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# Cespitulactones A and B, new diterpenoids from *Cespitularia taeniata*

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**Abstract**—Two new diterpenoids, designated cespitulactones A (**1**) and B (**2**), were isolated from a sample of the soft coral *Cespitularia taeniata* collected in Taiwan. Compound **1** possesses a novel structure with a bond cleavage between C-10 and C-11, and having a 14-membered lactone ring junction between C-10 and C-12. Their structures were elucidated on the basis of extensive spectroscopic analysis and chemical derivatization. The isolated compounds were also evaluated for cytotoxicity toward human cancer cell lines.


Cespitularins are rare diterpenoids known only from soft corals, especially members of the genus *Cespitularia*.[1,2] These marine organisms produce very interesting secondary metabolites, whose structures and biological activities are similar to those of taxane diterpenoids in the terrestrial plant genus *Taxus*.[3,4] Of particular interest is the recent discovery of a series of norditerpenes, which appear to be biogenically derived from geranylgeranyl pyrophosphate and 1S-verticillene via loss of a methyl unit.[5] The southern coast of Taiwan has long been a habitat of soft corals. Among them, specimens of *Cespitularia* are occasionally encountered and have different color variants similar to species of *Xenia*. The polyps of *Cespitularia* are like those of *Xenia,* but are not restricted to the branch ends. In the search for bioactive constituents from Taiwanese marine soft corals,[6–8] a novel diterpenoid designated cespitulactone A (**1**) with an unusual bond cleavage between C-10 and C-11, and having a 14-membered lactone ring connection between C-10 and C-12 has been isolated from *Cespitularia taeniata*. In addition, a new compound, cespitulactone B (**2**), together with cespitularin F (**3**), 6-*O*-acetylcespitularin F (**4**), cespitularine D (**5**), and flaccidoxide-13-acetate (**6**) was also isolated and characterized. In this paper, we describe the isolation, structure elucidation, presumed biogenetic pathway, and cytotoxicity of these new marine metabolites.

The soft coral (GSCII-14, wet wt 1.1 kg) collected in December, 2003, at a depth of 25 m was extracted with a mixture of $CH_2Cl_2$ and MeOH, and the extract was partitioned between EtOAc and $H_2O$ (1:1). The EtOAc-soluble fraction (6 g) was subjected to an Si gel column (*n*-hexane/EtOAc, 1:0–0:1) and HPLC (Si gel, *n*-hexane/EtOAc, 3:2; RP-$C_{18}$, MeOH/$H_2O$, 8:2) to furnish cespitulactones A (**1**, 32 mg), B (**2**, 20 mg), cespitularin F (**3**, 520 mg),[2] 6-*O*-acetylcespitularin F (**4**, 7 mg), cespitularin D (**5**, 16 mg),[2] and flaccidoxide-13-acetate (**6**, 35 mg).[9]

Cespitulactone A (**1**),[10] [α] −122° ($CH_2Cl_2$), was obtained as an amorphous powder and had the molecular formula $C_{19}H_{28}O_4$, as derived from its HRESIMS data indicating six degrees of unsaturation. The presence of hydroxyl, carbonyl, and lactonyl functions was evidenced by IR absorptions at 3442, 1737, and 1715 $cm^{-1}$. The $^1H$ NMR, $^{13}C$ NMR spectra (Table 1), and DEPT revealed that **1** contained a ketone carbonyl ($\delta$ 211.1), an ester ($\delta_C$ 169.7), a trisubstituted olefin, a 1,1-disubstituted olefin, one aliphatic quaternary carbon ($\delta_C$ 47.5), two oxygenated methine carbons ($\delta_C$ 68.7 and 72.2), six methylene carbons ($\delta_C$ 20.4, 26.3, 28.9, 33.4, 44.3, and 46.3), and three methyl groups ($\delta_C$ 16.9, 23.1, and 27.7; $\delta_H$ 1.71, 1.21, and 1.14). The corresponding proton and carbon

