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Cespitulins A–D, novel diterpenoids from Taiwanese *Cespitularia taeniata*

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ABSTRACT

Four novel diterpenoids, designated cespitulins A–D (**1**–**4**) were isolated and characterized from the Taiwanese soft coral *Cespitularia taeniata*. Their structures were determined by extensive spectroscopic analyses (^{1}H-^{1}H COSY, HMQC, HMBC, NOESY). Compounds **1**–**4** possess an unprecedented bond cleavage between C-9 and C-10 with a hemiacetalic lactone ring rather than a verticillane skeleton.

1. Introduction

Soft corals are widespread in shallow waters at the southern coast of Taiwan. Species of the genus *Cespitularia* are characterized by polyps on their soft branches with white, cream, blue, brown, or iridescent-green surfaces.[1] It is a rich source of secondary metabolites with novel, diverse chemical frameworks, and interesting biological activities such as antitumor, antibacterial, and immunomodulatory activities.[2–4] Soft corals belonging to *Cespitularia* were reported to produce various diterpenoids possessing sesquiterpene, caryophyllane, neodolabellane, dolabellane, cespitularane, cespitulactone, cespitulactam, norverticillane, and verticillane skeletons.[5–12] Due to carbon skeletons of these secondary metabolites being very close to the taxane diterpenoids isolated from terrestrial yew,[13,14] it is very interesting to investigate their structures and structure activity relationships of verticillane analogues.

Our previous chemical study of this species has resulted in the isolation of a series of verticillene diterpenes. Some of these compounds are nitrogen-containing molecules.[15–20] In our constant research programs oriented toward the investigation for bioactive natural products from Taiwanese soft corals of the genus *Cespitularia*, the EtOAc/CH_2Cl_2 extract of *C. taeniata* was investigated. This Lettter reports the chemical investigation of new secondary metabolites from *C. taeniata*. The examination of different chromatographic fractions of *C. taeniata* extract has led to the isolation of four novel diterpenoids, designated cespitulins A–D (**1–4**) (Fig. 1). The structures of these compounds were elucidated through detailed spectroscopic analyses, mainly 2D NMR experiments (^{1}H-^{1}H-COSY, HQMC, HMBC). The configuration at the chiral centers and the geometry of the double bonds were deduced from NOESY spectra and by application of molecular modeling.

2. Results and discussions

Compounds **1** and **2** were obtained as light yellow amorphous mixture, and both of them had a molecular formula $C_{20}H_{30}O_5$ as derived from its HR-ESI-MS and NMR spectroscopic data, indicating six degrees of unsaturation. The IR spectrum of compounds **1** and **2** suggested the presence of a diagnostic hydroxy (3385 cm^{-1}) and lactone (1738 cm^{-1}) groups. The ^{1}H NMR spectroscopic features of **1** is similar to that of **2** (Table 1), including a doublet at δ 5.44 (J = 7.8 Hz, H-7), a doublet of doublet at δ 4.52 (J = 14.4, 7.5 Hz, H-6), a pair of singlet at δ 4.91 and 4.93 in addition to three methyl singlets (δ 1.05, 1.26, 1.75). The comparison of ^{13}C NMR spectrum for both **1** and **2** displayed signal of an ester carbonyl (δ_C 170.0 s for **1** and **2**) two trisubstituted olefinic carbons (δ_C 137.7, 127.0, and δ_C 137.7, 127.1 for **1** and **2**, respectively), an exocyclic double bond (δ_C 145.6 s, 113.3 t, and δ_C 145.5 s, 113.3 t for **1** and **2**, respectively), and three corresponding methyl carbons (δ_C 20.7 q, 24.3 q, 14.0 q, and δ_C 20.3 q, 23.8 q, 14.0 q for **1** and **2**, respectively). Subsequently, the methylene of H-9 shifted downfield to δ_H 4.00 suggesting that a hydroxyl group is attached at C-9. Besides, a pair of tetrasubstituted olefinic carbons appeared at δ_C 136.4 and 158.6 for **1** and δ_C 136.4 and 158.9 for **2** were characterized. The assignment of compounds **1** and **2** was completely established by two-dimensional NMR experiments, in which the ^{1}H-^{1}H COSY experiment showed two sets of correlations (H-13/H-14, H-14/H-1, H-1/H-2, H-2/H-3 as well as the H-5/H-6, H-6/H-7) and these

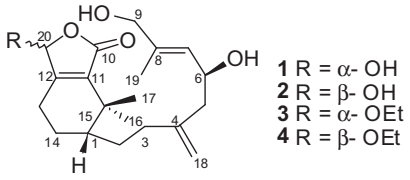

Figure 1. Compounds **1**–**4** isolated from the soft coral *Cespitularia taeniata*.

