Nitrogen-Containing Verticillene Diterpenoids from the Taiwanese Soft Coral *Cespitularia taeniata*

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Received June 28, 2007

Extensive column chromatography of the ethanolic extract of the soft coral *Cespitularia taeniata* collected in Taiwan has resulted in the isolation of eight new nitrogen-containing verticillene diterpenes, designated cespitulactams D−K (**1**−**8**), together with three known diterpenes, cespitulactams A (**9**) and B (**10**) and cespitularin F. In addition, one new derivative, 6-*O*-acetylcespitulactam F (**11**), was prepared from compound **3**. The structures of these compounds were elucidated on the basis of spectroscopic analyses, especially HRMS and 2D NMR experiments. The cytotoxicity against human oral epidermoid carcinoma (KB) and murine L1210 leukemia cell lines and antimicrobial activities of **1**−**8** and **11** were tested and evaluated. A biogenetic pathway for these novel diterpene alkaloids was also proposed.

Marine organisms have developed many secondary metabolites as chemical weapons for surviving during evolution. Soft corals of the genus *Cespitularia* are rich in novel and diverse chemical structures with interesting biological activities.[1−4] For example, sesquiterpenes such as (+) palustrol and cubebol were isolated from *C. subviridis* and *Cespitularia* sp., respectively.[5,6] Two novel diterpenes, 4β,5β-epoxyxeniaphylla-8(19),14-diene and decahydrocyclo- pentacycloundecenes, were isolated from an unknown species of *Cespitularia*.[7,8] Previously, we reported three novel diterpenes, cespitulactams A, B, and C, from *C. taeniata*, which was collected from the southern coast of Taiwan.[9] These nitrogen-containing tricyclic compounds are very close to the bicyclic taxane diterpenes that were found in terrestrial species of *Taxus*.[10−14] From a biogenetic point of view they are all derived from the same precursors, geranylgeranyl pyrophosphate and 1*S*-verticillene. Continued investigation of this species has resulted in the isolation of an additional eight new nitrogen-containing verticillene diterpenes, designated cespitulactams D−K (**1**−**8**). In addition, cespitulactams A (**9**) and B (**10**) and cespitularin F were also isolated, and one new derivative, 6-*O*-acetylcespitulactam F (**11**), was prepared. The structures of these compounds were established by extensive study of their spectroscopic data, especially 2D NMR and HRMS.

Results and Discussion

The ethanolic extract of *C. taeniata* was partitioned between H_2O and EtOAc. Extensive Si gel column and HPLC chromatography of the EtOAc-soluble extract using gradient solvent combinations yielded cespitulactams D (**1**), E (**2**), F (**3**), G (**4**), H (**5**), I (**6**), J (**7**), and K (**8**), together with the previously reported cespitulactams A (**9**) and B (**10**) and cespitularin F.

Cespitulactam D (**1**), $[\alpha]_D$ −52 (CH_2Cl_2), had a molecular formula of $C_{20}H_{29}NO_2$ deduced from HRESIMS (*m/z* 316.2274, $[M + H]^+$), indicating seven degrees of unsaturation. The UV absorption (λ_{max} 226 nm) and IR bands showed the presence of hydroxyl (3450 cm^{-1}), and conjugated lactam (1676 cm^{-1}) groups. The ^{1}H NMR data of **1** (Table 1) revealed the presence of three singlet methyl groups (δ 1.16, 1.37, 1.56), one exocyclic methylene (δ 4.80, 4.82), two heteroatom-bearing methines (δ 4.35, 4.43, both as brs), and an olefinic proton (δ 5.38, d, J = 7.6 Hz). To determine the proton sequences of **1**, a COSY spectrum (Figure 1) disclosed the connectivities of H-13 (δ 2.09 and 2.34)/H-14 (δ 1.69 and 2.06)/H-1 (δ 1.71)/H-2 (δ 2.30)/H-3 (δ 1.62), H-5 (δ 2.33)/H-6 (δ 4.35)/H-7 (δ 5.38), and H-9 (δ 2.09 and 2.34)/H-10 (δ 4.43). The ^{13}C NMR (Table 3) and DEPT spectra of **1** showed 20 carbon signals for three methyl carbons (δ 18.5, 24.9, and 34.9), six aliphatic methylene carbons (δ 18.3, 24.1, 31.8, 31.9, 42.3, and 43.7), three methine (δ 42.9, 58.3, and 68.3), one aliphatic quaternary carbon (δ 36.9), one olefinic methine (δ 134.0), one olefinic methylene (δ 113.3), four olefinic quaternary carbons (δ 131.9, 133.7, 146.7, and δ 162.7), and an amide carbonyl signal (δ 173.8). On the basis of the above data, the remaining three degrees of unsaturation suggested that compound **1** contains a tricyclic verticillene ring similar to that previously reported for cespitulactams.[9]

