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Tetrahedron Letters
Tetrahedron Letters 47 (2006) 6651–6655

New norditerpenoids from *Cespitularia hypotentaculata*

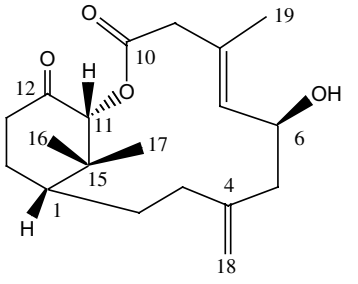
Ya-Ching Shen,[a,b,*] Jyun-Jhou Lin,[b] Ying-Ru Wu,[b] Jiun-Yang Chang,[b] Chang-Yih Duh[b] and Kuang Liang Lo[b]

[a]*School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan, ROC*
[b]*Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung 804, Taiwan, ROC*

Received 5 April 2006; revised 26 June 2006; accepted 30 June 2006
Available online 28 July 2006

Abstract—Four new norditerpenoids, designated as cespihypotins A (**1**), B (**2**), C (**3**) and D (**4**), were isolated from *Cespitularia hypotentaculata* Roxas (Xeniidae) that was collected in Taiwan. Compounds **1** and **2** are unprecedented structures having 13- and 14-membered lactone ring, respectively. Their structures were elucidated on the basis of extensive spectroscopic analysis. A plausible biogenetic pathway for compounds **1**–**4** was also proposed.

Norditerpenes possessing a 19 carbon skeleton are relatively rare compounds. Some of them have been reported from soft corals, especially members of the genus *Cespitularia*.[1] Biogenetically, they are derived from geranylgeranyl pyrophosphate and 1*S*-verticillene via loss of a methyl unit.[2,3] These marine organisms produce structures very similar to those of taxane diterpenoids in *Taxus*.[4,5] However, bicyclic norditerpenoids have never been found in terrestrial plants. Previously, three novel nitrogen-containing verticillene diterpenoids



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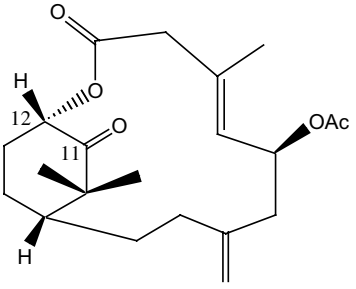

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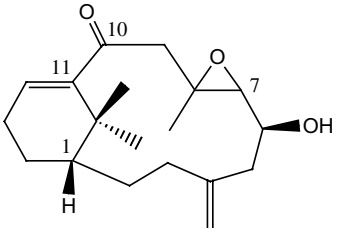

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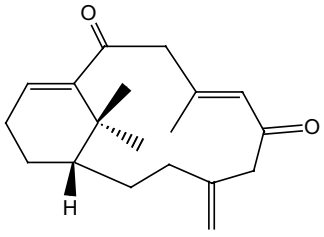

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* Corresponding author. Tel.: +886 7 5252000x5058; fax: +886 7 5255020; e-mail: ycshen@mail.nsysu.edu.tw

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doi:10.1016/j.tetlet.2006.06.178

named cespitulactams A, B and C were isolated from *Cespitularia taeniata*.[6] Our continuing investigation on the constituents of Taiwanese soft corals has led to the isolation of four novel compounds, cespihypotins A (**1**), B (**2**), C (**3**) and D (**4**), from *Cespitularia hypotentaculata* Roxas (Xeniidae). Of particular interest are compound **1** containing a novel 13-membered lactone ring and **2** possessing a rare 14-membered lactone ring. In this letter, we describe the isolation, structural elucidation and plausible biogenetic pathway for **1**–**4**.

The soft coral (0.8 kg, dry) collected at a depth of 20 m was extracted with a mixture of CH_2Cl_2 and MeOH, and the extract (63 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble portion (40 g) was subjected to a Si gel column (*n*-hexane/EtOAc, 50:1 to EtOAc/MeOH, 2:1) to yield fractions 1–12. Fraction 10 (7.13 g) was chromatographed on a LH-20 Sephadex resin column (CH_2Cl_2/MeOH, 1:1) to give a residue (4.89 g), which was separated by a Si gel column to afford S7-7. Application of fraction S7-7 (20 mg) on a HPLC column (Si gel, *n*-hexane/acetone, 4:1) furnished cespihypotin A (**1**, 5.5 mg). Fraction 5 (1.1 g) was chromatographed on a LH-20 Sephadex resin column (CH_2Cl_2/MeOH, 1:1) and a Si gel column (*n*-hexane/CH_2Cl_2/EtOAc, 3:1:1), and further HPLC (*n*-hexane/CH_2Cl_2/MeOH, 50:50:1 and *n*-hexane/EtOAc, 5:1) to give cespihypotin B (**2**, 3 mg). Separation of fraction 7 (3.29 g) by a Si gel column (*n*-hexane/EtOAc, 1:1), a LH-20 Sephadex resin column (CH_2Cl_2/MeOH, 1:1) and HPLC (Si gel, *n*-hexane/CH_2Cl_2/EtOAc, 3:1:1) yielded cespihypotins C (**3**, 3 mg) and D (**4**, 2 mg).

