Cespihypotins Q−V, Verticillene Diterpenoids from *Cespitularia hypotentaculata*

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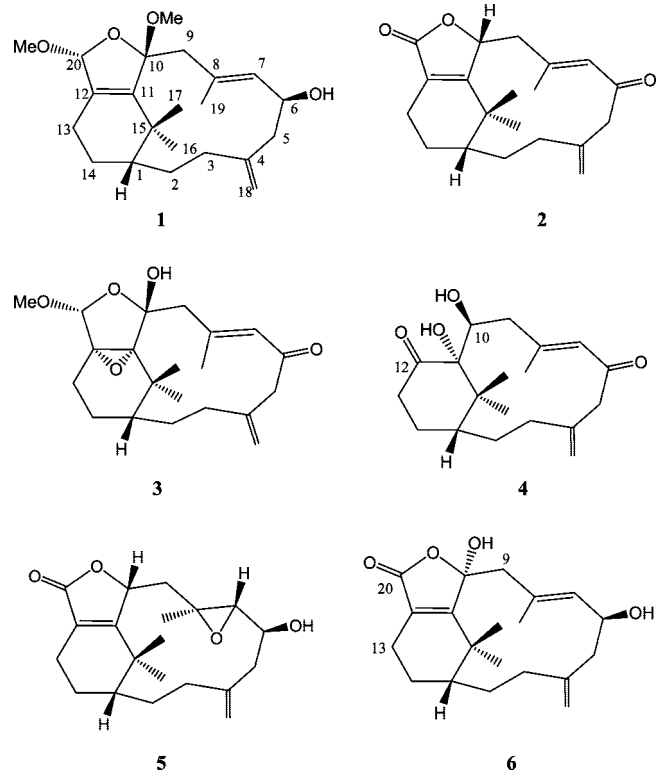
Chemical investigation of the soft coral *Cespitularia hypotentaculata* resulted in the isolation of six new diterpenes, cespihypotins Q−V (**1**−**6**). The new metabolites comprised five verticillane-type diterpenes and one nor-verticillane derivative. Their structures were determined through detailed spectroscopic analyses, especially HRESIMS and 2D NMR techniques. The relative configuration was deduced by interpretation of NOESY spectra. Cespihypotin T (**4**) exhibited significant cytotoxic activity against human Daoy and WiDr tumor cell lines.

Colonies of the soft coral *Cespitularia* (Xeniidae) have polyps on the soft branches with white, cream, blue, brown, or green surfaces.[1] Species of this genus produce various diterpenoids possessing cembrane, neodolabellane, cespitularane, and verticillane skeletons.[2−7] Some of these compounds showed cytotoxic and immunomodulatory activities.[8−10] The verticillenes from *Cespitularia* are mainly metabolites of bicyclo[9.3.1]diterpenes resembling the bicyclic taxanes isolated from terrestrial yew trees.[11] Some nor-verticillane derivatives have also been reported in this genus.[7,12] Chemical investigation of the nonpolar extract of *Cespitularia hypotentaculata* Roxas (Xeniidae) led to the isolation of six new diterpenes, cespihypotins Q−V (**1**−**6**). Five of the new metabolites, **1**−**3**, **5**, and **6**, are verticillane-type diterpenes, while **4** possesses a nor-verticillene skeleton. The structures of **1**−**6** were determined by detailed spectroscopic/spectrometric analyses, especially employing HRESIMS and 2D NMR techniques. The relative configuration of these compounds was deduced from interpretation of NOESY data. Compound **4** showed significant cytotoxic activity against human Daoy and WiDr tumor cell lines.



Results and Discussion

The HRESIMS of cespihypotin Q (**1**) revealed an $[M + Na]^+$ molecular ion peak at *m/z* 385.2351, corresponding to the molecular formula $C_{22}H_{34}O_4Na$ possessing six degrees of unsaturation. The IR spectrum displayed absorption bands diagnostic of hydroxyl (3418 cm^{-1}) and double-bond (1638 cm^{-1}) functionalities. The ^{13}C NMR data showed an exomethylene double bond (δ_C 146.9, 114.1), a trisubstituted double bond (δ_C 134.2 d, 133.6 s), and a tetrasubstituted double bond (δ_C 141.7 s, δ_C 136.5 s), all of which implied that **1** was a tricyclic compound. The ^1H NMR spectrum of **1** (Table 1) displayed an olefinic proton singlet at δ_H 5.43, two exomethylene singlets at δ_H 4.80 and 4.78, two methoxy groups (δ_H 3.16, 3.44), two oxymethines (δ_H 4.33, 5.10), and three methyl singlets (δ_H 1.15, 1.33, 1.60). In the HMBC spectrum of **1**, the exomethylene protons correlated to a quaternary carbon at δ_C 146.9 (C-4) and two $\underline{C}H_2$ at δ_C 34.0 and 44.2, indicating that the exomethylene functionality is located between two methylene groups (Figure 1). The oxymethine proton at δ_H 4.33 correlated with C-4 and two olefinic carbons of the trisubstituted double bond, while the vinyl methyl protons (δ_H 1.60) correlated with the latter carbons and also $\underline{C}H_2$ at δ_C 49.1 (C-9). Thus, the partial structure $-CH_2-C(CH_2)-\underline{C}H_2-CH(OH)-CH{=}C(CH_3)-CH_2-$ was deduced.

