

Novel Terpenoids from the Formosan Soft Coral *Cespitularia hypotentaculata*

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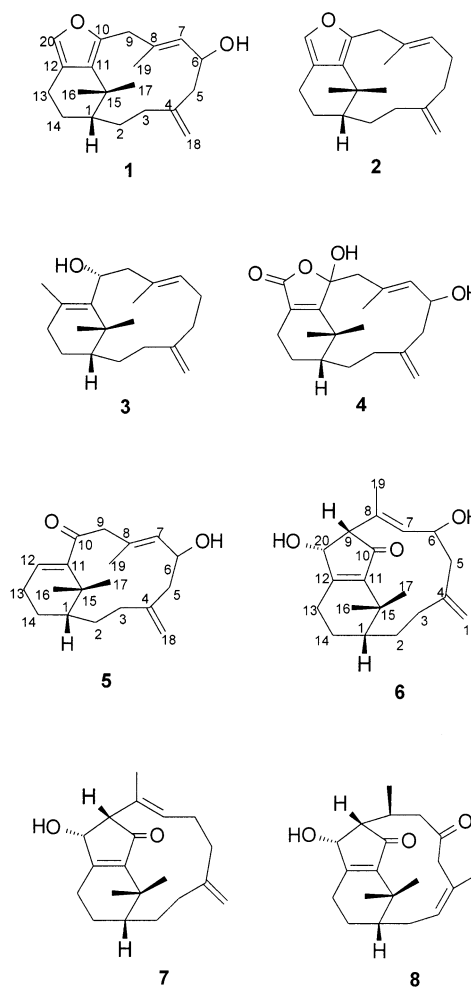
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Four new cytotoxic diterpenes, cespitularins A–D (**1–4**), having the verticillane skeleton, a new cytotoxic norditerpene, cespitularin E (**5**), that possesses a novel norverticillane skeleton, and three new diterpenes, cespitularins F–H (**6–8**), possessing a novel carbon skeleton named cespitularane, were isolated from the methylene chloride solubles of the Formosan soft coral *Cespitularia hypotentaculata*. The structures of cespitularins A–H (**1–8**) were elucidated by 1D and 2D NMR spectral analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

The genus *Cespitularia* has afforded diterpenes of cembrane and neodolabellane skeletons.^{1,2} As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Cespitularia hypotentaculata* Roxas (Xeniidae) was studied because CH₂Cl₂ extracts showed significant cytotoxicity to A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.^{3,4} Bioassay-guided fractionation resulted in the isolation of four new cytotoxic diterpenes, cespitularins A–D (**1–4**), that possess the verticillane skeleton, a new cytotoxic norditerpene, cespitularin E (**5**), having a novel norverticillane skeleton, and three new diterpenes, cespitularins F–H (**6–8**), possessing a novel carbon skeleton named cespitularane.

Results and Discussion

Cespitularin A (**1**) was isolated as a colorless amorphous solid. HREIMS, ¹³C NMR, and DEPT spectra established the molecular formula of **1** as C₂₀H₂₈O₂. Thus, seven degrees of unsaturation were determined for **1**. The IR spectrum of **1** indicated the presence of hydroxyl group(s) (ν_{\max} 3650 cm⁻¹). The presence of eight sp² hybridized carbon atoms in the molecule, as deduced from the ¹³C and DEPT NMR spectra (Table 2), corresponding to four carbon–carbon double bonds as the only multiple bonds, indicated compound **1** to be tricyclic. The ¹³C NMR singlet at δ 134.3 and a doublet at δ 129.9 that was correlated in the HMBC experiment (Table 3) with the ¹H NMR signal at δ 5.13 (br d, J = 7.5 Hz, 1H) together with the vinylic methyl signals at δ 1.67 (s, 3H) in the ¹H NMR spectrum and at δ 17.7 (q) in the ¹³C NMR spectrum were assigned to an *E*-trisubstituted double bond bearing a methyl group.⁵ HMQC correlation of δ_{H} 4.79 (s, 1H) and 4.80 (s, 1H) with δ_{C} 112.4 (t) as well as HMBC correlation of δ_{H} 4.79 (s, 1H) and 4.80 (s, 1H) with δ_{C} 147.1 (s), 29.8 (t), and 44.6 (t) indicated that **1** contained an exocyclic methylene. HMQC correlation of δ_{H} 7.05 (s, 1H) with δ_{C} 134.6 (d) and HMBC correlation of δ_{H} 7.05 (s, 1H) with δ_{C} 124.1 (s), 126.2 (s), and 144.5 (s) indicated the presence of an α,β,β' -trisubstituted furan. The geminal methyls at δ_{H} 1.45 (s, 3H) and 1.21 (s, 3H) showed HMBC correlations with δ_{C} 34.2 (s),



