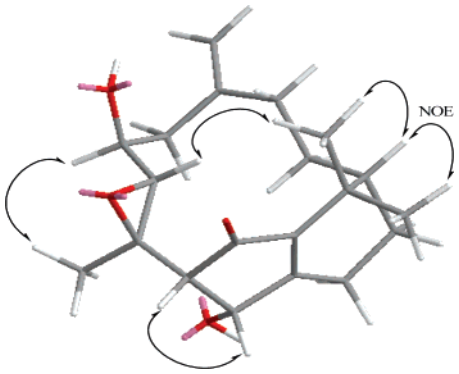

Figure 2. Computer-generated perspective model for **4** using MM2 force field calculations.

double bond bearing a methyl group. HMQC correlation of δ_H 4.92 (s, 1H) and 4.87 (s, 1H) with δ_C 115.6 (t) as well as HMBC correlation of δ_H 4.92 (s, 1H) and 4.87 (s, 1H) with δ_C 145.0 (qC), 31.6 (CH_2), and 47.0 (CH_2) indicated that **5** contained an exocyclic methylene. The geminal methyls at δ_H 1.32 (s, 3H) and 1.55 (s, 3H) showed HMBC correlations with δ_C 45.6 (qC), 89.1 (qC), and 45.5 (CH), indicating **5** contained a *gem*-dimethyl-bearing quaternary carbon adjacent to a methine carbon and an oxygenated quaternary carbon. The NMR features (Tables 2 and 3; Tables S4 and S5) of **5** resembled those of cespitularin E (**14**)[1] with the exception that the resonances for the double bond at C-11/C-12 were replaced by those of a tertiary hydroxyl at C-11 and a secondary hydroxyl at C-12. COSY correlation from H-12 to H-13 as well as HMBC correlations (Table S1) from H_3-16, 17 to C-15 and C-1 and from H-12 to C-10, C-11, C-13, C-14, and C-15 suggested these assignments. According to an analysis of NOESY correlations (Table 4) from H_3-16 to H-12 and from H_3-17 to H-7 as well as consideration of the Dreiding model of compound **5**, OH-11 and OH-12 should be located on the same side of the molecule. The structure of cespitularin M was thus assigned as **5**.

Cespitularin N (**6**) was assigned the molecular formula $C_{19}H_{30}O_4$, as shown by its HRESIMS and ^{13}C NMR spectroscopic data. The NMR features (Tables 2 and 3; Tables S4 and S5) of **6** were analogous to those of **5** except for the resonances in the vicinity of C-12. NOESY correlation (Table 4) from H_3-16 to OH-12 indicated that Me-16 and OH-12 occurred on the same side of the molecule. The structure of cespitularin N was thus established as **6**.

The molecular formula of cespitularin O (**7**) was obtained from HRESIMS and ^{13}C NMR spectroscopic data indicating seven degrees of unsaturation. The UV and IR spectra of **7** showed the presence of α,*β*-unsaturated lactone and hydroxy functionalities, respectively. The ^{1} H NMR spectrum (Table 3) of **7** exhibited

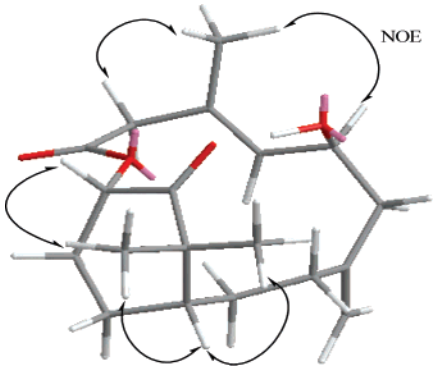

Figure 3. Computer-generated perspective model for **9** using MM2 force field calculations.

characteristic signals including a doublet at δ 5.40, two singlets at δ 4.81 and 4.85, a broad singlet at δ 5.24, and a multiplet at δ 4.38. The ^{13}C NMR spectrum (Table 2) of **7** showed signals of a conjugated ester carbon (δ 172.5), three methyl carbons (δ 34.3, 25.0, 18.3), and one quaternary carbon at δ 36.8 (C-15). The proton and carbon assignments were determined by COSY, HMQC, and HMBC. Detailed comparison of the ^{1}H and ^{13}C NMR spectral data (Tables 2 and 3; Tables S4 and S5) with those of cespitularins revealed that compound **7** is a 10-deoxy analogue of cespitularin D (**15**).[1] COSY correlation from H_2-9 to H-10 and HMBC correlations (Table S1) from H_2-9 to C-7, C-10, C-11, and C-19 help ascertain this assignment. NOESY correlations (Table 4) from H_3-16 to H-10, from H_3-19 to H-6, and from H_3-17 to H-7 indicated Me-16, Me-17, H-7, OH-6, and H-10 were on the same side of the molecule. Thus, cespitularin O can be formulated as **7**.