HMBC data further showed that each of the methyl singlets at δ_H 1.33 and 1.15 correlated with one another and with a quaternary carbon at δ_C 35.5 (C-15), $\underline{C}H$ at δ_C 44.4 (C-1), and a quaternary olefinic carbon at δ_C 141.7 (C-11). Thus, the quaternary carbon bearing two methyl groups was positioned between $\underline{C}H$ (C-1) and the quaternary olefinic carbon (C-11). COSY NMR connectivities between CH_2-3/H_2-2/H-1/H_2-14/H_2-13 and HMBC correlations of H-1/C-11, C-13, C-15 suggested that the two *gem*-methyls are attached to a quaternary carbon in a cyclohexene ring. In the HMBC spectrum, the $C\underline{H}_2$ singlet at δ_H 2.75 (H_2-9) correlated to C-8 (δ_C 133.6), the acetal carbon (δ_C 115.2), the vinyl methyl (δ_C 16.6, C-19), and C-11 (δ_C 141.7). The O-methyl protons at δ_H 3.16 correlated to the acetal carbon at δ_C 115.2, while the methoxy protons at δ_H 3.44 correlated to a second acetal carbon at δ_C 106.4 (C-20). The latter was bound to the oxymethine singlet at δ_H 5.10, which also correlated with the acetal carbon (δ_C 115.2, C-10), C-11, and C-13 (δ_C 19.0), thereby proving the presence of a 2,5-

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Table 1. ^{1}H NMR Data ($CDCl_3$, 500 MHz) for Compounds **1**−**6**[a]

C	1	2	3	4	5	6
1	1.46 m	1.70 m	1.60 m	1.90 m	1.89 m	1.58 m
2	1.51 m	2.32 dd (11.0,2.0)	1.31 m	1.75 m	2.13 m	1.88 m (2H)
	1.41 m	2.08 m	(2H)	1.35 m	2.04 m	
3	2.32 m	2.38 m	2.22 m	1.10 m	1.94 m	2.17 m
	2.15 m	2.22 m	2.14 m	2.06 m	1.57 m	1.90 m
5	2.45 m	3.22 d (12.5)	3.17 d (10.2)	2.63 d (14.5)	2.96 m	2.48 d
	2.30 m	2.94 d (12.5)	3.07 d (10.2)	2.13 br d (14.5)	2.48 dd (14.0,8.5)	(13.0)
						2.37 dd
						(13.0,6.7)
6	4.33 t (8.0)				3.41 dt (7.5,2.0)	4.49 br t (6.7)
7	5.43 d (8.0)	6.29 s	6.47 s	6.12 s	2.92 d (7.5)	5.37 d (6.7)
9	2.75 s (2H)	3.09 dd (14.0,3.5)	3.24 d (15.5)	2.46 dd (15.0,8.5)	2.25 m	2.80 d (13.5)
		2.73 d (14.0, 3.5)	2.50d (15.5)	2.31 d (15.0)	1.76 dd (15.0,3.0)	2.59 d (13.5)
10		5.30 br d (3.5)		3.77 t (8.5)	5.22 br s	
12						
13	2.26 m	2.18 m	1.72 m	2.40 m	2.39 m	2.27 m
	2.02 m	1.72 m	1.56 m	1.69 m	2.33 m	(2H)
14	2.20 m	1.59 m	1.75 m	2.37 m	2.30 m	1.52 m
	1.53 m	1.57 m	1.56 m	1.81 m	1.84 m	1.17 m
16	1.33 s	1.49 s	1.35 s	1.10 s	1.23 s	1.27 s
17	1.15 s	1.23 s	0.95 s	1.44 s	1.30 s	1.22 s
18	4.80 s	4.95 s	5.03 s	4.95 s	4.90 s	4.99 s
	4.78 s	4.87 s	4.89 s	4.87 s	4.81 s	4.91 s
19	1.60 s	2.03 s	2.12 s	1.96 s	1.22 s	1.85 s
20	5.10 s		4.43 s			
10-C$\underline{H}_3$O	3.16 s					
20-C$\underline{H}_3$O	3.44 s		3.47 s		2.11 s	
OH			3.30 br s	4.58 br s		

[a] Chemical shifts are in ppm; *J* values (Hz) are in parentheses.