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Table 1
^{1}H NMR spectroscopic data of **1**–**4** (mult, *J* in Hz)

No.	**1**[a]	**2**[a]	**3**[a]	**4**[b]
1	1.30 (m)	1.30 (m)	1.22 (m)	1.28 (m)
2	1.17, 1.67 (m)	1.17, 1.67 (m)	1.10, 1.70 (m)	1.19 (m), 1.71 (m)
3	2.00, 2.20 (m)	2.00, 2.20 (m)	1.98, 2.20 (m)	2.04 (dd, 8.5, 15.5) 2.28 (dm, 4.5)
5	2.20 (m)	2.20 (m)	2.20 (m)	2.25 (d, 7.0)
6	4.52 (dd, 7.6, 14.8)	4.52 (dd, 7.6, 14.8)	4.50 (m)	4.55 (dd, 8.5, 15.0)
7	5.45 (d, 8.4)	5.45 (d, 8.4)	5.44 (d, 8.8)	5.49 (dd, 1.5, 8.5)
9	4.00 (s)	4.00 (s)	3.99 (s)	4.04 (s)
12				
13	2.10, 2.45 (m)	2.10, 2.45 (m)	2.20, 2.28 (m)	2.42 (m) 2.12 (dd, 5.0, 19.0)
14	1.37, 1.95 (m)	1.37, 1.95 (m)	1.33, 1.85 (m)	1.38 (m) 1.90 (dm, 6.0)
16	1.26 (s)	1.26 (s)	1.27 (s)	1.30 (s)
17	1.00 (s)	1.02 (s)	1.00 (s)	1.05 (s)
18	4.87 (s)	4.90 (s)	4.85, 4.88 (s)	4.91, 4.93 (s)
19	1.70 (s)	1.70 (s)	1.70 (s)	1.74 (s)
20	5.81 (s)	5.83 (s)	5.53 (s)	5.57 (s)
21			3.68, 3.87 (m)	3.72, 3.90 (dq, 9.5, 7.5)
22			1.22 (t, 6.0)	1.28 (t, 7.5)

[a] Measured at 400 MHz.
[b] Measured at 500 MHz.

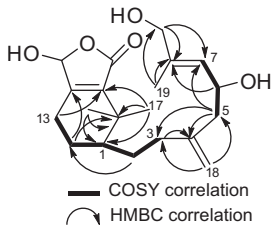


Figure 2. COSY and HMBC correlations of **1**.

two sets of proton sequences were further connected by the HMBC correlations of H-18/C-3, C-4, C-5 as shown in Figure 2. Furthermore, the HMBC correlations of Me-16/C-1, C-15, C-11, and H-14/C-15, C-12 (Fig. 2) clearly indicated that **1** possesses an 2′,2′-dimethyl cyclohexene moiety. The extraordinary bond cleavage between C-9 and C-10 was deduced from HMBC experiment, which clearly showed correlations of H_2-9/C-7, C-8, C-19 but lacked for correlations of H_2-9/C-10 (Fig. 2). In addition, compounds **1** and **2** possessed a hemiacetalic lactone ring system by virtue of HMBC correlations from H-20 to C-11, C-12, and C-10.[12] Thus, the gross structures of compounds **1** and **2** were determined.

A computer generated 3D chemical model for **1** by using MM2 force field calculation is illustrated in Figure 3, clarifying that compounds **1** and **2** have three asymmetric carbon centers (C-1, C-6, C-20). Based on previously published cespihypotins and biogenetic consideration, the configuration of the hydroxy at C-6 was tentatively assigned at β.[4,20] In the course of our investigation, **1** and **2** were in 1:1 amount mixture by comparing the intensity of ^{13}C-NMR data.

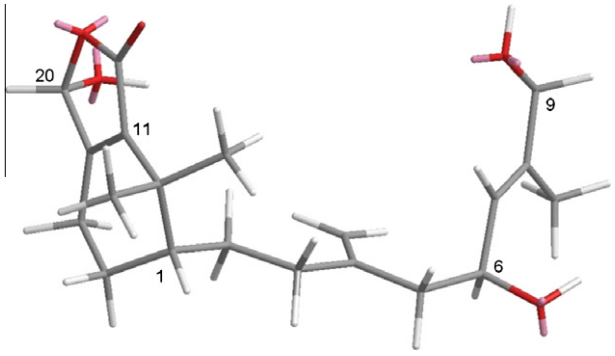


Figure 3. Computer-generated perspective model for **1** using MM2 force field calculation.