Table 1. ^{1}H NMR Data for Compounds **1**−**4** (chemical shifts are in ppm; *J* values in Hz are in parentheses)

position	**1**[a]	**2**[a]	**3**[b]	**4**[a]
1	1.71 m	1.73 m	1.58 m	1.54 m
2	2.30 m	2.29 m	2.19 m	2.24 m
3	1.62 m	1.57 m	1.56 m	1.53 m
5	2.33 m	2.45 m	2.37 m	2.40 m
6	4.36 brs	5.36 brs	4.23 m	4.36 m
7	5.38 d (7.6)	5.34 d (7.6)	5.52 d (8.6)	5.53 d (7.7)
9	2.66 d (14.1)	2.61 d (13.3)	2.83 d (13.8)	2.74 d (14.1)
	2.75 d (14.1)	2.78 (13.3)	2.94 d (13.8)	2.98 d (14.1)
10	4.43 brs	4.43 br		
13	2.09 m	2.09 m	2.06 m	2.11 m
	2.34 m	2.35 m	2.31 m	2.26 m
14	1.69 m	1.70 m	1.70m	1.60 m
	2.06 m	2.21 m	2.27 m	2.18 m
16	1.16 s	1.16 s	1.29 s	1.22 s
17	1.37 s	1.39 s	1.52 s	1.47 s
18	4.80 s	4.78 s	4.76 s	4.83 s
	4.82 s	4.79 s	4.80 s	4.83 s
19	1.56 s	1.57 s	1.54 s	1.55 s
1′				3.20 m
				3.51 m
2′				
Ac		2.02 s		1.16 t (6.9)

[a] Recorded in $CDCl_3$ at 300 MHz. [b] Recorded in CD_3OD at 300 MHz.

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10.1021/np078011u CCC: $37.00
Published on Web 12/06/2007

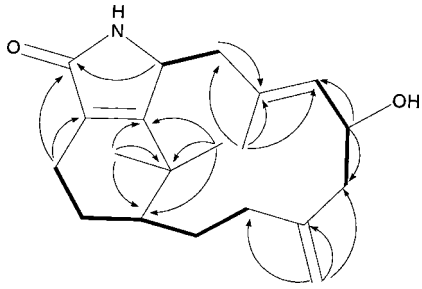
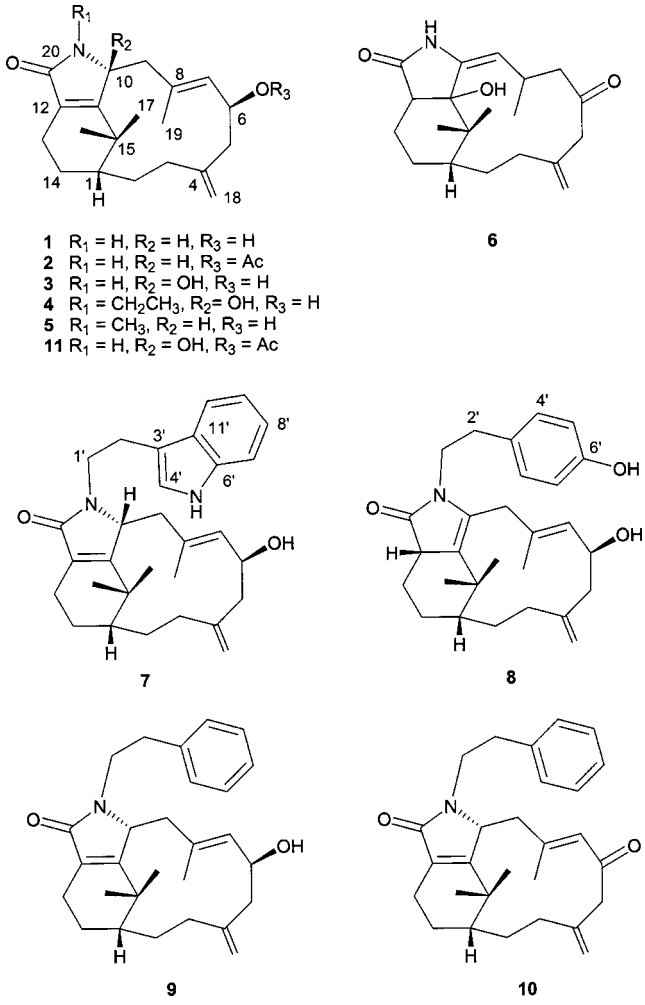


Figure 1. Selected $^1H-^1H$ COSY (bold lines) and HMBC correlations (arrows) of **1**.



The COSY spectrum assigned the hydroxyl group to C-6. Detailed analysis of the HMBC spectra (Figure 1) for **1** revealed the correlations of H-18/C-3, C-4, C-5 and H-19/C-7, C-8, C-9, as well as H-16, H-17/C-1, C-15, C-11, and further established the right hemisphere of structure **1**. The HMBC correlations of H-10/C-20 and H-13/C-12, C-20 indicated that **1** is a verticillene-type skeleton with an α,*β*-unsaturated *γ*-lactam ring. The relative stereochemistry of **1** was determined on the basis of NOESY correlations (Figure 2) and further confirmed by molecular modeling using MM2 minimized energy calculation (Figure 3). The NOESY spectra showed correlations of H-1/H-16/H-17, H-16/H-10, H-17/H-7, and H-7/H-10/H-9*β*, suggesting that H-1, H-10, Me-16, and Me-17 were in a *β*-orientation. In turn, the NOESY exhibited correlations between H-19/H-9α and H-19/H-6, in good agreement with the α-configuration of H-6.