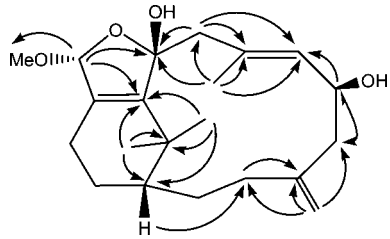


Figure 1. Key HMBC correlations of **1**.

dihydrofuran with methoxy substitution at C-10 and C-20. The previous data were in agreement with a 1*S*-verticillene-type diterpene with unsaturation at positions 4(18), 7(8), and 11(12), as well as a hydroxy group at C-6, *O*-methyl groups at C-10 and C-20, and an ether linkage between C-10 and C-20.

The relative configuration of **1** was established on the basis of biogenetic considerations, NOESY correlations, and a computer-generated perspective model using MM2 force field calculations. We assume that H-1 is on the β-face of molecule **1**, consistent with naturally occurring bicyclic verticilanes. NOESY correlations between H-1/H-16, H-17 and H-7/H-17 indicated that Me-16, Me-17, and H-20 are on the β-face. NOESY correlation between H-6/H_α-5 and H-19 suggested that H-6 was α-oriented. The absence of an NOE effect between H-7 and H-19 favored the *E*-geometry of the 7,8-double bond.

The HRESI mass spectrum of cespihypotin R (**2**) showed *m*/*z* 337.1779, $[M + Na]^+$, indicating the molecular formula $C_{20}H_{26}O_3$. The IR spectrum revealed absorption bands for hydroxy (3422 cm^{-1}), carbonyl(s) (lactone, 1750 cm^{-1}, a conjugated carbonyl, 1684 cm^{-1}), and double-bond (1616 cm^{-1}) functionalities. The ^{13}C NMR data showed two carbonyls (δ_C 198.9, 172.5), an exomethylene double bond (δ_C 143.6, 115.4), a trisubstituted double bond (δ_C 128.7 d, 152.1 s), and a double bond adjacent to a carbonyl (δ_C 127.3, 168.9), suggesting a tricyclic diterpene skeleton. HMBC correlations from the methylene protons at δ_H 3.22, 2.94 (each d, J = 12.5 Hz, H_2-5) and the olefinic C$\underline{H}$ at δ_H 6.29 (H-7) to the carbonyl carbon at δ_C 198.9 (C-6) and a correlation from H-5 to the exomethylene carbon (C-18) were observed, consistent with a carbonyl at C-6. Each of the methyl singlets at δ_H 1.23 and 1.49 showed correlations with one another and also with a quaternary carbon at δ_C 38.1 (C-15), $\underline{C}$H at δ_C 43.2 (C-1), and a quaternary olefinic carbon at δ_C 168.9 (C-11). The presence of an α,β-unsaturated-γ-lactone moiety was evident from the presence of a conjugated carbonyl carbon at δ_C 172.5 (C-20) and adjacent carbons at 127.3 (C−12), 168.9 (C−11), and 80.9 (C-10). COSY correlations were observed between the oxymethine proton at δ_H 5.30 (H-10) and the C$\underline{H}_2$ protons at δ_H 3.09, 2.73 (H_2-9). The HMBC spectrum displayed ^3J-correlations between the latter (H_2-9) and $\underline{C}H_3$ at δ_C 20.3 (C-19), $\underline{C}$H at δ_C 128.7 (C-7), and a quaternary carbon at δ_C 168.9 (C-11), and between methyl groups at δ_H 1.49 and 1.23 (H-16, H-17) and C-11, verifying the position of the attachment of the lactone ring. It is worthy to note that **2** is a 10-deoxy analogue of cespihypotin F, previously isolated from the same species.[10] Similar to compound **1**, NOESY correlations were observed between H-1/H-16, H-17; H-16/H-7, H-17, H_β-9; and H_β-9/H-10, indicating that H-1, H-10, Me-16, and Me-17 were on the β-face of the molecule. Additionally, NOESY correlation between Me-19/H_α-9 suggested that Me-19 was on the α-side of the molecule and that the geometry of the 7,8-double bond was *E*.