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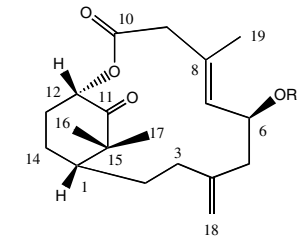
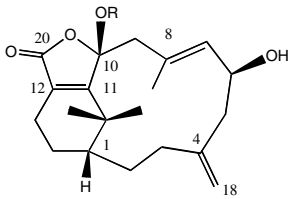
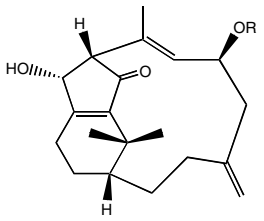


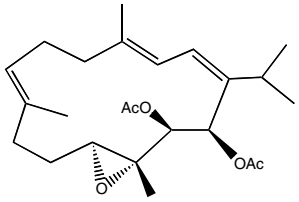
**1**, R = H
**7**, R = Bz
**8**, R = Ac
**9**, R = 4-Chloro-Bz



**2** R = Me
**5** R = H



**3**, R = H
**4**, R = Ac



**6**

assignments were further determined by COSY and HMQC experiments. The COSY spectrum revealed the presence of a simple continuous spin-system including correlations of H-9α ($\delta$ 2.84, d, $J$ = 13.5 Hz)/H-9β ($\delta$ 3.21, d, $J$ = 13.5 Hz)/Me-19/H-7/ H-6 ($\delta$ 4.45, td, $J$ = 8.7, 2.4 Hz)/H-18/H-5/H-3/ H-2/ H-1 and H-12/H-13/H-14/H-1. This partial structure was verified by HMBC data, which confirmed the connectivities of Me-16/C-1, C-15 and Me-17/C-1, C-15 and H-18/C-3, C-4, C-5 as well as H-6/C-4, C-5, C-7, C-8 (Table 1). The above findings account for 4 of the 6 degrees of unsaturation, indicating two more rings in structure **1**. Benzoylation of **1** yielded a monobenzoate, in which H-6 and C-6 were shifted to $\delta$ 5.75 and $\delta$ 72.0, respectively, as shown in the $^{1}$H and $^{13}$C NMR spectra of **7**.[11] The location of an ester carboxyl at C-10 and the assignment of a ketone at C-11 together with the connection between C-10 and the C-12 oxygen atom were assigned on the basis of HMBC correlations of $H_2$-9/C-10, H-12/C-10, H-12/C-11, Me-16/C-11, Me-17/C-11, and H-1/C-11, indicating that compound **1** possesses an unusual bicyclic norditerpenoid system. The relative stereochemistry of cespitulactone A (**1**) was determined by analyses of NOESY correlations. Assuming that **1** has the same absolute configuration at C-1 as other naturally occurring cespitularines and taxoids,[2,12] A NOESY experiment was performed to ascertain the relative stereochemistry of C-12, Me-16, Me-17, and C-6. The presence of mutual correlations between H-1, Me-16, Me-17, and H-12 agreed with all β-configuration, while H-6 was α-configuration. The NOESY correlation is summarized in Figure 1. The configuration of the hydroxyl at C-6 was further determined by Mosher's reactions[13] to yield compounds **10** and **11**.[14] The results, illustrated in Figure 2, suggested that the

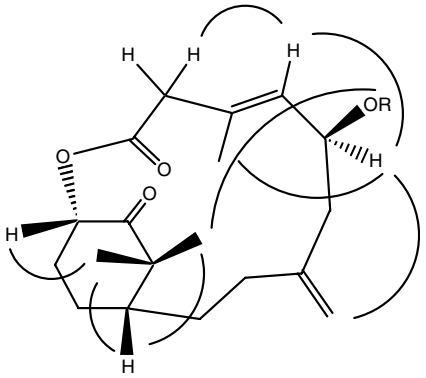
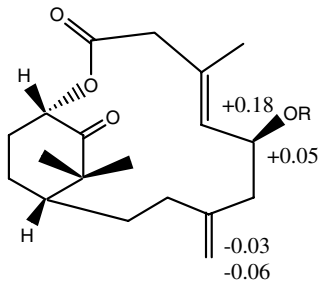