Cespihypotin A (**1**), [α] −43.4 (acetone), possessing a molecular formula $C_{19}H_{28}O_4$, was deduced from HRESIMS data.[7] The ^{1}H NMR spectrum of **1** exhibited signals including a doublet at δ 5.44 ($J = 8.4$ Hz), a two proton singlet at δ 5.03, a triplet at δ 4.60 ($J = 6.3$ Hz), a pair of doublets at δ 2.94 and 3.22 ($J = 14.8$ Hz) in addition to three methyl singlets (δ 0.99, 1.12, 1.73). The ^{13}C NMR spectrum of **1** showed signals of a ketone (δ 206.9), an ester carbonyl (δ 169.3), a trisubstituted olefinic carbon (δ 134.7, 129.1), an exocyclic double bond (δ 146.2, 114.2) and three methyl carbons (δ 25.3, 24.6, 17.3). The proton and carbon assignments were determined by the COSY and HMQC, the former established the partial structures as illustrated in Figure 1. HMBC data revealed correlations of H-11 (δ 4.44)/C-1,C-10,C-12 (δ 206.9); H-16/C-1,C-11 (δ 84.5); H-17/C-1,C-11; and correlations among the right hemisphere of **1** as indicated in Table 1. The stereochemistry of **1** was determined by NOESY experiment (Fig. 2), in which correlations were observed between H-11 and both the methyl groups H-16/H-17 and between H-1 and H-16/H-17. These findings established the β-orientation of both H-1 and H-11. The cross peaks among H-6, H-7 and H-19 were consistent with the α-configuration of H-6 in **1**. A computer generated 3D chemical model for cespihypotin A shown in Figure 4 by using MM2 force field calculation agreed with the assigned structure of **1**. The configuration of the hydroxyl at C-6 was further determined by Mosher's reactions.[8] It was suggested that the C-6 has the *S* configuration as illustrated in Figure 5.

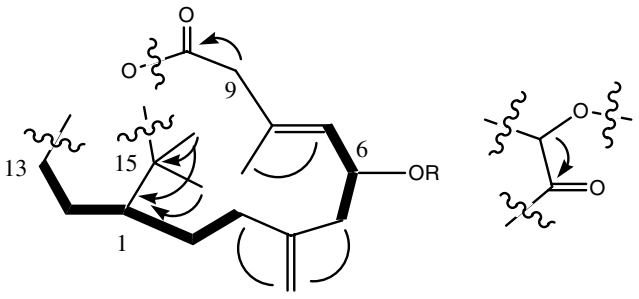


Figure 1. Partial structures of **1** and **2** established by COSY (curve, alylic correlation) and HMBC (arrow).

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

No.	δ_H (mult, *J*, Hz)	δ_C	HMBC ^{1}H–^{13}C	COSY ^{1}H–^{1}H
1	1.30 (m)	42.3	11, 14, 15	2, 14
2	1.90 (m), 2.05 (m)	26.3		3
3	2.02 (m)	39.0		2
4		146.2		
5	2.38 (m), 2.50 (m)	42.1	3, 4, 6, 7	6
6	4.60 (t, 6.3)	70.1	4, 5, 8	7
7	5.44 (d, 8.4)	134.7		6, 19
8		129.1		
9α	2.94 (d, 14.8)	45.8	7, 8, 10, 19	9β
9β	3.22 (d, 14.8)			9α
10		169.3		
11	4.44 (s)	84.5	1, 10, 12	
12		206.9	11	
13	2.55 (m), 2.13 (m)	33.1	12	14
14	1.95 (m), 1.73 (m)	29.4	12	1, 13
15		42.0		
16	0.99 (s)	25.3	1, 11, 15, 17	
17	1.12 (s)	24.6	1, 11, 15, 16	
18	5.03 (s)	114.2	3, 4, 5	3, 5
19	1.73 (s)	17.3	7, 8, 9	7

[a] Chemical shifts (δ) in parts per million, *J* values in hertz are in parentheses. Assignment was made using HMQC and HMBC techniques.

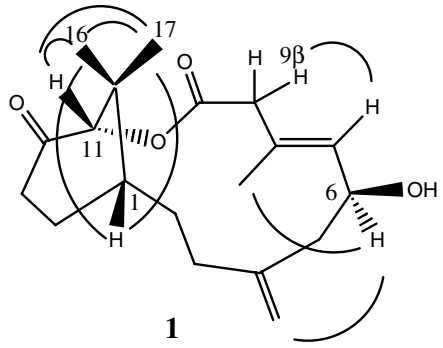


Figure 2. Key NOESY correlations and relative stereochemistry of **1**.