A molecular formula of $C_{21}H_{30}O_5$ was assigned to cespihypotin S (**3**) based on interpretation of HRESIMS data [*m*/*z* 385.1993 ([M + Na]$^+$)]. The IR spectrum of **3** also exhibited absorption bands for hydroxy (3419 cm^{-1}), conjugated carbonyl (1699 cm^{-1}), and double bond (1635 cm^{-1}) groups. Recorded NMR data (Tables 1 and 2) were in accordance with a verticillene-type diterpene with signals indicating the same sequence of C_1−C_9 as that of **2**. In the HMBC spectrum of **3**, each of the methyl groups (δ_H 1.35 and 0.95) correlated to the methine carbon at δ_C 43.5 (C-1), a quaternary carbon at δ_C 38.2 (C-15), and an oxyquaternary carbon at δ_C 73.1 (C-11). The methylene protons at δ_H 3.24 and 2.50 (each d, J = 15.0 Hz, H_2-9) correlated to C-7, C-8, and C-19 (δ_C 17.5), as well as the OH-bearing acetal carbon at δ_C 93.7 (C-10). The oxymethine proton at δ_H 4.43 was assigned to H-20 based upon its HMQC correlation with the methine carbon (δ_C 109.7) and also upon a correlation with the O-methyl carbon (δ_C 56.6), C-10, an oxyquaternary carbon (δ_C 73.1, C-11), and the methylene carbon at C-13 (δ_C 31.3), respectively. Thus, C-20 was linked to C-10 through an

Table 2. ^{13}C NMR Data ($CDCl_3$, 125 MHz) for Compounds **1**−**6**[a]

C	1	2	3	4	5	6	cespitularin D
1	44.4 d	43.2 d	43.5 d	43.3 d	42.1 d	42.9 d	43.7 d
2	32.6 t	17.1 t	24.3 t	28.3 t	31.3 t	19.2 t	18.1 t
3	34.0 t	34.8 t	37.7 t	32.1 t	31.9 t	34.5 t	31.7 t
4	146.9 s	143.6 s	143.2 s	145.8 s	144.7 s	146.1 s	146.1 s
5	44.2 t	54.0 t	54.9 t	42.5 t	41.0 t	43.6 t	43.6 t
6	68.7 d	198.9 s	199.0 s	207.0 s	71.2 d	68.4 d	68.3 d
7	134.2 d	128.7 d	128.8 d	121.6 d	70.4 d	133.2 d	136.0 d
8	133.6 s	152.1 s	132.5 s	151.5 s	61.2 s	134.6 s	131.0 s
9	49.1 t	42.8 t	40.9 t	46.0 t	40.9 t	50.9 t	48.7 t
10	115.2 s	80.9 d	93.7 s	68.9 d	79.0 d	105.7 s	108.8 s
11	141.7 s	168.9 s	73.1 s	81.5 s	169.2 s	162.2 s	167.8 s
12	136.5 s	127.3 s	78.1 s	213.2 s	128.1 s	133.9 s	129.1 s
13	19.0 t	24.8 t	31.3 t	18.2 t	19.0 t	20.5 t	32.3 t
14	25.1 t	33.0 t	33.8 t	31.2 t	23.4 t	25.7 t	23.9 t
15	35.5 s	38.1 s	38.2 s	49.5 s	35.9 s	34.7 s	37.2 s
16	24.5 q	25.4 q	25.9 q	22.7 q	33.9 q	22.7 q	24.2 q
17	35.5 q	34.4 q	23.7 q	26.0 q	23.9 q	28.7 q	34.0 q
18	114.1 t	115.4 t	116.4 t	113.9 t	113.9 t	114.8 t	114.0 t
19	16.6 q	20.3 q	19.2 q	20.9 q	19.2 q	17.9 q	17.2 q
20	106.4 d	172.5 s	109.7 d		172.4 s	169.6 s	171.5 s
10-OMe	49.7 q						
20-OMe	55.3 q		56.6 q				

[a] s = C, d = CH, t = CH_2, q = CH_3, Multiplicities and assignments made by HMQC and HMBC techniques.