The difference appeared on C-20 (δ_c 96.6, 92.2, respectively) indicating that it was obtained as an inseparable epimeric mixture due to the hemiacetalic inversion. Based on the above detailed explanation, the gross structures of both **1** and **2** are assumed to be as shown in Figure 1 and designated as cespitulins A (**1**) and B (**2**).

In the less polar fraction, cespitulin C (**3**) was isolated as an amorphous yellow powder, $[\alpha]_D^{22}$ +28.3 (*c* = 0.25, CH_2Cl_2), which had the molecular formula $C_{22}H_{34}O_5$ as derived from HR-ESI-MS,

Table 2
^{13}C NMR spectroscopic data of **1**–**4**

No.	δ_C			
	1[a]	**2**[a]	**3**[a]	**4**[b]
1	44.1 d	44.3 d	44.1 d	44.5 d
2	26.5 t	26.4 t	26.4 t	26.5 t
3	34.2 t	34.4 t	33.8 t	34.3 t
4	145.6 s	145.5 s	145.6 s	145.6 s
5	43.9 t	43.9 t	43.9 t	44.0 t
6	65.8 d	65.8 d	65.7 d	65.7 d
7	127.1 d	127.0 d	127.1 d	127.2 d
8	137.7 s	137.7 s	137.6 s	137.6 s
9	67.7 t	67.8 t	67.8 t	67.9 t
10	170.0 s	170.0 s	169.8 s	170.0 s
11	136.4 s	136.4 s	136.8 s	136.9 s
12	158.6 s	158.9 s	157.0 s	157.4 s
13	22.7 t	22.4 t	22.7 t	22.6 t
14	22.8 t	22.9 t	22.9 t	22.9 t
15	33.8 s	33.7 s	34.2 s	33.8 s
16	24.3 q	23.8 q	24.2 q	23.6 q
17	20.7 q	20.3 q	20.3 q	20.6 q
18	113.3 t	113.3 t	113.3 t	113.3 t
19	14.0 q	14.0 q	14.0 q	14.0 q
20	96.6 d	92.2d	101.5 d	101.1 d
21			65.8 t	65.7 t
22			15.0 q	15.0 q

[A] Measured at 100 MHz.
[b] Measured at 125 MHz.

indicating six degrees of unsaturation. The IR spectrum revealed that this compound has a hydroxyl (3444 cm^{-1}) and a lactone (1731 cm^{-1}) group. The ^1H- and ^{13}C NMR spectra of **3** (Tables 1 and 2) were superimposable to those of **2** except that **3** contains signals of *O*-ethyl protons at δ_H 3.68 m, 3.87 m and 1.22 t (J = 6.0 Hz) and carbons at δ_C 65.8 t and 15.0 q.

However, the acetalic proton shifted upfield to δ_H 5.53s (H-20) in **3** and its corresponding carbon shifted downfield to δ_C 101.5. The ^1H-^1H COSY of **3** determine three sets of correlations (Fig. 4). Moreover, the HMBC of **3** revealed correlations between H-21 and C-20.

Furthermore, the relative stereochemistry of **3** was determined by the NOESY correlations (Fig. 5). Assuming that **3** was β-configuration of H-1 the same with cespihypotins,[4,20] NOESY experiments would assign the configuration of H-20. The presence of mutual correlations between H-1/H-13β, H-14β, Me-17; H-14α/H-13α, H-20 and the absence of correlation between H-20 and H-13β suggested that the configuration of H-20 is α-oriented. On the other hand, the absence of correlation between H-7 and Me-19 suggested the *E* geometry of C-7 and C-8.

Compound **4** was isolated as a light yellow powder. Its IR, ^1H- and ^{13}C-NMR spectroscopic data (Tables 1 and 2) are almost same as those of **3**, while its physical property is different in some manners. Compound **4** has a specific rotation $[\alpha]_D^{25}$ −36.8 (*c* 0.25, CH_2Cl_2). On the HPLC separtions, using RP-C_{18}, (MeOH/CH_3CN/H_2O, 5:1:4, flow: 2 ml/min), compounds **3** and **4** were separated at two different retention times, 42.6 and 46.6 mins, respectively. Also, some differences were shown by comparing the HMBC correlations of **4** [H-18/C-3, C-5, H-3, H-5/C-4, Me-19/C-7, C-8, C-9, H-9 / C-7, C-8, H-20/C-10, C-11, C-12, C-21, H-13/C-11, C-12, H-21/C-20] (Fig. 6) with those of **3**.