**Figure 1.** Key NOESY correlations and relative stereochemistry of **1** and **7**.

**Table 1.** $^{1}$H and $^{13}$C NMR data, HMBC and COSY correlations of **1**[a]

| Position | $\delta_H$ (mult, $J$, Hz) | $\delta_C$ | HMBC $^{1}$H–$^{13}$C | COSY $^{1}$H–$^{1}$H |
|---|---|---|---|---|
| 1 | 1.85 (m) | 43.0 | 11, 15 | 2, 14 |
| 2 | 1.56 (m), 1.65 (m) | 28.9 | 1 | 1, 3 |
| 3 | 1.80 (m), 1.95 (m) | 33.4 | | 2 |
| 4 | | 146.2 | | |
| 5 | 2.28 (m), 2.47 (m) | 44.3 | 3, 4, 18 | 6 |
| 6 | 4.45 (td, 8.7, 2.4) | 68.7 | 4, 5, 7, 8 | 5, 7 |
| 7 | 5.38 (d, 8.7) | 134.3 | | 6, 19 |
| 8 | | 130.3 | | |
| 9α | 2.84 (d, 13.5) | 46.3 | 7, 8, 10, 19 | 9β |
| 9β | 3.21 (d, 13.5) | | | 9α |
| 10 | | 169.7 | | |
| 11 | | 211.1 | | |
| 12 | 5.06 (dd, 5.7, 3.9) | 72.2 | 10, 11, 13, 14 | 13 |
| 13 | 2.40 (m), 1.85 (m) | 26.3 | 11 | 12, 14 |
| 14 | 1.90 (m), 1.60 (m) | 20.4 | | 1, 13 |
| 15 | | 47.5 | | |
| 16 | 1.21 (s) | 27.7 | 1, 11, 15, 17 | |
| 17 | 1.14 (s) | 23.1 | 1, 11, 15, 16 | |
| 18 | 4.83 (s), 4.85 (s) | 112.2 | 3, 4, 5 | 3, 5 |
| 19 | 1.71 (s) | 16.9 | 9 | 7 |

[a] Data were recorded in $CDCl_3$ on 300 MHz; chemical shifts ($\delta$) and coupling constant are given in parts per million and hertz, respectively.




**10**, R = *R*-MPTA
**11**, R = *S*-MPTA

**Figure 2.** $\delta_S$–$\delta_R$ values (ppm) for Mosher's reaction products **10** and **11**.

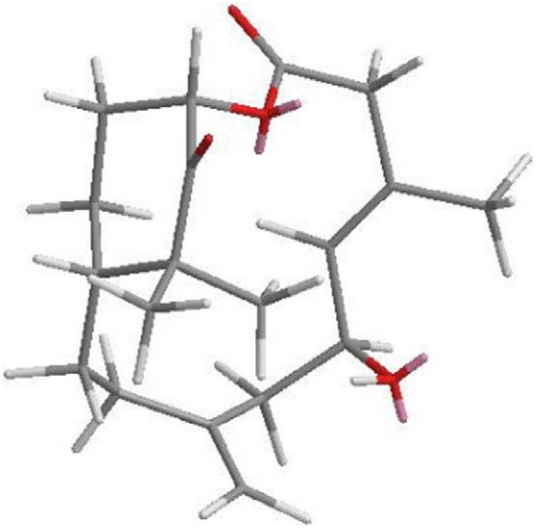
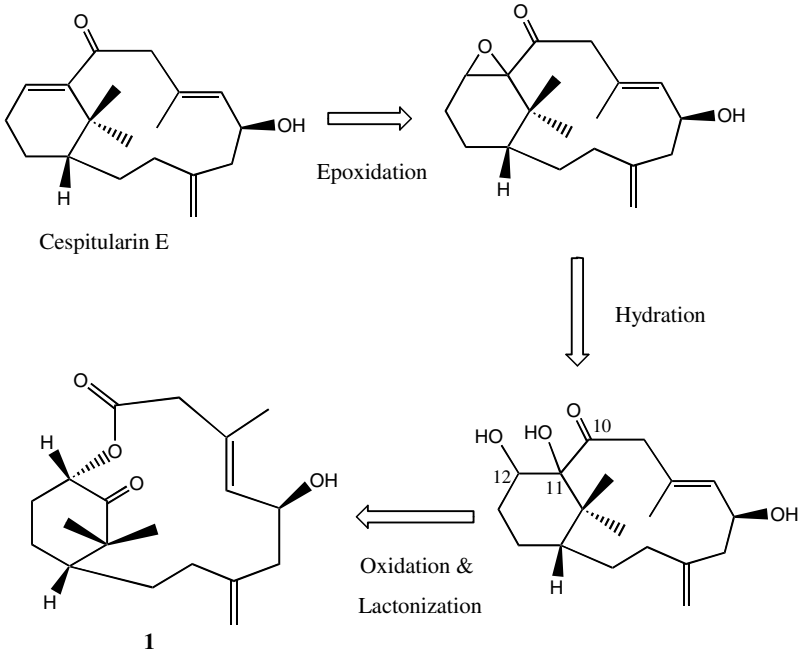