43.1 (d), and 126.2 (s), indicating **1** contained a gem-dimethyl-bearing quaternary carbon which was adjacent to a methine carbon and a quaternary olefinic carbon. Measurement of the ¹³C–¹³C homonuclear shift correlation 2D spectrum (INADEQUATE) (Figure 1) of **1** together with COSY, HMQC, and HMBC experiments established its chemical structure and enabled also the assignment of all resonances in the NMR spectra. The relative stereochemistry of **1** was deduced from 2D NOESY experiment (Table 4), which indicated that Me-16, Me-17, H-7, and H-1 were on one side of the molecule, while Me-19 was on the

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Table 1. ¹H NMR Spectral Data^a of **1–8** in CDCl₃

	1	2	3	4	5	6	7^b	8
1	1.87 m	1.79 m	1.53 m	1.68 m	1.72 m	1.67 m	1.69 m	1.73 m
2	1.39 m	1.46 m	1.50 m	2.30 m	1.50m	1.89 m	1.60 m	1.97 m
	1.69 m	2.11 m	2.16 m		1.98 m	2.28 m	1.94 m	2.03 m
3	1.65 m	2.17 m	2.00 m	1.54 m	2.69 dd (11.0, 10.2)	2.01 m	1.96 m	5.52 t (6.0)
5	1.79 m 2.16 dd (13.0, 3.0) 2.35 dd (13.0, 6.5)	2.13 m	2.30 m 1.94 m	2.36 m	1.91 m 2.27 m	2.43 m 1.96 dd (13.2, 3.0)	2.22 m 1.75 m	2.92 d (13.5)
6	4.44 m	1.93 m	2.14 m	4.38 dt (7.5, 11.8)	2.47 dd (11.0, 9.8) 4.44 dt (7.5, 2.1)	2.49 dd (1.2, 4.5) 4.52 m	2.29 m 2.10 m	2.99 d (13.5)
7	5.13 d (7.5)	2.14 m 4.47 br d (12.0)	5.19 dd (10.5, 3.9)	5.50 d (8.1)	5.14 d (6.0)	4.94 m	2.31 m 5.16 t (4.2)	2.28 m
8								2.82 m
9α	3.21 d (14.7)	3.21 d (18.6)	2.95 t (12.0)	2.97 s	3.44 d (15.6)	3.27 d (6.0)	3.29 d (3.3)	2.65 m
9β	3.49 d (14.7)	3.35 d (18.6)	2.30 m		3.05 d (15.6)			2.54 d (6.3)
10			4.59 dd (12.3, 4.2)					
12					6.21 dd (3.6, 3.3)			
13	2.57 m	2.60 dt (7.2, 0.9)	1.97 m	2.22 m	2.32 m	2.37 m	2.68 dt (12.6, 5.7)	2.35 m
			2.72 dd (14.7, 9.3)			2.65 m	2.37 m	2.45 m
14α	1.12 m	1.30 m	1.58 m	1.73 m	2.26 m	1.71 m	1.84 m	2.07 m
14β	2.23 m	2.25 m		2.24 m	1.75 m	2.09 m	2.36 m	1.61 m
16	1.21 s	1.17 s	0.91 s	1.31 s	1.28 s	1.14 s	1.14 s	1.09 s
17	1.45 s	1.33 s	1.12 s	1.47 s	1.16 s	1.37 s	1.35 s	1.43 s
18	4.78 s	4.60 s	4.60 s	4.83 s	4.82 s	4.93 s	4.74 s	1.86 s
	4.79 s	4.65 s	4.68 s	4.85 s	4.84 s		4.81 s	
19	1.67 s	1.65 s	1.64 s	1.60 s	1.75 s	1.84 s	1.78 s	1.25 d (7.2)
20	7.05 s	7.09 s	1.95 s			4.89 m	4.84 dd (5.1, 3.9)	4.60 dd (12.0, 6.4)
OH-20							2.26 d (3.9)	5.64 d (12.0)