Cespitulactam E (**2**), $[\alpha]_D$ −193 (CH_2Cl_2), had a molecular formula of $C_{22}H_{31}NO_3$ and eight degrees of unsaturation, as determined by HRESIMS (*m/z* 358.2383, $[M + H]^+$) and NMR

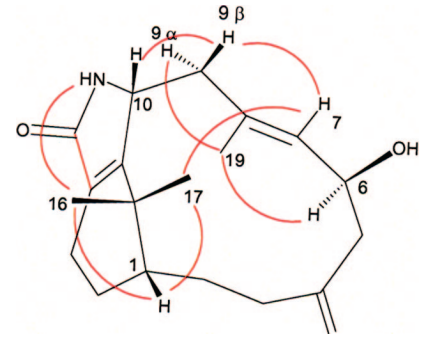


Figure 2. Selected NOESY correlations for **1**.

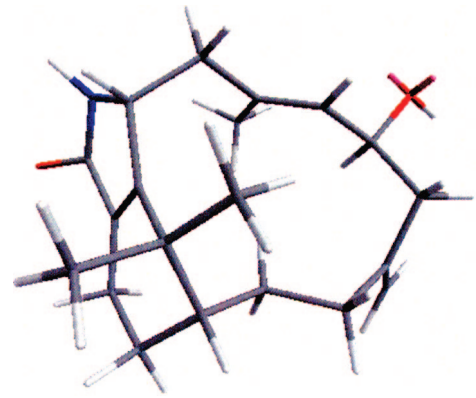


Figure 3. Computer-generated perspective model for **1** using a force field calculation.

data. The UV, IR, and ^1H and ^{13}C NMR data of **2** were similar to those of **1**, suggesting a close analogue of **1**. The differences between them were shifts for H-6 (*δ* 5.36 in **2**; *δ* 4.35 in **1**), H-2′ (*δ* 2.02, s in **2**), C-6 (*δ* 71.5 in **2**; *δ* 68.3 in **1**), C-1′ (*δ* 169.9 in **2**), and C-2′ (*δ* 21.3 in **2**). The structural assignment was further elucidated by COSY, HMQC, and HMBC spectra. Acetylation of **1** yielded a product identical with **2**, establishing the relative configuration of **2**.

Cespitulactam F (**3**), $[\alpha]_D$ −156 (MeOH), had a molecular formula of $C_{20}H_{29}NO_3$ and seven degrees of unsaturation, as derived from HRESIMS (*m/z* 332.2227, $[M + H]^+$) and NMR data. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **1**, suggesting that **3** was an analogue of **1**. Detailed comparison of the ^1H NMR spectrum of **3** with that of **1** revealed that the signal for H-10 in **1** was missing in **3**. Instead, a hydroxyl group appeared at C-10. The ^1H chemical shift of H-9 was shifted downfield from *δ* 2.66 and 2.75 in **1** to *δ* 2.74 and 2.98, respectively, in **3**. Moreover, the ^{13}C chemical shifts of C-9 and C-10 were also shifted downfield from *δ* 42.3 and 58.3 in **1** to *δ* 48.6 and 92.1 in **3**, respectively. The HMBC spectrum of **3** showed correlations of H-9/C-10, confirming the additional hydroxyl group at C-10. Upon acetylation, **3** afforded the monoacetate **11**, which exhibited an additional three-proton acetyl singlet at *δ* 2.02. The HRESIMS of **11** revealed a molecular ion at *m/z* 396.2149, $[M + Na]^+$, consistent with a molecular formula of $C_{22}H_{31}NO_4Na$. On the basis of these results, the structure of **3** was elucidated for cespitulactam F.

Cespitulactam G (**4**), $[\alpha]_D$ −204 (CH_2Cl_2), had the composition $C_{22}H_{33}NO_3$, as deduced by a combination of HRESIMS and NMR spectra. The ^1H and ^{13}C NMR data of compound **4** were very similar to those of **3**, except for proton signals at *δ* 3.20 (H-1′), 3.51 (H-1′), and 1.16 (H-2′) and carbon signals at *δ* 57.9 (C-1′) and 15.3 (C-2′). The HMBC correlation of H-1′/C-10 (*δ* 93.8) indicated that compound **4** possessed an ethyl group on the nitrogen atom instead of a proton in **3**. The assignment of ^1H and ^{13}C NMR data of **4** was accomplished by COSY, HMQC, and HMBC experiments. Therefore, the structure **4** was established for cespitulactam G.