**Figure 3.** Computer-generated perspective model for **1** using MM2 force field calculation.

C-6 has the *S*-configuration. Thus, the absolute stereochemistry of **1** was proposed as shown. A computer-modeled structure of **1** was generated by CS Chem 3D version 9.0 using MM2 force field calculation for energy minimization as shown in Figure 3.

Compound **2** possesses the molecular formula $C_{21}H_{30}O_4$, as deduced from the HRESIMS data, indicating 7 degrees of unsaturation.[15] The UV and IR spectra of **2** showed the presence of α,β-unsaturated lactone and hydroxy functionalities, respectively. The $^1$H NMR spectrum of **2** exhibited characteristic signals including a doublet at $\delta$ 5.51, two singlets at $\delta$ 4.83 and $\delta$ 4.85, and a multiplet at $\delta$ 4.37. The $^{13}$C NMR spectrum of **2** showed signals of a conjugated ester carbon ($\delta$ 172.1), three methyl carbons ($\delta$ 33.7, 24.5, 17.1), and two quaternary carbons at $\delta$ 111.0 (C-10) and 37.5 (C-15). The proton and carbon assignments were determined by the COSY and HMQC. Detailed comparison of the $^1$H and $^{13}$C NMR spectral data with those of cespitularins revealed that compound **2** is a close analog of cespitularin D, different in the additional methoxyl group ($\delta$ 3.28 and $\delta$ 50.8). Further, the HMBC experiment revealed correlation of MeO/C-10 and H-9/C-10, indicating that the methoxy group was located at C-10. The cross peaks between the methoxy and Me-16 observed in the NOESY established the relative configurations of **2**.

A plausible biogenetic pathway of **1** was proposed as shown in Scheme 1 based on biosynthesis of taxane diterpenes and recently published norditerpenes such as cespitularin E.[2] Compound **1** might be derived from the norditerpenoid, cespitularine E, via epoxidation, hydration, oxidation, and lactonization that involves bond cleavage between C-10 and C-11, and subse-




**Scheme 1.** Plausible biogenetic pathway of **1**.

quent attack of the C-12 hydroxy on the carbonyl at C-10.

This paper describes the first isolation of the novel diterpenoid **1** from *Cespitularia taeniata*, which belongs to the family Xeniidae. To study the structure activity relationship of compound **1**, two additional derivatives cespitulactone A monoacetate (**8**)[16] and cespitulactone A 4-chlorobenzoate (**9**)[17] were prepared for antitumor testing. Human cancer cell lines were chosen to test compounds **1**–**9** for in vitro cytotoxicity. As a result, compound **1** exhibited significant cytotoxicity against human cervical epitheloid carcinoma (HeLa) and colon adenocarcinoma (DLD-1) cancer cells with $IC_{50}$ of 3.69 and 9.98 μg/mL, respectively. Flaccidoxide-13-acetate (**6**) showed mild activity against human medulloblastoma (Daoy) and colon (WiDr) cancer cells at 16.9 and 13.8 μg/mL, respectively. The other derivatives were inactive (>20 μg/mL), suggesting that the hydroxyl at C-6 in **1** is critical in cytotoxicity.