Cespitularin P (**8**) proved to have the molecular formula $C_{21}H_{30}O_4$ from its HRESIMS and ^{13}C NMR spectroscopic data. The NMR features (Tables 2 and 3; Tables S4 and S5) of **8** showed some similarity to those of cespitularin D (**15**),[1] except for the replacement of the tertiary hydroxyl by a tertiary methoxyl in **8**. HMBC correlations (Table S1) from OMe-10 to C-10 and from H_2-9 to C-7, C-8, C-10, C-11, and C-19 enabled the correct positioning of the methoxyl group. NOESY correlations (Table 4) from H_3-16 to OMe-10, from H_3-19 to H-6, and from H_3-17 to H-7 indicated Me-16, Me-17, H-7, OH-6, and OMe-10 occurred on the same side of the molecule. Thus, cespitularin P can be formulated as **8**.

Cespitularin Q (**9**) was proved to have the molecular formula $C_{19}H_{28}O_4$ by HRESIMS and ^{13}C NMR spectroscopic data, indicating six degrees of unsaturation. The presence of hydroxyl, carbonyl, and lactonyl functions was indicateded by IR absorptions at 3560, 1732, and 1716 cm^{-1}. The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) and DEPT revealed that **1** contained a ketone carbonyl (δ 211.2), an ester (δ_C 169.7), a trisubstituted olefin, a 1,1-disubstituted olefin, one aliphatic quaternary carbon (δ_C 47.7), two oxygenated methine carbons (δ_C 68.8 and 72.3), six methylene carbons (δ_C 20.5, 26.4, 29.1, 33.6, 44.4, and 46.4), and three methyl groups (δ_C 17.0, 23.2, and 27.8; δ_H 1.74, 1.23, and 1.17). The NMR features (Tables 2 and 3; Tables S4 and S5) of **9** were analogous to those of cespitularin E (**14**)[1] with the exception that the conjugated ketone was replaced by an ester bond between C-10 and C-12 and a ketone at C-11.[1] COSY correlation from H-13 to H-12 and H-14 as well as HMBC correlations (Table S1) from H_3-16, 17 to C-11, C-15, and C-1, from H_2-9 to C-7, C-8, C-10, and C-19, and from H-12 to C-10, C-11, C-13, and C-14 confirmed this assignment. NOESY correlation (Table 4) from H_3-16 to H-12 indicated that Me-16 and H-12 were on the same side of the molecule. The computer-modeled structure of **9** was generated by CS Chem 3D version 9.0 using MM2 force field calculations for energy minimization, as shown in Figure 3. The result was consistent with the stereochemistry of **9** as established by NOESY experiments. The structure of cespitularin Q was thus formulated as **9**.

Cespitolide (**10**) gave the molecular formula $C_{20}H_{28}O_3$ as revealed by HRESIMS and ^{13}C NMR spectroscopic data. The NMR features of **10** were similar to those of 9-deoxy-7,8-epoxyisoxeniaolide A,[2] expect for the absence of a tertiary hydroxyl at C-15. COSY correlations from H_3-16, 17 to H-15 and from H-15 to H-14 as well as HMBC correlations from H_3-16, 17 to C-15, 14 supported this assignment. NOESY correlations from H-11a to H-1β (δ 4.15) and Me-18 showed that these protons occurred on the same face on the ring system (β). NOESY correlation from H-4a to H-8 showed that these protons occurred on the α-face on the ring system. A NOESY correlation from H-4a to H-12 supported the *Z*-geometry at C-4 (12). The structure of cespitolide A was thus formulated as 9,15-dideoxy-7,8-epoxyisoxeniaolide A.

Compound **11** has the molecular formula $C_{27}H_{46}O_4$ as deduceded by HRESIMS and ^{13}C NMR spectroscopic data. The NMR features of **11** resembled those of (24*S*)-methyl-3β,11-dihydroxy-5β,6β-epoxy-9,11-secocholestan-9-one,[3] except for the absence of the methyl at C-24. COSY correlation from H_2-11 to H_2-12 as well as HMBC correlations from H_3-18 to C-12, C-13, and C-17 and from H_3-19 to C-1, C-5, C-9, and C-10 confirmed that **11** was a 9,11-secosteroid. NOESY correlations from H-8 to Me-18 and Me-19 and from Me-19 to H-4β and H-7β indicated that these protons occurred on the same face (β) of the molecule. NOESY correlations from H-14 to H-11 indicated that H-14 and H-11 occurred on the opposite face (α). The relative stereochemistry at C-20 was deduced by comparison with spectra data of known 9,11-secocholestanes[4] and confirmed by NOESY correlations from H-20 to Me-18 and from Me-21 to H-11 and H-12. The structure of **11** was thus established as 3β,11-dihydroxy-5β,6β-epoxy-9,11-secocholestan-9-one.