Table 2. ^{1}H NMR Data for Compounds **5**−**8** (chemical shifts are in ppm; *J* values in Hz are in parentheses)

position	**5**[a]	**6**[b]	**7**[a]	**8**[b]
1	1.68 m	1.50 m	1.65 m	1.38 m
2	2.27 m	1.24 m	2.22 m	1.01 m
		1.84 m	2.40 m	1.51 m
3	1.59 m	1.95 m	1.58 m	1.95 m
				2.22 m
5	2.37 m	3.03 d (14.9)	2.32 m	2.19 m
		3.19 d (14.9)		2.62 m
6	4.34 m		4.31 m	4.44 m
7	5.40 d (7.7)	2.41 dd (3.3, 16.7)	5.32 d (7.5)	5.42 d (8.1)
		2.69 dd (3.3, 16.7)		
9	2.68 d (14.0)	4.68 d (10.5)	2.52 d (14.2)	2.82 d (15.5)
	2.72 d (14.0)		2.62 d (14.2)	3.29 d (15.5)
10	4.11 brs		3.91 brs	
11		2.38 m		
12				2.93 m
13	2.08 m	1.46 m	2.07 m	1.26 m
	2.38 m		2.33 m	2.09 m
14	1.70 m	2.05 m	1.70 m	1.38 m
	2.23 m		2.22 m	2.02 m
16	1.14 s	1.14 s	0.95 s	1.17 s
17	1.39 s	1.26 s	1.27 s	1.44 s
18	4.80 s	4.84 s	4.79 s	4.86 s
	4.82 s	5.00 s	4.80 s	4.90 s
19	1.41 s	1.10 d (6.8)	1.38 s	1.60 s
1′	3.02 s		3.31 m	3.29 m
			4.28 m	4.15 m
2′			3.05 m	2.69 m
4′			7.00 s	6.99 d (8.3)
5′			8.20 s (NH)	6.74 d (8.3)
7′			7.36 d (7.8)	
8′			7.18 m	
9′			7.12 m	
10′			7.61 d (7.6)	

[a] Recorded in $CDCl_3$ at 300 MHz. [b] Recorded in $CDCl_3$ at 500 MHz.

The molecular formula $C_{21}H_{31}NO_2$ of **5** ($[\alpha]_D$ −112, CH_2Cl_2), determined from HRESIMS, showed 30 units less than that of **4**. The ^{1}H and ^{13}C NMR spectra of cespitulactam H (**5**) were very similar in both chemical shifts and coupling constants to those of cespitulactam D (**1**) and **4**, except for the methyl signals of H-1′ (δ 3.02) and C-1′ (δ 27.1). The HMBC correlations between H-1′/C-10 (δ 62.8) and between H-10 (δ 4.11)/C-1′ indicated that the proton on the nitrogen atom in **1** was replaced by a methyl group in **5** rather than an ethyl group in **4**.

Cespitulactam I (**6**), $[\alpha]_D$ −3 (CH_2Cl_2), was isolated as a colorless, amorphous solid that had a molecular formula of $C_{20}H_{29}NO_3$, as deduced from HRESIMS ($[M + H]^+$ *m/z* 332.2225). Compound **6** was thus an isomer of **3**. The IR absorption at 3281 and 1659 cm^{-1} indicated the presence of hydroxyl and lactam groups. Data from the ^{13}C NMR and DEPT spectra (Table 3) suggested a ketone (δ 208.1), an amide carbonyl signal (δ 177.5), an exocyclic methylene (δ 116.3), an olefinic methine (δ 110.4), two olefinic quaternary carbons (δ 143.6 and 142.1), and an oxygen-bearing quaternary carbon at δ 79.3. The ^{1}H NMR data of **6** (Table 2) indicated the presence of three methyl groups (δ 1.10, d, J = 6.8 Hz; δ 1.14, s; δ 1.26, s), a pair of exocyclic methylene protons (δ 4.84, 5.00), and a methine proton (δ 4.68). The COSY spectrum of **6** revealed two separated proton sequences, including H-12/H-13/H-14/H-1/H-2 and H-7/H-8/H-19. The HMBC spectrum established connectivities of H-5/C-2, C-3, C-18, C-6 and H-16/C-1, C-15, C-11, as well as H-9/C-8, C-10, C-11. The relative configuration of **6** was determined by NOESY, which agreed with a β-configuration for H-1, H-12, Me-16, and Me-17. This was also supported by molecular modeling using a force field calculation and by comparison to those data of cespitulactams **1**–**5**. Accordingly, structure **6** was assigned.

The HRESIMS of cespitulactam J (**7**) gave a molecular ion peak at *m/z* 459.3009 ($M + H^+$), consistent with the molecular formula $C_{30}H_{38}N_2O_2$ and 13 degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **7** were similar to those of **1**, except that **7** contains an indole substituent (a fragment of $C_{10}H_{10}N$ and six degrees of unsaturation) on the nitrogen atom. Detailed analysis of the ^{1}H and ^{13}C NMR spectra of **7** revealed that the substituent was a 3-ethylindole (δ_H 8.20, s; 7.61, d; 7.36, d; 7.18, m; 7.12, m and 7.00, s; δ_C 40.3 CH_2 and 24.3 CH_2).[4] The HMBC correlation of H-1′ (δ 4.28)/C-10 (δ 60.6) indicated that the proton at the nitrogen in **1** was replaced by the 3-ethylindole group in **7**. The assignments of ^{1}H and ^{13}C NMR data of **7** were accomplished by COSY, HMQC, and HMBC experiments. Consequently, structure **7** was established for cespitulactam J.