^a Recorded in CDCl₃ at 300 MHz, unless stated otherwise. ^b Measured in CDCl₃ at 500 MHz.**Table 2.** ¹³C NMR Spectral Data^a (δ) of **1–8** in CDCl₃

	1	2	3	4	5	6	7^b	8
1	43.1 d	44.8 d	44.9 d	43.7 d	43.1 d	41.8 d	42.0 d	42.1 d
2	31.4 t	32.5 t	27.7 t	18.1 t	30.7 t	31.0 t	22.3 t	31.2 t
3	29.8 t	29.2 t	38.4 t	31.7 t	30.7 t	22.6 t	32.2 t	129.0 d
4	147.1 s	152.7 s	149.8 s	146.1 s	147.0 s	145.8 s	150.6 s	127.0 s
5	44.6 t	32.9 t	33.6 t	43.6 t	44.7 t	45.7 t	37.4 t	46.9 t
6	68.7 d	36.5 t	31.9 t	68.3 d	69.3 d	66.8 d	29.6 t	213.3 s
7	129.9 d	127.4 d	128.8 d	136.0 d	133.2 d	132.4 d	130.7 d	47.2 t
8	134.3 s	134.3 s	127.5 s	131.0 s	130.8 s	133.7 s	130.2 s	25.3 d
9	37.8 t	36.4 t	48.3 t	48.7 t	51.1 t	62.6 d	62.5 d	57.5 d
10	144.5 s	147.0 s	69.0 d	108.8 s	202.2 s	204.1 s	202.9 s	205.2 s
11	126.2 s	125.3 s	137.4 s	167.8 s	148.0 s	146.6 s	145.8 s	143.2 s
12	124.1 s	123.1 s	129.9 s	129.1 s	135.5 d	169.2 s	169.2 s	168.0 s
13	17.7 t	16.1 t	33.9 t	32.6 t	24.0 t	22.1 t	21.5 t	20.8 t
14	25.9 t	26.0 t	33.0 t	23.9 t	23.0 t	28.5 t	28.7 t	22.3 t
15	34.2 s	34.5 s	38.9 s	37.2 s	35.4 s	33.7 s	33.8 s	35.4 s
16	33.7 q	33.6 q	28.5 q	34.0 q	32.9 q	30.6 q	30.4 q	31.5 q
17	26.3 q	25.8 q	33.9 q	24.2 q	24.6 q	23.9 q	23.6 q	25.3 q
18	112.4 t	109.2 t	106.6 t	114.0 t	112.6 t	112.4 t	109.5 t	27.1 q
19	17.7 q	17.6 q	20.0 q	17.2 q	19.0 q	19.5 q	18.3 q	23.0 q
20	134.6 d	134.8 d	24.1 q	171.5 s		73.8 d	73.2 d	72.4 d

^a Recorded in CDCl₃ at 75 MHz, unless stated otherwise. ^b Measured in CDCl₃ at 125 MHz.

opposite side of the molecule. The relative stereochemistry of the secondary hydroxyl at C-6 was not determined due to the flexibility of the 13-membered ring. From the aforementioned data, cespitularin A can be formulated as **1**.

Cespitularin B (**2**) was isolated as a colorless amorphous solid, whose molecular formula, C₂₀H₂₈O, was revealed by HREIMS and NMR spectra. The NMR features (Tables 1 and 2) of **2** were quite similar to those of **1**. The only difference was the absence of the secondary hydroxyl at

C-6 in **2**. The relative stereochemistry of **2** was deduced from a 2D NOESY experiment (Table 4), which indicated that Me-16, Me-17, H-7, and H-1 were on one side of the molecule, while Me-19 was on the opposite side of the molecule. The structure of cespitularin B is thus assigned as **2**.