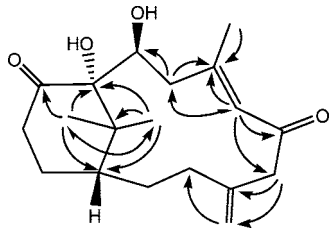


Figure 2. Key HMBC correlations of **4**.

ether bond as part of the tetrahydrofuran ring. An epoxy ring involving two oxyquaternary carbons at δ_C 73.1 (C-11) and 78.1 (C-12) was suggested to account for a seventh degree of unsaturation. This was proven by HMBC correlations from H-16 and H-17 to C-11 and from H-20 to C-11 and C-12. The NOESY correlations between H-1/H-20, H-17; O<u>H</u>-10/H_β-9; H-20/OC<u>H</u>$_3$; and H-17/H-7, H_β-9, H-16 indicated that H-1, H-7, Me-16, Me-17, H-20, and O<u>H</u>-10 were located on the β-face of the molecule. The geometry of the 7,8-double bond was deduced to be *E*, the same as that of **1** and **2**.

The molecular formula of cespihypotin T (**4**) was established as $C_{19}H_{28}O_4$ (*m*/*z* 343.1883 $[M + Na]^+$), suggesting a norditerpene skeleton. The IR spectrum revealed absorptions for hydroxy (3446 cm^{-1}), carbonyl (1716 cm^{-1}), conjugated carbonyl (1685 cm^{-1}), and double-bond (1618 cm^{-1}) functionalities. The ^{13}C NMR data revealed an exomethylene, trisubstituted double bond, a carbonyl carbon (δ_C 213.2), and a conjugated carbonyl carbon (δ_C 207.0), suggesting a bicyclic structure. The HMBC spectrum showed correlations between C<u>H</u>$_2$-18/C-5 (δ_C 42.5); H-7 (δ_H 6.12)/C-5, carbonyl carbon (δ_C 207.0), C-8 (δ_C 151.5), C<u>H</u>$_2$-9 (δ_C 46.0), C-19 (δ_C 20.9); H-19/C-7 (δ_C 121.6, d), C-8, C-9; and H-9/oxymethine (δ_C 68.9, C-10) (Figure 2). This allowed the assignment of a carbonyl carbon at C-6, as well as a 7,8-double bond and a hydroxy group at C-10. In the COSY spectrum the oxymethine proton at δ_H 3.77 (H-10) correlated to a signal at δ_H 2.46 (H-9), and in the HMBC spectrum the proton correlated to the oxyquaternary carbon at δ_C 81.5 (C-11). Both H-16 and H-17 (δ_H 1.10 and 1.44) exhibited HMBC correlations to C-1 (δ_C 43.3) and C-11 (δ_C 81.5). This led to the assignment of the latter carbonyl carbon at C-12 and a hydroxy group at C-11 in a cyclohexanone ring. The NOESY spectrum of **4** (Figure 3) revealed correlations between H-1/H-16, H-17; H-7/H-10, H_α-2; H_β-2/H-16; H_α-9/H-10; and H-19/H-16, H-17, H_β-9, indicating that H-1, H-16, H-17, and H-19 were located

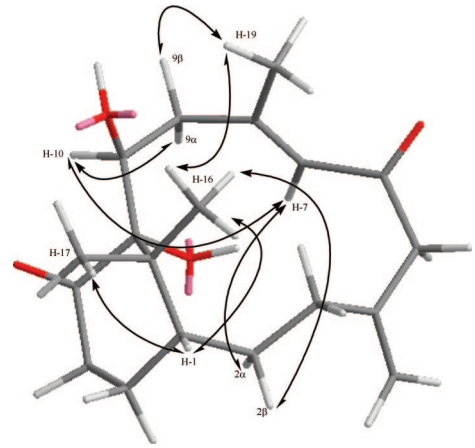


Figure 3. Key NOESY correlations of **4**.

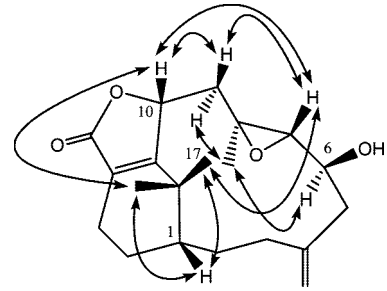


Figure 4. Selected NOESY correlations of **5**.

on the β-face and H-10 was α-oriented. As in the other metabolites, the geometry of the 7,8-double bond was assigned as *E*.