*Cytotoxicity assay*. The bioassay used against human cervical epitheloid carcinoma (HeLa), colon adenocarcinoma (DLD-1, WiDr), and medullocarcinoma (Daoy) cancer cells was based on a MTT assay method. The assay procedure was carried out as previously described.[18]

## Acknowledgment

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10. Amorphous powder, $[\alpha]_D^{25}$ −122 (*c* 1.0, $CH_2Cl_2$); IR (neat) $\nu_{max}$ 3442, 2928, 1737, 1715, 1639, 1443, 1258, 1023, 896, 736 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 235 nm; FABMS *m/z* 341 $[M+Na-2H]^+$; EIMS *m/z* (rel int) 321 ($[M+H]^+$, 0.1), 111 (10), 93 (8.1), 91 (9.6), 84 (83), 83 (100), 69 (31), 55 (87); HRESIMS *m/z* 343.1883 ($C_{19}H_{28}O_4Na$, calcd 343.1885).
11. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.85 (m, H-1), 1.60 (2H, m, H-2), 2.40 (2H, m, H-3), 2.50–2.60 (2H, m, H-5), 5.75 (1H, td, *J* = 9.3, 2.7 Hz, H-6), 5.49 (1H, d, *J* = 9.0 Hz, H-7), 2.90 (1H, d, *J* = 13.8 Hz, H-9α), 3.22 (1H, d, *J* = 13.8 Hz, H-9β), 5.10 (1H, m, H-12), 1.90 (1H, m, H-13), 2.40 (1H, m, H-13), 1.65 (2H, m, H-14), 1.25 (3H, s, H-16), 1.18 (3H, s, H-17), 4.92 (1H, s, H-18), 4.98 (1H, s, H-18), 1.86 (3H, s, H-19), 8.03 (2H, d, *J* = 7.5 Hz), 7.43 (2H, t, *J* = 7.5 Hz), 7.54 (1H, d, *J* = 7.5 Hz); $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 43.1 (d, C-1), 28.8 (t, C-2), 33.8 (s, C-3), 145.5 (s, C-4), 43.1 (t, C-5), 72.0 (d, C-6), 133.2 (d, C-7), 130.6 (s, C-8), 46.5 (t, C-9), 169.6 (s, C-10), 211.2 (s, C-11), 72.2 (d. C-12), 26.2 (t, C-13), 20.2 (t, C-14), 47.8 (s, C-15), 27.7 (q, C-16) 23.2 (q, C-17), 112.7 (t, C-18), 17.3 (q, C-19), 165.7 (s, COO), 133.0 (s), 128.4 (d), 129.6 (d), 130.0 (d); FABMS *m/z* 447 $[M+Na]^+$.
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14. Compound **10**: $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.72 (1H, td, *J* = 8.6, 2.3 Hz, H-6), 5.13 (1H, m, H-7), 5.14 (1H, overlap, H-12), 0.93, 0.92 (6H, s, H-16, -17), 4.76 (1H, s, H-18), 4.84 (1H, s, H-18), 1.64 (3H, s, H-19).
Compound **11**: $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.77 (1H, td, *J* = 8.5, 2.2 Hz, H-6), 5.31 (1H, d, *J* = 8.4 Hz, H-7), 5.13 (1H, m, H-12), 0.94, 0.92 (6H, s, H-16, -17), 4.73 (1H, s, H-18), 4.76 (1H, s, H-18), 1.64 (3H, s, H-19).