Compound **12** was isolated as a colorless, amorphous solid, whose molecular formula, $C_{30}H_{50}O_4$, was revealed by HRESIMS and ^{13}C NMR spectroscopic data. Detailed comparison of ^{1}H and ^{13}C NMR spectral data of **12** with **11** revealed that **12** differed from **11** in the side chain. The relative stereochemistry of the side chain was deduced by comparison with known 9,11-secogorgostans.[5] The structure of **12** was thus determined as 3β,11-dihydroxy-5β,6β-epoxy-9,11-secogorgostan-9-one.

Compound **7** showed cytotoxicty against P-388 cells with an ED_{50} of 3.4 μg/mL. Compound **11** exhibited cytotoxicity against HT-29 cells with an ED_{50} of 1.0 μg/mL. The other isolated compounds were not cytotoxic to P-388 and HT-29 cells.

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. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C in $CDCl_3$ using TMS as internal standard. ESIMS spectra were obtained with a Bruker APEX II. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *C. hypotentaculata* was collected at Green Island, off Taiwan, in March 2004, at a depth of 6 m and was stored for 1 month in a freezer until extraction. A voucher specimen, NSUGN-067, was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *C. hypotentaculata* were freeze-dried to give 0.55 kg of a solid, which was extracted with CH_2Cl_2/acetone (2.0 L × 3). After removal of solvent in vacuo, the residue (8.58 g) was chromatographed over a column containing silica gel 60 using *n*-hexane/EtOAc and EtOAc/MeOH mixtures as eluting solvents. Elution with *n*-hexane gave fractions containing **3**, that with *n*-hexane/EtOAc (80:20) gave fractions containing **9**, that with *n*-hexane/EtOAc (70:30) gave fractions containing **10**, that with *n*-hexane/EtOAc (65:35) gave fractions containing **6**, that with

n-hexane/EtOAc (60:40) gave fractions containing **7**, **8**, and **1**, that with *n*-hexane/EtOAc (25:75) gave fractions containing **5**, that with EtOAc gave fractions containing **2** and **4**, and that with MeOH/EtOAc (98:2) gave fractions containing **11** and **12**. Compound **2** was further purified by silica gel column chromatography, by eluting with CH_2Cl_2/MeOH (99:1). Compounds **1**, **4**, **7**, **8**, and **10** were further purified by silica gel column chromatography, by eluting with *n*-hexane/acetone (9:1). Compound **3** was further purified by silica gel column chromatography, by eluting with CH_2Cl_2/acetone (1:1). Compound **5** was further purified by silica gel column chromatography, by eluting with CH_2Cl_2/MeOH (99:1). Compound **6** was further purified by passage over RP-C_{18} HPLC column chromatography, by using MeOH/H_2O (7:3) as solvent system. Compound **9** was further purified by silica gel column chromatography, by eluting with *n*-hexane/acetone (85:15). Finally, compounds **11** and **12** were further purified by RP-C_{18} HPLC separation, by eluting with MeOH/H_2O (88:12).

Cespitularin I (1): oil (3 mg); $[\alpha]^{25}_D$ −19 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 232 (4.2) nm; IR ν_{max} 3520, 1725, 1680 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 337.1782 (calcd for $C_{20}H_{26}O_3Na$, 337.1780).

Cespitularin J (2): oil (3 mg); $[\alpha]^{25}_D$ +31 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 235 (4.1) nm; IR ν_{max} 3580, 1745, 1600 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 381.2046 (calcd for $C_{22}H_{30}O_4Na$, 381.2042).

Cespitularin K (3): oil (3 mg); $[\alpha]^{25}_D$ −28 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 231 (4.3) nm; IR ν_{max} 1728, 1682 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 379.1888 (calcd for $C_{22}H_{28}O_4Na$, 379.1885).

Cespitularin L (4): oil (2 mg); $[\alpha]^{25}_D$ +12 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 234 (4.0) nm; IR ν_{max} 3550, 1750, 1650 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 355.1884 (calcd for $C_{20}H_{28}O_4Na$, 355.1885).