The molecular formula of cespitulactam K (**8**) was determined as $C_{28}H_{38}NO_3$ on the basis of HRESIMS at *m/z* 436.2851 $[M + H]^+$ coupled with ^{1}H and ^{13}C NMR data (Tables 2 and 3). The IR absorption bands at 3312 and 1669 cm^{-1} indicated the presence of hydroxyl and lactam moieties, respectively. The above data for **8** were identical with those of cespitulactam C except that the α,β-unsaturated γ-lactam in cespitulactam C was replaced by a β,γ-unsaturated γ-lactam in **8**. This was supported by HMBC correlations of H-9 (δ 2.82, d, J = 15.5 Hz; δ 3.29, d, J = 15.5 Hz)/C-10 (δ 135.8, qC), C-11 (δ 126.1, qC), and H-17 (δ 1.44, s)/C-11, as well as H-12 (δ 2.93, m)/C-11. The appearance of a β,γ-unsaturated γ-lactam moiety in **8** could also be confirmed by the COSY cross-peaks of H-12/H-13 (δ 1.26 and 2.09). The relative configuration of **8** was determined by a NOESY experiment (Figure 4) and further by molecular modeling using the MM2 force field calculation. The NOESY correlations between H-1 (δ 1.38)/H-16 (δ 1.17 s) and H-16/H-12 indicated that H-1, H-12, Me-16, and Me-17 were all in the β-orientation. NOESY correlations between H-19 (δ 1.60)/H-17 and H-6 (δ 4.44)/H-19 agreed with the α-disposition of H-6.

A biogenetic pathway for these novel diterpenoids was proposed as shown in Scheme 1 based on the recently published biosynthesis of cespitulactams.[9] Compounds **1**, **4**, and **7** may be derived from intermediates **a** and **b** via amino transfer and amide formation. The occurrence of the amino, ethylamine, and tryptamine side chains in **1**, **4**, and **7** may be explained by incorporation of NH_3, alanine, and tryptamine to the intermediate **a**, respectively.

The cytotoxic activity of **1**−**8** and **11** was tested *in vitro* using human oral epidermoid carcinoma (KB) and murine L1210 leukemia cell lines. Compound **8** was active against both tumor cells at 3.7 and 5.1 μg/mL respectively, while others were inactive. All of the isolated diterpene alkaloids were also evaluated for antimicrobial activities against four selected microorganisms including *Micrococcus luteus*, *Staphylococcus aureus*, *Trichophyton mentagrophytes*, and *Crytococcus neoformans*.[15] Compound **4** exhibited potent antimicrobial activity against *Trichophyton mentagrophytes* (IFM45110) with an MIC value of 2.08 μg/mL. Compounds **1**, **7**, and **8** showed significant antimicrobial activity against *M. luteus* (IFM2066) and *C. neoformans* (IFM46914) (**6**–**8**) and *T. mentagrophytes* (**2** and **7**) with MIC values of 4.16 μg/mL. All compounds were inactive toward *Staphylococcus aureus* (209P).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were recorded using a Horiba FT-720 spectrophotometer. The ^{1}H and ^{13}C NMR spectra as well as 2D NMR spectra (COSY, HMQC, HMBC, and NOESY) were recorded in $CDCl_3$ (or CD_3OD) using Bruker DRX NMR spectrometers operating at 300 or 500 MHz for ^{1}H and 75 or 125 MHz for ^{13}C using the $CDCl_3$ solvent peak as internal standard (δ 7.26 for ^{1}H and δ 77.0 for ^{13}C). 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. Silica gel 60 (Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) were used for column chromatography.

Animal Material. *Cespitularia taeniata* May was collected in Green Island, Taiwan, in March 2004. This soft coral was identified by one

Table 3. ^{13}C NMR Data for Compounds **1**−**8** (assignments were made using HMQC and HMBC techniques)