The establishment of the relative three-dimensional structure was mainly deduced by virtue of the comparison with **3**. Some significant differences were observed in the NOESY spectrum of **4** (Fig. 7). The cross peaks between H-1/Me-17, H-13β, H-14β, and H-20/H-13β, H-13α confirmed the proton assignment between C-13 and C-14, therefore, H-20 was in the β position.

A molecular model of structure **4** was generated by CS Chem 3D version 9.0 using MM2 force field calculation for energy minimization (ChemBioUltra Calculation program) as shown in Figure 7. The result was consistent with the NOESY experiment. Based on the above interpretation, the structure of **4** was shown in Figure 1, and the name cespitulin D was given.

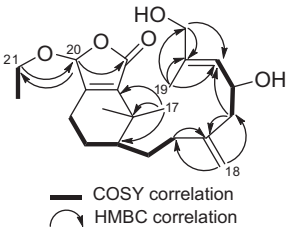


Figure 4. COSY and HMBC correlations of **3**.

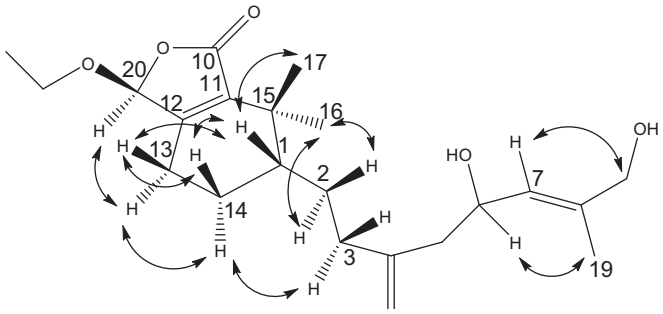


Figure 5. Selective NOESY correlations of **3**.

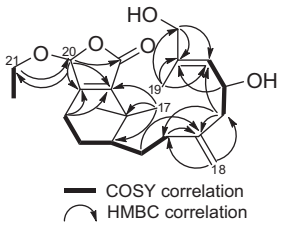


Figure 6. COSY and HMBC correlations of **4**.

Compounds **1–4** represent new verticilene-like diterpenoids having an unprecedented bond cleavage between C-9 and C-10 with a hemiacetalic lactone ring rather than a verticillane skeleton. However, these compounds were inactive as tested for their in vitro cytotoxicity against human tumor cells. Compounds **1** and **2** may be biogenetically derived from cespihypotin V[20] through steps of dehydration, oxidation leading to double bond cleavage, and reduction of ketone and aldehyde groups to **1** and **2** as illustrated in Scheme 1.

3. Experimental

3.1. General

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were recorded using a Horiba FT-720 spectrophotometer. The ^1H 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 ^1H and 75 or 125 MHz for ^{13}C using the $CDCl_3$ solvent peak as internal standard (δ 7.26 for ^1H 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. Lichrosorb Si-60 (Merck) and Lichrosorb RP-18 (Merck) were used for HPLC column.

3.2. Animal material

Cespitularia taeniata May was collected in Green Island, Taiwan, in March 2004. This soft coral was identified by one of the authors (YCS). A voucher specimen (GSC-1) was deposited in the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

3.3. Extraction and isolation

The soft coral (1.1 kg) was freeze-dried, powdered, and extracted with mixture of CH_2Cl_2/EtOH (1:1), and the crude extract was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble fraction (100 g) was subjected to a Si gel column (*n*-hexane/EtOAc, 15:1–0:1; EtOAc/MeOH, 50:1–2:1) to give fractions 1–12. Fraction 10 (540 mg) was separated with a Sephadex LH-20 column (MeOH) to yield a residue (400 mg), which then chromatographed extensively on a Si gel column (*n*-hexane/EtOAc, 3:1), LH-20 Sephadex resin (CH_2Cl_2/MeOH, 1:1) and HPLC (Si gel, *n*-hexane-acetone, 4:1; RP-C_{18}, MeOH/CH_3CN/H_2O, 6:1:3) to furnish a mixture residue, cespitulins A and B (**1** and **2**, 1 mg), cespitulactam C (12 mg),[7] and cespitularin F (95 mg).[6] Fraction 9 (2.5 g) was separated with a LH-20 Sephadex resin (CH_2Cl_2/MeOH, 1:1), Si gel column and HPLC (Si gel, *n*-hexane-acetone, 4:1; RP-C_{18}, MeOH/