Cespihypotin U (**5**) analyzed for the molecular formula $C_{20}H_{28}O_4$ by interpretation of HRESIMS data [*m*/*z* 355.1887 ($[M + Na]^+$)]. The IR spectrum of **5** indicated the presence of hydroxy (3440 cm^{-1}), lactone (1750 cm^{-1}), and double-bond (1630 cm^{-1}) functionalities. The methylene protons at δ_H 2.96 and 2.48 (H_2-5) showed HMBC correlations to the methylene carbons at δ_C 113.9 (C-18) and 31.9 (C-3) and also the oxymethine carbon at δ_C 70.4 (C-7), while in the COSY spectrum a correlation with the oxymethine proton at δ_H 3.41 (H-6) was observed, consistent with oxygenation at C-6. An oxymethine carbon at δ_C 70.4, an oxyquaternary carbon at δ_C 61.2, and a methine proton at δ_H 2.92 (H-7) were diagnostic of a 7,8-epoxy ring on the basis of the following HMBC correlations: H-7/C-5 (δ_C 41.0), C-8 (δ_C 61.2), C-19 (δ_C 19.2); H_2-9/ C-7, C-8, C-19; and H-19 (δ_H 1.22)/C-7, C-8, C-9 (40.9). Comparison of NMR data at C-10, C-11, C-12, and C-20 with the corresponding data from **2** indicated that **5** contained a similar lactone ring. The proposed structure was confirmed by analysis of COSY data that showed connectivities between H-5/H-6/H-7 and between H-9/H-10. These assignments were also shown by the following HMBC correlations: H-1/C-15, H-16, H-17; H-16/C-1, C-11, C-15, C-17; and H-17/C-1, C-11, C-15, C-16. Observed NOESY correlations between H-1/H-16, H-17; H-7/H_β-9, H-10, H-17; H_β-9/H-17; H-10/H-7, H_β-9, H-16; and H-19/H-6, H_α-9 were in agreement with a β-orientation for H-7 and H-10 and an α-orientation for H-6 and H-19 (Figure 4).

Cespihypotin V (**6**) had the molecular formula $C_{20}H_{28}O_4$, as deduced from HRESIMS data showing *m*/*z* 355.1882 $[M + Na]^+$. The IR spectrum of **6** revealed the presence of hydroxy, lactone, and double-bond functions. The ^{13}C NMR data were almost identical to those of cispitularin D[7] with the exception of significant differences of signals attributable to C-9 to C-13, and C-16, C-17, and C-20 (Table 2). Moreover, the methylene protons at C-9 resonated at δ_H 2.80 and 2.59 (each d, $J = 13.5$ Hz) versus a singlet at δ_H 2.97 (2H, s) in the case of cispitularin D. In addition, the

Table 3. Cytotoxic Activities (ED_{50}, μM) of Compounds **1**−**6**[a]

compound	Daoy[b]	WiDr[c]
cespihypotin Q (**1**)	>55	>55
cespihypotin R (**2**)	>55	50
cespihypotin S (**3**)	40	54
cespihypotin T (**4**)	9.3	7.5
cespihypotin U (**5**)	>60	>60
cespihypotin V (**6**)	60	>60

[a] ED_{50} of standard mitomycin C: Daoy 0.21 μM, WiDr 0.18 μM. [b] Daoy: human medulloblastoma. [c] WiDr: human colon adenocarcinoma.

COSY and HMBC correlations from **6** also confirmed a structure at C-6 similar to that in cispitularin D. It was suggested that the two compounds differ only in the configuration of C-10. The NOESY spectrum of **6** revealed correlations between H-1/H-16, H-17; H-6/H-19; and H-7/H-17 and the absence of a correlation between H-7/H-19, indicating H-16, H-17, and H-1 were located at the β-face of the molecule, H-6 and H-19 were on the α-face, and the 7,8-double bond geometry was *E*.

The *in vitro* cytotoxic activity of the new metabolites was evaluated against human Daoy (medulloblastoma) and WiDr (colon adenocarcinoma) tumor cell lines. Cespihypotin T (**4**) exhibited significant cytotoxicity against Daoy and WiDr cell lines with ED_{50} values of 9.3 and 7.5 μM, respectively, while the other metabolites were weakly active or inactive, as illustrated in Table 3.

Marine soft corals of *Cespitularia* are rich in verticillene diterpenoids with diverse structures and functionalities. The current study has reported six new compounds isolated from *C. hypotentaculata*. Among them, cespihypotin T (**4**), which belongs to the norditerpene class with a keto and two adjacent hydroxy groups, showed significant cytotoxic activity against human tumor cells. A plausible biogenetic pathway of compounds **1**−**6** is proposed as illustrated in Scheme 1 based on recently published diterpenoids.[10,12] 1*S*-Verticillene may be considered to produce intermediate **a**, which might be an important precursor leading to all the isolated diterpenes **1**−**6**. Some derivatives of intermediate **a**, which have been recently isolated from *Cespitularia* spp.,[10,13] are quite significant from a biogenetic point of view.