Cespitularin M (5): oil (3 mg); $[\alpha]^{25}_D$ −10 (*c* 0.1, CH_2Cl_2); IR ν_{max} 3558, 1742, 1664 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 345.2048 (calcd for $C_{19}H_{30}O_4Na$, 345.2041).

Cespitularin N (6): oil (2 mg); $[\alpha]^{25}_D$ +18 (*c* 0.2, CH_2Cl_2); IR ν_{max} 3562, 1745, 1659 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 345.2046 (calcd for $C_{19}H_{30}O_4Na$, 345.2041).

Cespitularin O (7): oil (3 mg); $[\alpha]^{25}_D$ −41 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 222 (4.1) nm; IR ν_{max} 3550, 1750, 1640 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 339.1937 (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

Cespitularin P (8): oil (4 mg); $[\alpha]^{25}_D$ −116 (*c* 0.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 222 (4.1) nm; IR ν_{max} 3560, 1752, 1715 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 369.2047 (calcd for $C_{21}H_{30}O_4Na$, 369.2042).

Cespitularin Q (9): oil (2 mg); $[\alpha]^{25}_D$ +42 (*c* 0.2, CH_2Cl_2); IR ν_{max} 3560, 1732, 1716 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 343.1889 (calcd for $C_{19}H_{28}O_4Na$, 343.1885).

Cespitolide (10): oil (10 mg); $[\alpha]^{25}_D$ +35 (*c* 0.1 , CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 246 (4.1) nm; IR ν_{max} 1740, 1660 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.99 (1H, dd, J = 15.0, 11.1 Hz, H-13), 6.16 (1H, m, H-12), 6.15 (1H, m, H-14), 5.15 (1H, br s, H-19), 5.03 (1H, br s, H-19), 4.15 (1H, dd, J = 5.1, 11.0 Hz, H-1β), 3.68 (1H, t, J = 11.5 Hz, H-1α), 3.15 (1H, m, H-4a), 3.02 (1H, m, H-8), 2.45 (1H, m, H-10), 2.42 (1H, m, H-11a), 2.36 (1H, m, H-10), 2.28 (1H, m, H-9), 2.22 (1H, m, H-6), 2.19 (1H, m, H-5), 2.18 (1H, m, H-15), 1.78 (1H, m, H-5), 1.48 (1H, m, H-9), 1.36 (3H, s, H_3-18), 1.21 (1H, m, H-6), 1.06 (3H, d, J = 6.7 Hz, H_3-17), 1.06 (3H, d, J = 6.7 Hz, H_3-16); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.6 (qC, C-3), 153.0 (CH, C-14), 148.9 (qC, C-11), 138.8 (CH, C-13), 130.4 (qC, C-4), 130.4 (qC, C-4), 120.9 (CH, C-12), 115.9 (CH_2, C-19), 71.0 (CH_2, C-1), 62.9 (CH, C-8), 59.2 (qC, C-7), 49.4 (CH, C-11a), 42.0 (CH, C-4a), 39.8 (CH_2, C-6), 36.4 (CH_2, C-5), 32.0 (CH, C-15), 31.6 (CH_2, C-10), 25.6 (CH_2, C-9), 22.0 (CH_3, C-17), 22.0 (CH_3, C-16), 16.9 (CH_3, C-18); HRESIMS *m*/*z* 339.1931 (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