	1[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[c]	**7**[a]	**8**[c]
1	42.9 CH	42.2 CH	45.7 CH	44.8 CH	43.1 CH	43.6 CH	43.0 CH	47.6 CH
2	18.3 CH_2	18.3 CH_2	18.8 CH_2	17.4 CH_2	18.4 CH_2	22.7 CH_2	18.4 CH_2	34.0 CH_2
3	31.9 CH_2	31.9 CH_2	33.9 CH_2	32.5 CH_2	32.0 CH_2	35.1 CH_2	31.8 CH_2	37.5 CH_2
4	146.7 C	145.8 C	148.7 C	146.2 C	146.6 C	143.6 C	146.7 C	146.1 C
5	43.7 CH_2	42.9 CH_2	45.2 CH_2	44.0 CH_2	43.8 CH_2	53.2 CH_2	43.8 CH_2	46.9 CH_2
6	68.3 CH	71.5 CH	69.9 CH	68.3 CH	68.4 CH	208.1 C	68.3 CH	70.2 CH
7	134.0 CH	135.9 CH	136.5 CH	135.3 CH	133.9 CH	49.1 CH_2	133.7 CH	130.9 CH
8	133.7 C	129.6 C	133.2 C	133.2 C	133.7 C	29.3 CH	133.6 C	131.0 C
9	42.3 CH_2	40.6 CH_2	49.4 CH_2	48.6 CH_2	38.6 CH_2	110.4 CH	38.3 CH_2	33.8 CH_2
10	58.3 CH	58.3 CH	92.1 C	93.8 C	62.8 CH	142.1 C	60.6 CH	135.8 C
11	131.9 C	132.0 C	132.0 C	131.7 C	132.0 C	79.3 C	131.8 C	121.6 C
12	162.7 C	162.7 C	165.1 C	160.7 C	160.2 C	49.8 CH	160.4 C	46.5 CH
13	31.8 CH_2	31.7 CH_2	33.9 CH_2	34.5 CH_2	32.3 CH_2	30.6 CH_2	32.2 CH_2	27.0 CH_2
14	24.1 CH_2	24.0 CH_2	25.0 CH_2	24.9 CH_2	24.3 CH_2	24.0 CH_2	24.2 CH_2	29.7 CH_2
15	36.9 C	36.8 C	38.3 C	37.7 C	36.8 C	40.9 C	36.7 C	40.2 C
16	34.9 CH_3	34.8 CH_3	34.7 CH_3	34.2 CH_3	35.1 CH_3	29.0 CH_3	34.8 CH_3	30.7 CH_3
17	24.9 CH_3	24.9 CH_3	25.0 CH_3	24.8 CH_3	25.3 CH_3	22.7 CH_3	25.1 CH_3	27.6 CH_3
18	113.3 CH_2	113.9 CH_2	114.2 CH_2	114.7 CH_2	113.6 CH_2	116.3 CH_2	113.4 CH_2	115.2 CH_2
19	18.5 CH_3	18.5 CH_3	17.1 CH_3	17.4 CH_3	16.9 CH_3	20.4 CH_3	17.0 CH_3	180.5C
20	173.8 C	174.0 C	174.1 C	172.1 C	170.8 C	177.5 C	171.0 C	15.9CH_3
1′				57.9 CH_2	27.1 CH_3		40.3 CH_2	41.7 CH_2
2′				15.3CH_3			24.3 CH_2	34.5 CH_2
3′							113.2 C	129.8 C
4′							121.9 CH	130.0 CH
5′								115.5 CH
6′							136.2 C	154.9 C
7′							111.2 CH	
8′							122.1 CH	
9′							119.5 CH	
10′							118.6 CH	
11′							127.4 C	

[a] Recorded in $CDCl_3$ at 75 MHz. [b] Recorded in CD_3OD at 75 MHz. [c] Recorded in $CDCl_3$ at 125 MHz.

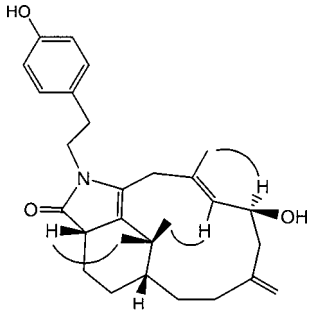
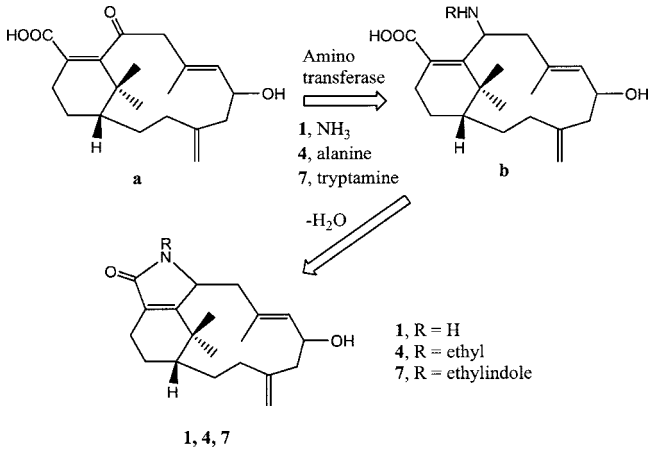


Figure 4. Key NOESY correlations for **8**.