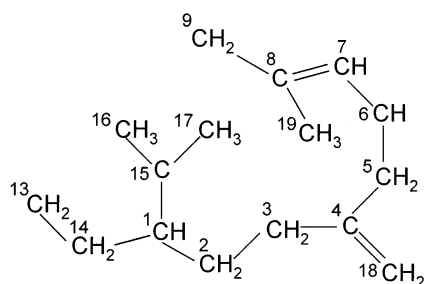
Cespitularin C (**3**) was isolated as a colorless amorphous solid and analyzed for C₂₀H₃₂O by HREIMS and NMR spectral data (Tables 1 and 2). Spectroscopic data of **3** were analogous to those of **2** with the exception that the

Table 3. Selected HMBC Correlations of **1–8**

H	1	2	3	4	5	6	7	8
1	C-2, 11, 13, 16, 17	C-2, 14	C-14	C-14	C-14	C-2, 14	C-15	C-2, 14
2	C-3, 4	C-14	C-14	C-14	C-1, 13, 15	C-1, 14	C-4	C-1
3	C-5	C-2	C-5, 18	C-5		C-2	C-2, 4, 18	
5	C-3, 4, 6, 7, 18	C-4, 6	C-3	C-3, 4, 6, 7, 18	C-3, 4, 6, 7, 18	C-3, 4, 6, 7, 18	C-3, 4, 7, 18	C-6
6						C-4, 8		
7					C-8, 9	C-6	C-9, 19	C-6, 8, 9
9	C-7, 8, 10, 19	C-7, 10	C-8, 10, 11, 19	C-7, 8, 10, 11, 19	C-7, 8, 10, 11, 12, 19	C-7, 8, 10, 11, 12, 19, 20	C-7, 8, 10, 11, 19	C-7, 8, 10, 11, 12, 20
10			C-8, 9, 11, 12, 15					
12					C-10, 11, 13, 14, 15			
13	C-1, 11, 12, 14, 20	C-1, 11, 14, 20	C-12	C-1, 14, 20	C-12, 14	C-11, 12	C-1, 11, 12, 14	C-11
14	C-1, 12, 13, 15	C-12, 13	C-1	C-12		C-1	C-1, 12, 13, 15	C-12
16	C-1, 11, 15, 17	C-1, 11, 15, 17	C-1, 11, 15, 17	C-1, 11, 15, 17	C-1, 11, 15, 17	C-1, 11, 15, 17	C-1, 11, 15, 17	C-1, 11, 15, 17
17	C-1, 11, 15, 16	C-1, 11, 15, 16	C-1, 11, 15, 16	C-1, 11, 15, 16	C-1, 11, 15, 16	C-1, 11, 15, 16	C-1, 11, 15, 16	C-1, 11, 15, 16
18	C-3, 4, 5		C-4, 5	C-3, 4	C-3, 4, 5	C-3, 4, 5	C-3, 5	C-3, 4, 5
19	C-7, 8, 9	C-7, 9	C-8, 9	C-7, 8, 9	C-7, 8, 9	C-7, 8, 9	C-7, 8, 19	C-7, 8, 9
20	C-10, 11, 12		C-11, 12, 13			C-9, 11	C-12	

Table 4. Selected NOE Correlations of **1–8**

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

**Figure 1.** 2D INADEQUATE correlations of **1**.

resonances for the trisubstituted furan were replaced by a tetrasubstituted olefin bearing a methyl group and a secondary hydroxyl at C-10. HMBC correlations (Table 3) between H-20 and C-12, C-11, and C-13; H-10 and C-8, C-9, C-11, C-12, and C-15; and H-16, 17 and C-11 clearly positioned the tetrasubstituted olefin and the secondary hydroxyl. The relative stereochemistry of **3** was deduced from a 2D NOESY experiment (Table 4), which indicated that H-10, Me-19, Me-16, Me-17, and H-1 were on one side of the molecule, while Me-20, H-7, and H-9 α were on the opposite side of the molecule. The structure of cespitularin C is thus formulated as **3**.

Cespitularin D (**4**) was isolated as an colorless amorphous solid of molecular formula $C_{20}H_{28}O_4$, as indicated by HREIMS and ^{13}C NMR (Table 2) spectral methods. The NMR features of **4** were also analogous to those of **1**.