3β,11α-Dihydroxy-5β,6β-epoxy-9,11-secocholestan-9-one (11): colorless, amorphous solid (18 mg); $[\alpha]^{25}_D$ −8 (*c* 0.1, CH_2Cl_2); IR ν_{max} 3300, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.87 (1H, m, H-3), 3.87 (1H, m, H-3), 3.76 (1H, m, H-11), 3.67 (1H, m, H-11), 3.11 (1H, br s, H-6), 2.67 (1H, m, H-8), 2.56 (1H, m, H-14), 2.45 (1H, m, H-7β), 2.27 (1H, m, H-7α), 2.11 (1H, t, J = 12.1 Hz, H-4β), 1.98 (1H, m, H-2α), 1.90 (1H, m, H-1α), 1.80 (1H, m, H-1β), 1.77 (1H, m, H-16β), 1.77 (1H, m, H-12), 1.59 (1H, m, H-15α), 1.58 (1H, m, H-17), 1.55 (1H, m, H-12), 1.52 (1H, m, H-2β), 1.51 (1H, m, H-25), 1.45 (1H, m, H-22), 1.44 (1H, m, H-4α), 1.41 (1H, m, H-15β), 1.40 (1H, m, H-20), 1.38 (1H, m, H-16α), 1.31 (3H, s, H_3-19), 1.20 (1H, m, H-24), 1.20 (1H, m, H-23), 1.15 (1H, m, H-24), 0.94 (3H, d, J = 6.5 Hz, H_3-21), 0.87 (3H, d, J = 6.4 Hz, H_3-27), 0.87 (3H, d, J = 6.4 Hz, H_3-26), 0.68 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.7 (qC, C-9), 68.0 (CH, C-3), 65.6 (qC, C-5), 58.8 (CH_2, C-11), 58.2 (CH_2, C-6), 49.6 (CH_2, C-17), 46.6 (qC, C-10), 45.7 (qC, C-13), 45.2 (CH, C-14), 41.1 (CH_2, C-12), 39.5 (CH_2, C-24), 38.9 (CH, C-8), 38.5 (CH_2, C-4), 35.5 (CH, C-20), 34.1 (CH_2, C-22), 30.5 (CH_2, C-25), 22.6 (CH_3, C-2), 22.5 (CH_2, C-15), 19.7 (CH_3, C-19), 19.5 (CH_3, C-21), 18.2 (CH_3, C-18); HRESIMS *m*/*z* 457.3293 (calcd for $C_{27}H_{46}O_4Na$, 457.3294).

3β,11α-Dihydroxy-5β,6β-epoxy-9,11-secogorgostan-9-one (12): colorless, amorphous solid (5 mg); $[\alpha]^{25}_D$ −16 (*c* 0.1, CH_2Cl_2); IR ν_{max} 3450, 1715 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.86 (1H, m, H-3), 3.79 (1H, m, H-11), 3.72 (1H, m, H-11), 3.11 (1H, br s, H-6), 2.66 (1H, m, H-8), 2.59 (1H, m, H-14), 2.45 (1H, m, H-7β), 2.27 (1H, m, H-7α), 2.11 (1H, m, H-4β), 2.02 (1H, m, H-16β), 2.02 (1H, m, H-2α), 1.86 (1H, m, H-1α), 1.85 (1H, m, H-12), 1.75 (1H, m, H-1β), 1.65 (1H, m, H-17), 1.60 (1H, m, H-25), 1.55 (1H, m, H-2β), 1.55 (1H, m, H-15α), 1.51 (1H, m, H-12), 1.41 (1H, m, H-4α), 1.35 (1H, m, H-15β), 1.35 (1H, m, H-16α), 1.31 (3H, s, H-19), 1.03 (3H, s, H_3-21), 1.01 (1H, m, H-20), 0.95 (3H, d, J = 7.0 Hz, H_3-26), 0.93 (3H, d, J = 7.4 Hz, H_3-28), 0.89 (3H, s, H_3-29), 0.86 (3H, d, J = 6.6 Hz, H_3-27), 0.68 (3H, s, H_3-18), 0.48 (1H, dd, J = 8.4, 4.9 Hz, H-30), 0.28 (1H, m, H-24), 0.23 (1H, m, H-22), −0.13 (1H, t, J = 4.9 Hz, H-30); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.8 (qC, C-9), 68.0 (CH, C-3), 65.4 (qC, C-5), 58.8 (CH_2, C-11), 58.2 (CH, C-6), 50.8 (CH, C-24), 50.7 (CH, C-17), 46.6 (qC, C-10), 45.9 (qC, C-13), 45.3 (CH, C-14), 41.3 (CH_2, C-12), 38.8 (CH, C-8), 38.6 (CH_2, C-4), 34.7 (CH, C-20), 32.1 (CH_2, C-22), 32.0 (CH, C-25), 30.5 (CH_2, C-2), 28.6 (CH_2, C-1), 28.2 (CH_2, C-16), 26.2 (CH_2, C-7), 25.9 (CH_2, C-23), 22.6 (CH_2, C-15), 22.3 (CH_3, C-26), 21.4 (CH_2, C-30), 21.4 (CH_3, C-27), 21.0 (CH_3, C-21), 19.7 (CH_3, C-19), 18.1 (CH_3, C-18), 15.2 (CH_3, C-28), 14.4 (CH_3, C-29); HRESIMS *m*/*z* 497.3609 (calcd for $C_{30}H_{50}O_4Na$, 497.3607).

Cytotoxicity Testing. P-388 cells were kindly supplied by Dr. J. M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; HT-29 cells were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[6]

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Supporting Information Available: HMBC correlations of **1**−**9** and comparison of NMR data for **1**−**4** and **13** as well as **5**−**9**, **14**, and **15** are available free of charge via the Internet at http://pubs.acs.org.

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