Scheme 1. Proposed Biogenetic Pathway of Compounds **1**, **4**, and **7**



of the authors (Y.-C.S.). A voucher specimen (GSC-1) was deposited in the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The whole animals of *C. taeniata* (wet, 13.5 kg) were extracted with EtOH (20 L) at room temperature and concentrated under reduced pressure to afford a crude extract (21 g). The residue was partitioned between H_2O and EtOAc to yield an EtOAc-soluble fraction (15 g). A portion (14.5 g) was chromatographed on a Si gel column (150 g) and initially eluted with *n*-hexane (100%, 2 L), *n*-hexane−EtOAc (10:1 to 1:5, each 1 L), and finally MeOH (100%, 1 L) to give 16 fractions. Fraction 15 (1.2 g) was further chromatographed on a Si gel column (15 g) and eluted with *n*-hexane−CH_2Cl_2−MeOH (20:15:1) to yield fraction 15-2, cespitulactam D (**1**, 286 mg), and other fractions 15-1 to 15-6. Fraction 15-6 (155 mg) was further purified with a RP-HPLC column (MeOH−H_2O, 6:4) to give cespitulactam F (**3**, 18 mg). Separation of fraction 15-1 (220 mg) by HPLC using a solvent mixture of *n*-hexane−CH_2Cl_2−MeOH (10:10:1) yielded fractions 15-1-a (56 mg) and 15-1-b (27 mg). Fraction 15-1-a was further purified by RP-HPLC (MeOH−H_2O−CH_3CN, 65:35:5) to afford cespitulactam E (**2**, 5 mg). Fraction 15-1-b was also separated with RP-HPLC (MeOH−H_2O, 6:4) to give cespitulactam J (**7**, 11 mg). Fraction 13 (203 mg) was applied on a NP-HPLC column (Si gel) and developed with *n*-hexane−CH_2Cl_2−MeOH (10:10:1) to yield fractions 13-1 and 13-2 and cespitulactam B (**10**, 44 mg). Fraction 13-1 was further purified with a RP-HPLC column (MeOH−H_2O, 7:3) to afford cespitularin F (10 mg), cespitulactam G (**4**, 14 mg), cespitulactam H (**5**, 7 mg), and an impure fraction 13-1-a (3.3 mg). This fraction was further applied to a RP-HPLC column and developed with MeOH−H_2O−CH_3CN (65:35:5) to yield cespitulactam K (**8**, 7.5 mg). Fraction 10 (430 mg) was separated by HPLC and developed with *n*-hexane−CH_2Cl_2−MeOH (10:10:1) to yield cespitulactam A (**9**, 57 mg) and fraction 10− (41.0 mg). Fraction 10-6 was finally purified by HPLC using MeOH−H_2O (8:2) to yield cespitulactam I (**6**, 2 mg).

Cespitulactam D (1): colorless, amorphous solid; $[\alpha]^{25}_D$ −52 (*c* 0.08, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 226 (4.52) nm; IR (neat) υ_{max} 3314, 2931, 2870, 1676, 1448, 1382, 1360, 1266, 1186, 1144, 1031, 893, 736 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2, respectively; EIMS *m*/*z* 315 (7), 260 (5), 232 (11), 216 (18), 176 (16), 162 (23), 160 (20), 148 (18), 136 (31), 91 (20), 83 (36); HRESIMS *m*/*z* 316.2274 ($[M + H]^+$, calcd for $C_{20}H_{30}NO_2$, 316.2276).

Cespitulactam E (2): colorless, amorphous solid; $[\alpha]^{25}_D$ −193 (*c* 1.37, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 228 (5.11) nm; IR (neat) υ_{max} 3229, 2929, 2872, 1728, 1693, 1443, 1368, 1241, 1119, 1019, 954,

898, 736 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2, respectively; EIMS *m/z* 357 (3), 313 (1), 298 (2), 188 (5), 176 (5), 162 (10), 160 (20), 136 (16), 122 (15), 107 (21), 91 (25); HRESIMS *m/z* 358.2383 ($[M + H]^+$, calcd for $C_{22}H_{32}NO_3$, 358.2382).

Cespitulactam F (3): pale white powder; $[\alpha]^{25}_D$ −156 (*c* 1.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (4.48) nm; IR (neat) υ_{max} 3349, 2925, 1685, 1457, 1260, 1033, 735 cm^{-1}; ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Tables 1 and 2, respectively; EIMS *m/z* 331 (1), 313 (2), 280 (4), 247 (5), 232 (13), 176 (14), 162 (15), 152 (20), 133 (23), 122 (29), 107 (60); HRESIMS *m/z* 332.2227 ($[M + H]^+$, calcd for $C_{20}H_{30}NO_3$, 332.2226).

Cespitulactam G (4): colorless, amorphous solid; $[\alpha]^{25}_D$ −204 (*c* 1.50, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 226 (4.61) nm; IR (neat) υ_{max} 3269, 2971, 2828, 1694, 1441, 1414, 1386, 1267, 1118, 1071, 992, 895, 736 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2, respectively; EIMS *m/z* 359 (0.4), 326 (2), 312 (3), 275 (11), 260 (22), 246 (10), 232 (9), 204 (10), 175 (16), 152 (15), 133 (18); HRESIMS *m/z* 360.2536 ($[M + H]^+$, calcd for $C_{22}H_{34}NO_3$, 360.2539).