15. Amorphous powder, $[\alpha]_D^{25}$ −61 (*c* 2.0, $CH_2Cl_2$); IR (neat) $\nu_{max}$ 3461, 2927, 1765, 1637, 1445, 1280, 1112, 1017, 893 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 222 nm; $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.65 (m, H-1), 1.45 (1H, m, H-3), 2.40 (1H, m, H-5), 4.37 (1H, dt, *J* = 7.8, 2.7 Hz, H-6), 5.51 (1H, d, *J* = 7.8 Hz, H-7), 2.84 (1H, d, *J* = 14.1 Hz, H-9α), 3.02 (1H, d, *J* = 14.1 Hz, H-9β), 1.45 (1H, m, H-13), 1.25 (3H, s, H-16), 1.44 (3H, s, H-17), 4.83 (1H, s, H-18), 4.85 (1H, s, H-18), 1.62 (3H, s, H-19), 3.28 (3H, s, OMe); $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 44.0 (d, C-1), 17.8 (t, C-2), 32.2 (s, C-3), 146.6 (s, C-4), 43.8 (t, C-5), 68.3 (d, C-6), 135.8 (d, C-7), 131.2 (s, C-8), 46.7 (t, C-9), 111.0 (s, C-10), 166.2 (s, C-11), 130.0 (s. C-12), 32.2 (t, C-13), 24.4 (t, C-14), 37.5 (s, C-15), 33.7 (q, C-16) 24.5 (q, C-17), 114.6 (t, C-18), 17.1 (q, C-19), 172.1 (s, C-20), 50.8 (q, OMe); FABMS *m/z* 369 $[M+Na]^+$; EIMS *m/z* (rel int) 321 ($[M+H]^+$, 0.1), 191 (0.2), 177 (0.2), 111 (2.7), 105 (4.7), 91 (13.4), 84 (88), 83 (100), 55 (81); HRESIMS *m/z* 369.2044 ($[M+Na]^+$, calcd for $C_{21}H_{31}O_4$ Na, 369.2042).
16. Cespitulactone A monoacetate (**8**): $[\alpha]_D^{25}$ −189 (*c* 0.05, $CH_2Cl_2$); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.85 (m, H-1), 1.61 (2H, m, H-2), 1.90 (2H, m, H-3), 2.36 (2H, m, H-5), 5.48 (1H, td, *J* = 9.1, 2.6 Hz, H-6), 5.35 (1H, d, *J* = 8.4 Hz, H-7), 2.89 (1H, d, *J* = 13.3 Hz, H-9α), 3.20 (1H, d, *J* = 13.3 Hz, H-9β), 5.09 (1H, m, H-12), 1.86 (1H, m, H-13), 1.90 (1H, m, H-13), 1.75 (2H, m, H-14), 1.23 (3H, s, H-16), 1.16 (3H, s, H-17), 4.91 (1H, s, H-18), 4.88 (1H, s, H-18), 1.79 (3H, s, H-19), 2.03 (3H, s, OAc); ESIMS *m/z* 385 $[M+Na]^+$.
17. Cespitulactone A 4-chlorobenzoate (**9**): $[\alpha]_D^{25}$ −8 (*c* 0.05, $CH_2Cl_2$); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.92 (m, H-1), 1.57 (2H, m, H-2), 2.45 (2H, m, H-3), 2.52 (2H, m, H-5), 5.73 (1H, td, *J* = 9.2, 2.6 Hz, H-6), 5.48 (1H, d, *J* = 9.9 Hz, H-7), 2.91 (1H, d, *J* = 13.2 Hz, H-9α), 3.23 (1H, d, *J* = 13.2 Hz, H-9β), 5.10 (1H, m, H-12), 1.95 (1H, m, H-13), 2.45 (1H, m, H-13), 1.60 (2H, m, H-14), 1.25 (3H, s, H-16), 1.18 (3H, s, H-17), 4.92 (1H, s, H-18), 4.97 (1H, s, H-18), 1.85 (3H, s, H-19), 7.96 (2H, d, *J* = 8.1 Hz), 7.41 (1H, d, *J* = 8.1 Hz); ESIMS *m/z* 481, 483 $[M+Na]^+$.
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