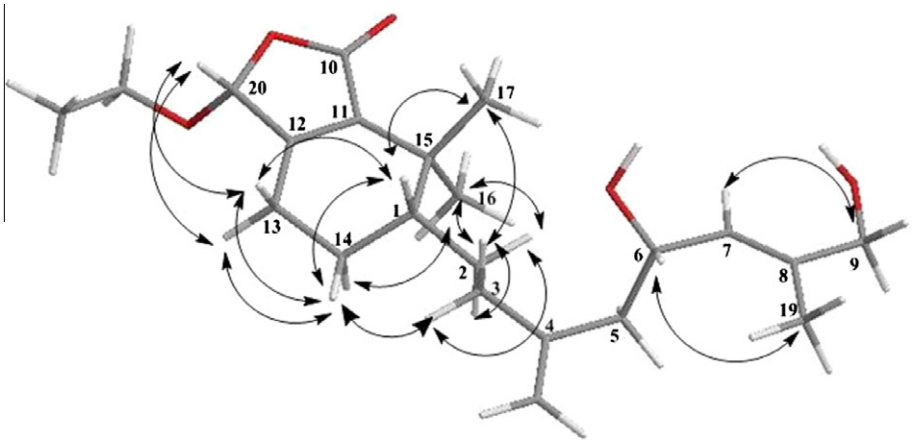
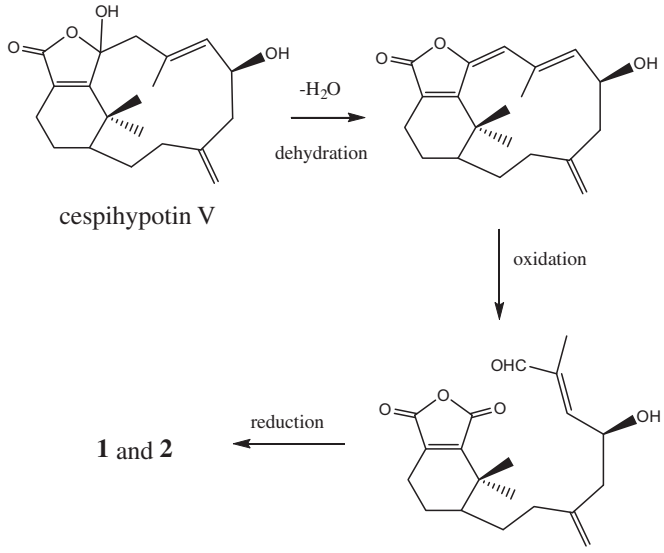


Figure 7. NOESY correlations of **4**.



Scheme 1. Plausible biogenetic pathway to compounds **1** and **2**.

CH_3CN/H_2O, 5:1:4) to furnish cespitulin C (**3**, 5 mg), cespitulin D (**4**, 5 mg), and cespitulactam A (11 mg).[7]

Cespitulins A and B (**1** and **2**): light yellow powder. $[\alpha]_D^{25}$ +15.8 (*c* 0.2, CH_2Cl_2). UV (CH_2Cl_2) λ_{max} (log ε): 220 nm. IR (neat) υ_{max}: 3385, 1738 cm^{-1}. ^1H- and ^{13}C NMR ($CDCl_3$): see Tables 1 and 2, respectively. HR-ESI-MS *m/z* 373.1990 ($[M+Na]^+$, calcd for $C_{20}H_{30}O_5Na$, 373.1991).

Cespitulin C (**3**): light yellow powder. $[\alpha]_D^{22}$ +28.3 (*c* 0.25, CH_2Cl_2). UV (CH_2Cl_2) λ_{max} (log ε): 220 nm. IR (film) υ_{max} 3444, 1731 cm^{-1}. ^1H- and ^{13}C- NMR ($CDCl_3$): see Tables 1 and 2, respectively. HR-ESI-MS *m/z* 401.2301 ($[M+Na]^+$, calcd for $C_{22}H_{34}O_5Na$, 401.2304).

Cespitulin D (**4**): light yellow powder. $[\alpha]_D^{25}$ −36.8 (*c* 0.25, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε): 220 nm. IR (neat) υ_{max} 3390, 1757 cm^{-1}. ^1H- and ^{13}C-NMR ($CDCl_3$): see Tables 1 and 2, respectively. HR-ESI-MS *m/z* 401.2302 ($[M+Na]^+$, calcd for $C_{22}H_{34}O_5Na$, 401.2304).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.10.057.

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