Analyses of 2D NMR data revealed that **4** possessed the same carbocyclic skeleton as **1**. However, there was a significant difference that indicated the presence of a γ -hydroxy- α,β -unsaturated- γ -lactone [δ_C 171.5 (s), 129.1 (s), 167.8 (s), 108.8 (s)] in **4** instead of a α,β,β' -trisubstituted furan. HMBC correlations (Table 3) between H-16, H-17 and C-11; H-13 and C-14, C-1, C-20; H-9 and C-10, C-11, C-8, C-7, C-19; and H-19 and C-7, C-8, C-9 clearly positioned the γ -hydroxy- α,β -unsaturated- γ -lactone. The relative stereochemistry of **4** 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 is on the opposite side of the molecule. The relative stereochemistry of the secondary hydroxyl at C-6 was not determined due to the flexibility of the 13-membered ring. The structure of cespitularin D is thus formulated as **4**.

Cespitularin E (**5**) was analyzed for $C_{19}H_{28}O_2$ by HREIMS and NMR spectral data. The IR and UV spectra showed the presence of an α,β -unsaturated ketone (1690 cm^{-1} ; 232 nm) and hydroxyl (3660 cm^{-1}) moieties. Spectroscopic data of **5** were analogous to those of **1** with the exception that the resonances for the trisubstituted furan were replaced by the α,β -unsaturated ketone at C-11(α), C-12(β), and C-10(carbonyl), which was proved by HMBC correlations (Table 3) between H-12 and C-10, C-11, C-13, C-14; H-16, 17 and C-11; H-9 and C-10, C-19, C-8, C-7 as well as COSY correlation between H-12 and H-13. The relative stereochemistry of **5** 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 is on the opposite side of the molecule. The relative stereochemistry of the secondary hydroxyl at C-6 was not determined due to the flexibility of the 13-membered ring. The structure of cespitularin E is thus formulated as **5**.

Cespitularin F (**6**) has the molecular formula $C_{20}H_{28}O_3$ as determined by HREIMS and NMR spectral data (Tables 1 and 2). The IR spectrum showed the presence of hydroxyl (3610 cm^{-1}) and α,β -unsaturated ketone (1730 cm^{-1}) moieties. The UV absorption at λ_{\max} 236 nm suggested the presence of an α,β -unsaturated ketone. The NMR spectra of **6** were analogous to those of **5** except that the resonances for the α,β -unsaturated ketone moiety and the methylene α (C-9) to the ketone in **5** were replaced by an α,β -unsaturated- γ -hydroxycyclopentanone (δ_H 4.89 m, 3.27 d, δ_C 204.1 s, 146.6 s, 169.2 s, 73.8 d, 62.6 d) in **6**. COSY cross-peaks between H-9 (δ 3.27, d) and H-20 (δ 4.89, m), as well as HMBC correlations (Table 3) between H-9 and C-7, C-8, C-19, C-11, C-12, C-20; H-20 and C-9, C-11; H-16, 17, and C-11; and H-13 and C-11, C-12 clearly positioned the α,β -unsaturated- γ -hydroxycyclopentanone at C-11 (α), C-12 (β), C-20 (γ), C-9 (δ), and C-10 (carbonyl). The relative stereochemistry of **6** 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 is on the opposite side of the molecule. According to an analysis of NOE correlation from H-7 to H₃-17 and consideration of a Dreiding model of compound **6**, H-9 and H-20 should be located on the β -face of tetrahydroindenone ring. The relative stereochemistry of the secondary hydroxyl at C-6 was not determined due to the flexibility of the 13-membered ring. The structure of cespitularin F is thus formulated as **6**.

Cespitularin G (**7**) was isolated as a colorless amorphous solid, whose molecular formula, $C_{20}H_{28}O_2$, was revealed by HREIMS and NMR spectra (Tables 1 and 2). The IR spectrum of **7** indicated the presence of an α,β -unsaturated ketone (1735 cm^{-1}) and a hydroxyl group (3640 cm^{-1}). The UV absorption at λ_{\max} 234 nm suggested the presence of an α,β -unsaturated ketone. The NMR features of compound **7** were quite similar to those of compound **6**. The only difference was the absence of the secondary hydroxy at C-6 in **7**. The relative stereochemistry of **7** 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 is on the opposite side of the molecule. According to an analysis of NOE correlation from H-7 to H₃-17 and consideration of a Dreiding model of compound **7**, H-9 and H-20 should be located on the β -face of the tetrahydroindenone ring. The structure of cespitularin G is thus formulated as **7**.