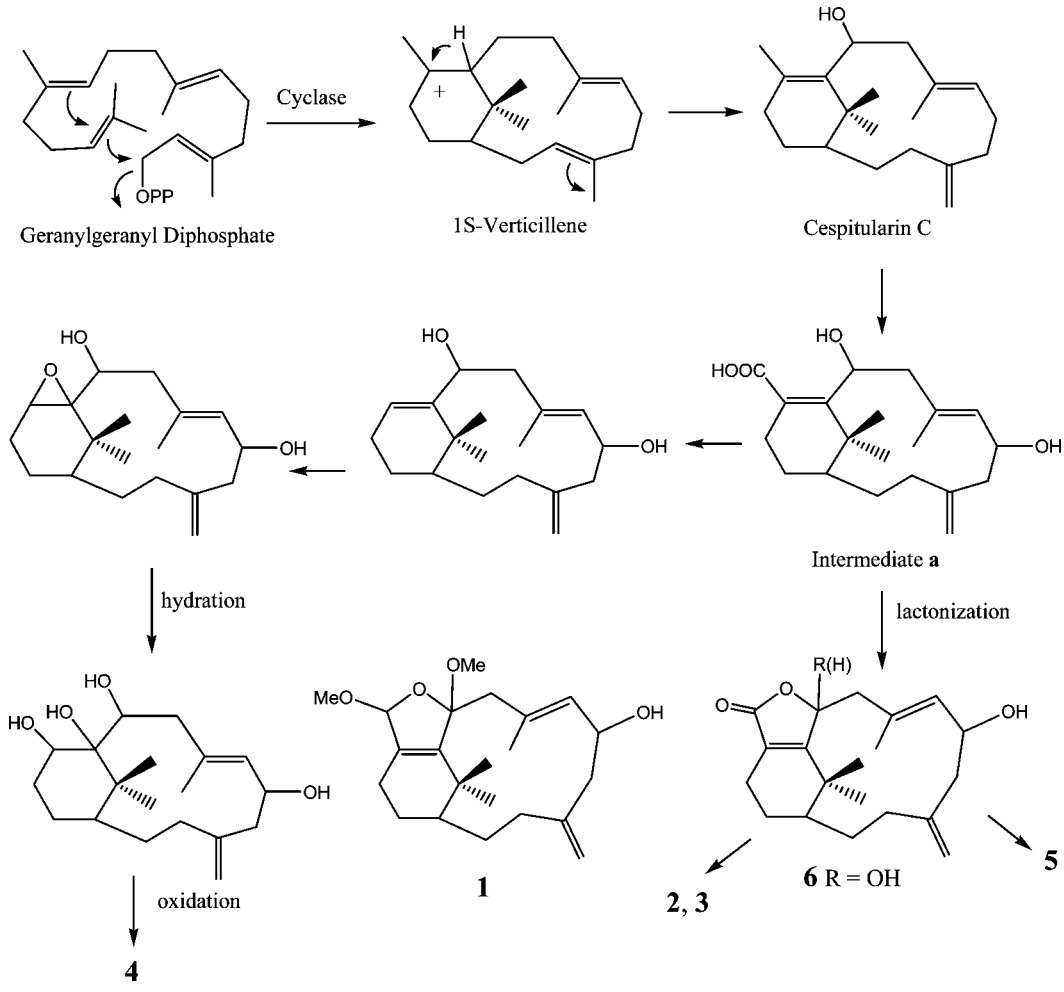
Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were measured on a Hitachi U-3210 spectrophotometer. The ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on a Varian Unity INOVA 500 FT-NMR spectrometer at 500 MHz for ^{1}H and 125 for ^{13}C, respectively using TMS as internal standard. The chemical shifts are given in δ (ppm) and coupling constants in Hz. Low-resolution ESIMS and HRESIMS were run on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Merck) was used for column chromatography (CC), and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used for separation. LiChrospher Si 60 (5 μm, 250-10, Merck) and LiChrospher 100 RP-18e (5 μm, 250−10, Merck) were used for NP-HPLC and RP-HPLC (Hitachi), respectively.

Animal Material. The soft coral *Cespitularia hypotentaculata* Roxas (Xeniidae) was collected at Green Island, off the eastern coast of Taiwan, in December 2004, by scuba diving at a depth of 15 m. The fresh coral was immediately frozen after collection and kept at −20 °C until processed. This species was identified by one of the authors (Y.-C.S.). A voucher specimen (NTUO-5) was deposited at the School of Pharmacy, College of Medicine, National Taiwan University, Taiwan.