Cespitulactam H (5): colorless, amorphous solid; $[\alpha]^{25}_D$ −112 (*c* 0.7, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 228 (4.52) nm; IR (neat) υ_{max} 3378, 2926, 1663, 1443, 1387, 1250, 1077, 1027, 894, 734, 669 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 2 and 3, respectively; EIMS *m/z* 329 (4), 328 (3), 316 (4), 244 (2), 232 (2), 143 (100), 130 (81), 117 (9), 103 (9), 91 (19), 83 (28); HRESIMS *m/z* 330.2431 ($[M + H]^+$, calcd for $C_{21}H_{32}NO_2$, 330.2433).

Cespitulactam I (6): colorless, amorphous solid; $[\alpha]^{25}_D$ −3 (*c* 0.2, CH_2Cl_2); IR (neat) υ_{max} 3380, 2925, 2870, 1703, 1680, 1457, 1375, 1288, 1050, 981, 735, 668 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 2 and 3, respectively; EIMS *m/z* 331 (3), 316 (2), 232 (1), 214 (3), 206 (2),192 (5), 180 (18), 151 (19), 138 (17), 112 (14), 68 (23); HRESIMS *m/z* 332.2225 ($[M + H]^+$, calcd for $C_{20}H_{30}NO_3$, 332.2226).

Cespitulactam J (7): colorless, amorphous solid; $[\alpha]^{25}_D$ −119 (*c* 1.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 274 (2.84), 221(4.88) nm; IR (neat) υ_{max} 3281, 2926, 1659, 1451, 1340, 1223, 1010, 894, 740, 668 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 2 and 3, respectively; EIMS *m/z* 458 (6), 328 (3), 316 (4), 244 (2), 232 (2), 143 (100), 130 (81), 117 (9), 103 (9), 91 (19), 83 (28); HRESIMS *m/z* 459.3009 ($[M + H]^+$, calcd for $C_{30}H_{39}N_2O_2$, 459.3011).

Cespitulactam K (8): colorless, amorphous solid; $[\alpha]^{25}_D$ +53 (*c* 0.75, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 274 (3.30), 229 (4.89) nm; IR (neat) υ_{max} 3312, 2928, 2865, 1669, 1515, 1412, 1384, 1264, 1170, 1013, 889, 827, 736 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 2 and 3, respectively; EIMS *m/z* 435 (6), 351 (3), 328 (14), 256 (4), 244 (24), 230 (4), 216 (6), 160 (8), 120 (100), 107 (80), 77 (56); HRESIMS *m/z* 436.2851 ($[M + H]^+$, calcd for $C_{28}H_{38}NO_3$, 436.2852).

6-*O*-acetylcespitulactam F (11). Compound **3** (10.3 mg) was treated with Ac_2O/pyridine (1:1) for 9 h at room temperature. To the reaction mixture was added water (10 mL) and extracted with EtOAc (10 mL × 3) to yield 11.1 mg of 6-*O*-acetylcespitulactum F (**11**) as a white powder: ^1H NMR ($CDCl_3$, 300 MHz) 5.48 (1H, d, J = 8.6 Hz, H-7), 5.32 (1H, m, H-6), 4.79 (2H, s, H-18), 3.00 (1H, d, J = 14.0, H-9), 2.78 (1H, d, J = 14.0, H-9), 2.45 (2H, dd, J = 8.4, 13.1 Hz, H-5), 2.29 (1H, m, H-13), 2.23 (1H, m, H-14), 2.18 (2H, m, H-2), 2.07 (1H, m, H-13), 1.71 (1H, m, H-14), 1.60 (1H, m, H-1), 1.56 (2H, m, H-3), 1.50 (3H, s, H-17), 1.31 (3H, s, H-16); ^{13}C NMR ($CDCl_3$, 75 MHz) 174.2 (C-20), 163.4 (C-11), 145.7 (C-4), 133.6 (C-7), 131.4 (C-12), 131.3 (C-8), 114.4 (C-18), 90.9 (C-10), 71.4 (C-6), 48.4 (C-9), 44.1 (C-1), 40.6 (C-5), 37.2 (C-15), 35.5 (C-13), 34.4 (C-16), 32.1 (C-3), 24.3 (C-17), 23.9 (C-14), 17.8 (C-2), 17.3 (C-19); EIMS *m/z* 373 (1), 313 (2), 280 (4), 247 (5), 232 (13), 176 (14), 162 (15), 152 (20), 133 (23), 122 (29), 107 (60); HRESIMS *m/z* 396.2149 ($[M + Na]^+$, calcd for $C_{22}H_{31}NO_4Na$, 396.2151).

Cytotoxicity Assay. [16,17] Human oral epidermoid carcinoma (KB) and mouse leukemia L1210 cell lines were used. These cells were cultured in an incubator at 37 °C for 48 h in 1 mL of medium containing various concentrations of test compounds dissolved in 0.6% Me_2SO. The IC_{50} values were obtained by plotting the logarithm of the concentration of the test compound versus the growth rate of the treated cells.

Acknowledgment. The authors thank the National Science Council, Republic of China (NSC-95-2323-B-002-018), for financial support. C.-L. Ho, R.-R. Wu, and S.-C. Yu of NSC Southern NMR and MS Instrument Center are acknowledged for measurement of high-resolution NMR (500 MHz) and MS data.

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NP078011U