Cespitularin H (**8**) was isolated as a colorless amorphous solid, whose molecular formula, $C_{20}H_{28}O_3$, was revealed by HREIMS and NMR spectra (Tables 1 and 2). The IR spectrum of **8** indicated the presence of a hydroxyl at 3650 cm^{-1} and ketones at 1730 and 1715 cm^{-1} . The UV absorption at λ_{\max} 235 nm suggested the presence of an α,β -unsaturated ketone. The NMR features of compound **8** were analogous to those of compound **6** except that the exomethylene at C-4 and the olefinic methyl at C-8 in **6** were replaced by a cis olefinic methyl (δ_H 1.86 s; δ_C 27.1 q) and a secondary methyl (δ_H 1.25 d; δ_C 23.0 q), respectively. The relative stereochemistry of **8** was deduced from a 2D NOESY experiment (Table 4), which indicated that Me-16, Me-17, H-7 β , and H-1 are on one side of the molecule.

Table 5. Cytotoxicity^a of **1–8**

compound	cell lines ED ₅₀ ($\mu\text{g/mL}$)		
	A549	HT-29	P-388
1	8.42	9.76	3.66
2	7.96	9.25	3.23
3	0.12	8.86	0.01
4	>50	>50	3.86
5	0.034	17.10	4.66
6	16.11	>50	>50
7	>50	>50	>50
8	9.32	23.69	>50

^a For significant activity of pure compounds, an ED₅₀ of $\leq 4.0\text{ }\mu\text{g/mL}$ is required.

According to an analysis of a NOE correlation from H-7 to H₃-17 and consideration of a Dreiding model of compound **8**, H-9 and H-20 should be located on the β -face of the tetrahydroindenone ring. The NOE between Me-19 and H-9 allowed us to locate Me-19 at the β -equatorial position. The NOE between Me-18 and H-3 confirmed the *Z*-configuration at $\Delta^{3,4}$. The structure of cespitularin H is thus formulated as **8**.

The cytotoxicity of cespitularins A–H (**1–8**) is shown in Table 5. Cespitularin C exhibited potent cytotoxicity against P-388 and A549 cells. Cespitularins A, B, and D showed moderate cytotoxicity against P-388 cells. Cespitularin E exhibited potent cytotoxicity against A549 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 ^1H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^1H and 125 MHz for ^{13}C , 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 F₂₅₄, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *C. hypotentaculata* was collected at Orchid Island, off Taiwan, in February 2001, at a depth of 10 m and was stored for 1 month in a freezer until extraction. A voucher specimen, NSUGN-046, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *C. hypotentaculata* were freeze-dried to give 0.62 kg of a solid, which was extracted with CH_2Cl_2 (2.0 L \times 3). After removal of solvent in vacuo, the residue (8.82 g) was chromatographed over Si gel 60 using *n*-hexane and *n*-hexane–EtOAc mixtures of increasing polarity. Elution by *n*-hexane–EtOAc (9:1) afforded fractions containing **2**. Elution by *n*-hexane–EtOAc (6:1) afforded fractions containing **7** and **8**. Elution by *n*-hexane–EtOAc (4:1) afforded fractions containing **3**. Elution by *n*-hexane–EtOAc (2:1) afforded fractions containing **1**. Elution by *n*-hexane–EtOAc (1:2) afforded fractions containing **5**. Elution by *n*-hexane–EtOAc (1:9) afforded fractions containing **4**. Elution by EtOAc afforded fractions containing **6**. Compound **2** was further purified by Si gel column chromatography, by eluting with *n*-hexane–EtOAc (49:1). Compounds **7** and **8** were obtained by C₁₈ HPLC column chromatography, by using MeOH–H₂O (1:1) as solvent system. Compound **3** was obtained by Si gel column chromatography, by eluting with *n*-hexane– CH_2Cl_2 (4:1). Compound **1** was obtained by Si gel column chromatography, by eluting with *n*-hexane– CH_2Cl_2 (2:1). Compound **5** was further purified by Si gel column

chromatography, by eluting with *n*-hexane–EtOAc (2:1). Compound **4** was further purified by Si gel column chromatography, by eluting with *n*-hexane–EtOAc (10:1). Compound **6** was further purified by Si gel column chromatography, by eluting with *n*-hexane–EtOAc (1:1).