Extraction and Isolation. The soft coral (wet, 8 kg) was extracted with CH_2Cl_2/MeOH (1:1, 3 × 10 L) at rt, and the extract was

Scheme 1. Plausible Biogenetic Pathway to Compounds **1**−**6**



concentrated under vacuum. The crude extract (40 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble portion was subjected to a flash column (silica gel, *n*-hexane/EtOAc 100:0 → 0:100). The fraction eluted with *n*-hexane/EtOAc (3:1) was separated on a Sephadex LH-20 column using CH_2Cl_2/MeOH (1:1) to furnish four fractions (S_1-S_4). Fractionation of S_3 (1.2 g) was done with a silica gel column eluting gradiently with *n*-hexane/EtOAc/MeOH (100:0:0 → 0:8:2) (F_1-F_{30}). Fraction F_8, eluted with *n*-hexane/EtOAc/MeOH (18:18:1), was chromatographed on a silica gel column using gradient *n*-hexane/CH_2Cl_2/MeOH. A fraction eluted with the previous solvent system (ratio 20:18:1) was further subjected to separation on NP-HPLC using an *n*-hexane/acetone (4:1) solvent system to yield **1** (7 mg) and **2** (8 mg), while another fraction, eluted with a solvent ratio 18:18:1, was subjected to NP-HPLC using *n*-hexane/acetone (9:2) as the eluent followed by separation on RP-HPLC using MeOH/H_2O/MeCN (70:25:5) to yield **3** (12 mg), **4** (7 mg), **5** (8 mg), and **6** (6 mg).

Cespihypotin Q (1): $[\alpha]^{25}_D$ −25.4 (*c* 2.0, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3418 (OH), 2934 (C−H), 1638 (double bond), 1268, 1108, 997, 736 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* 385.2351 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_4Na$, 385.2355).

Cespihypotin R (2): $[\alpha]^{25}_D$ −34.6 (*c* 2.0, acetone); IR (CH_2Cl_2) ν_{max} 3422 (OH), 2934 (C−H), 1750 (lactone), 1684 (conj. C=O), 1616 (double bond), 1224, 1080, 898, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* 337.1779 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_3Na$, 337.1780).

Cespihypotin S (3): $[\alpha]^{25}_D$ −4.4 (*c* 0.05, acetone); IR (CH_2Cl_2) ν_{max} 3419 (OH), 2933 (C−H), 1699 (conj. C=O), 1635 (double bond), 1266, 1057, 991, 749 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* 385.1993 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_6Na$, 385.1991).

Cespihypotin T (4): $[\alpha]^{25}_D$ +42.9 (*c* 0.075, acetone); IR (CH_2Cl_2) ν_{max} 3446 (OH), 2926 (C−H), 1716 (C=O), 1685 (conj. C=O), 1618 (double bond), 1275, 1110, 994, 909, 743 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* 343.1883 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_4Na$, 343.1885).

Cespihypotin U (5): $[\alpha]^{25}_D$ −26.4 (*c* 0.37, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3440 (OH), 2937 (C−H), 1750 (lactone), 1630 (double bond), 1226, 1036, 903, 736 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* 355.1887 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1885).

Cespihypotin V (6): $[\alpha]^{25}_D$ −58 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3417 (OH), 2926 (C−H), 1750 (lactone), 1650 (double bond), 1267, 902, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* 355.1882 $[M + Na]^+$, (calcd for $C_{20}H_{28}O_4Na$, 355.1885).

Cytotoxicity Assay. Cytotoxicity was determined against Daoy (human medulloblastoma) and WiDr (human colon adenocarcinoma) tumor cells and was based on a modified MTT assay method.[14−16] The cells were cultured in RPMI-1640 medium supplemented with serum in 5% CO_2 incubated at 37 °C. Test samples and standard were prepared at concentrations of 1, 10, 20, and 40 μg/mL. After seeding 2880 cells/well in a 96-well microplate for 4 h, 20 μL of sample or standard agent was placed in each well and incubated at 37 °C for 3 days, and then 20 μL of MTT was added for 5 h. After removing the medium and adding DMSO (200 μL/well) into the microplate with shaking for 10 min, the formazan crystals (the product of MTT reacting with dehydrogenase existing in mitochondria) were redissolved and absorbance was measured on a model MR 7000 microtiter plate reader (Dynatech International Corporation, Edgewood, NY) at a wavelength of 550 nm. The ED_{50} values were defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance.

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