Cespitularin A (1): amorphous solid (360 mg); mp 71–72 °C; $[\alpha]_D^{25} -140.1^\circ$ (*c* 0.12, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 202 (2.8) nm; IR (KBr) ν_{\max} 3650 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 300 [M]⁺ (55), 285 (20), 271 (6), 257 (3), 229 (14), 201 (29), 189 (12), 175 (18), 161 (42), 147 (56), 91 (100); HREIMS *m/z* 300.2093 (calcd for C₂₀H₂₈O₂, 300.2082).

Cespitularin B (2): amorphous solid (5 mg); mp 62–63 °C; $[\alpha]_D^{25} -20.6^\circ$ (*c* 0.08, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 204 (3.9) nm. ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 284 [M]⁺ (26), 255 (6), 241 (5), 229 (14), 201 (29), 189 (12), 159 (8), 147 (46), 91 (100); HREIMS *m/z* 284.2144 (calcd for C₂₀H₂₈O, 284.2133).

Cespitularin C (3): amorphous solid (26 mg); mp 66–68 °C; $[\alpha]_D^{25} -62.3^\circ$ (*c* 0.10, CHCl₃); IR (KBr) ν_{\max} 3620 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 288 [M]⁺ (9), 273 (18), 255 (5), 245 (7), 227 (4), 205 (7), 177 (11), 161 (16), 149 (61), 135 (71), 109 (100); HREIMS *m/z* 288.2460 (calcd for C₂₀H₃₂O, 288.2445).

Cespitularin D (4): oil (4 mg); $[\alpha]_D^{25} -169.6^\circ$ (*c* 0.23, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 220 (4.1) nm; IR (KBr) ν_{\max} 3630, 1750 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 332 [M]⁺ (1), 314 (8), 269 (1), 248 (2), 245 (1), 233 (5), 220 (5), 205 (9), 187 (4), 177 (16), 107 (45), 85 (100); HRFABMS *m/z* 333.2056 (calcd for C₂₀H₂₉O₄, 333.2058).

Cespitularin E (5): resinous oil (6 mg); $[\alpha]_D^{25} +122.3^\circ$ (*c* 0.22, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 232 (4.2) nm; IR (KBr) ν_{\max} 3660, 1690 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 288 [M]⁺ (1), 270 (1), 256 (1), 221 (1), 192 (3), 176 (5), 154 (45), 137 (100), 107 (66); HRFABMS *m/z* 289.2158 (calcd for C₁₉H₂₈O₂, 289.2168).

Cespitularin F (6): resinous oil (18 mg); $[\alpha]_D^{25} +39.8^\circ$ (*c* 0.21, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 236 (4.3) nm; IR (KBr) ν_{\max} 3610, 1730 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 316 [M]⁺ (13), 299 (40), 298 (23), 283 (17),

270 (10), 255 (13), 227 (18), 215 (13), 201 (14), 187 (17), 119 (21), 91 (100); HRFABMS *m/z* 316.2057 (calcd for C₂₀H₂₈O₃, 316.2031).

Cespitularin G (7): resinous oil (1 mg); $[\alpha]_D^{25} -63.6^\circ$ (*c* 0.16, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 234 (4.2) nm; IR (KBr) ν_{\max} 3640, 1735 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 300 [M]⁺ (3), 282 (2), 272 (1), 257 (1), 223 (1), 229 (2), 199 (3), 189 (3), 149 (8), 119 (9), 55 (100); HREIMS *m/z* 300.2090 (calcd for C₂₀H₂₈O₂, 300.2082).

Cespitularin H (8): amorphous solid (3 mg); mp 120–121 °C; $[\alpha]_D^{25} -93.6^\circ$ (*c* 0.19, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 235 (4.0) nm; IR (KBr) ν_{\max} 3650, 1730, 1715 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 316 [M]⁺ (7), 298 (7), 283 (5), 257 (6), 229 (10), 203 (12), 161 (18), 105 (17), 91 (24), 69 (100); HREIMS *m/z* 316.2046 (calcd for C₂₀H₂₈O₃, 316.2031).

Cytotoxicity Testing. P-388 cells were kindly supplied by Prof. J. M. Pezzuto, Department of Medicinal Chemistry and Pharmacognosy, 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.⁵

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