Cespihypotin B (**2**), [α] −97 (EtOAc), had a molecular formula $C_{21}H_{30}O_5$ as derived from HRESIMS data.[9] The ^{1}H NMR spectrum of **2** exhibited a trisubstituted olefinic proton (δ 5.34, H-7), two oxygenated methine

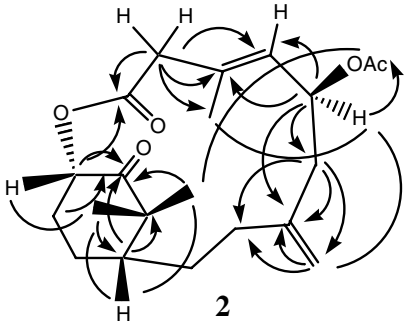


Figure 3. Selective HMBC (arrow) and NOESY (curve) correlations **2**.

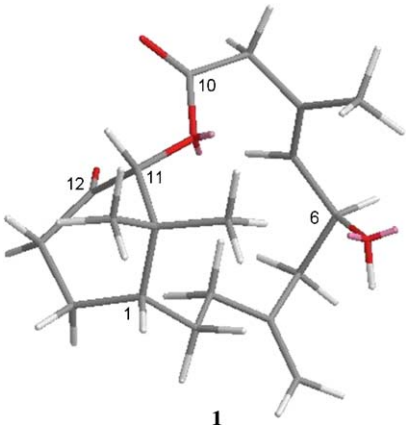
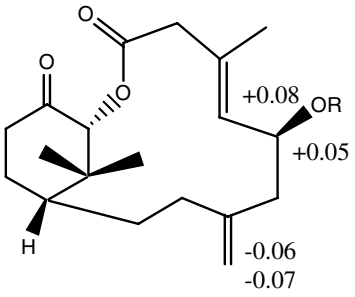


Figure 4. Computer-generated perspective models for **1** using MM2 force field calculation.



5, R = *R*-MPTA
6, R = *S*-MPTA

Figure 5. $\delta_S - \delta_R$ values for Mosher's reaction products.

signals (δ 5.48, H-6; δ 5.07, H-12), two exocyclic olefinic singlets (δ 4.90, 4.87, H-18) and three methyl singlets (δ 1.23, 1.15, 1.78, H-16, H-17, H-19). As in **1**, the ^{13}C NMR spectra revealed the presence of a ketone carbonyl (δ 211.1), an ester (δ_C 169.6), a trisubstituted olefin (δ 133.1, 130.1), a 1,1-disubstituted olefin (δ 145.5, 112.5), two oxygenated methine carbons (δ_C 71.4, C-7; 72.2, C-12), in addition to an acetyl group (δ 170.2 and 21.4). The COSY spectrum of **2** established the connectivities of H-9α (δ 2.88, d, $J = 13.5$ Hz)/H-9β (δ 3.20, d, $J = 13.5$ Hz), /H-19/H-7/H-6, /H-18/H-5/H-3/H-2/H-1 and H-12/H-13/H-14/H-1 which are same as that of **1** (Fig. 1). The HMBC data of **2** located the acetyl group at C-6 because the correlation of H-6/OAc was clearly observed. However, HMBC correlations of H_2-9/C-10, H-12/C-10, H-12/C-11, H-16/C-11, H-17/C-11 and H-1/C-11 assigned the ester carboxyl at C-10 and the ketone at C-11. The configuration of cespihypotin B (**2**) was elucidated by analyses of NOESY correlations (Fig. 3). The presence of mutual correlations between H-1, H-16, H-17 and H-12 agreed with all β-configuration, while H-6 was α-configuration.

Cespihypotin C (**3**) had the molecular formula $C_{19}H_{28}O_3$, as deduced from HRESIMS and DEPT NMR.[10] The UV absorption and IR bands indicated the presence of an α,β-unsaturated ketone and a hydroxyl functionality. The ^1H NMR spectral data (Table 2) revealed three olefinic singlets (δ_H 6.33, 4.92 and 4.84) and two oxygenated methine protons at δ_H 4.15 (H-6) and 3.10 (H-7) in addition to three methyl singlets at δ_H 1.57, 1.30 and 1.19. The ^{13}C NMR spectrum exhibited 19 carbons, in which six methylene groups (δ_C 53.1, 42.2, 32.3, 29.7, 24.0 and 22.7) were observed in the DEPT spectra of **3**. The two oxygenated methine carbon signals at δ_C 70.0 and 66.5 were assigned to C-6 and C-7, respectively, whereas the oxygenated quarternary carbon at δ_C 60.4 was attributed to C-8. The COSY spectrum of **3** revealed correlations of H-1/H-2/H-3, H-1/H-14/H-13/H-12 and H-5/H-6/H-7. The HMBC correlations between the carbonyl (δ_C 201.1) and the methylene AB quartet (δ_H 2.87 and 2.85, $J = 15$ Hz, H-9), and the olefinic proton (δ_H 6.33, H-12) indicated the carbonyl at C-10. The methyl proton at δ_H 1.57 (H-19) was correlated to C-9 (δ_C 53.1), C-8 and C-7, whose proton was, in turn, correlated to C-6, thus confirming the hydroxyl group at C-6 and an epoxide ring between C-7 and C-8. The HMBC correlations between

Table 2. ^1H and ^{13}C NMR data, HMBC and COSY correlations of **3**[a]

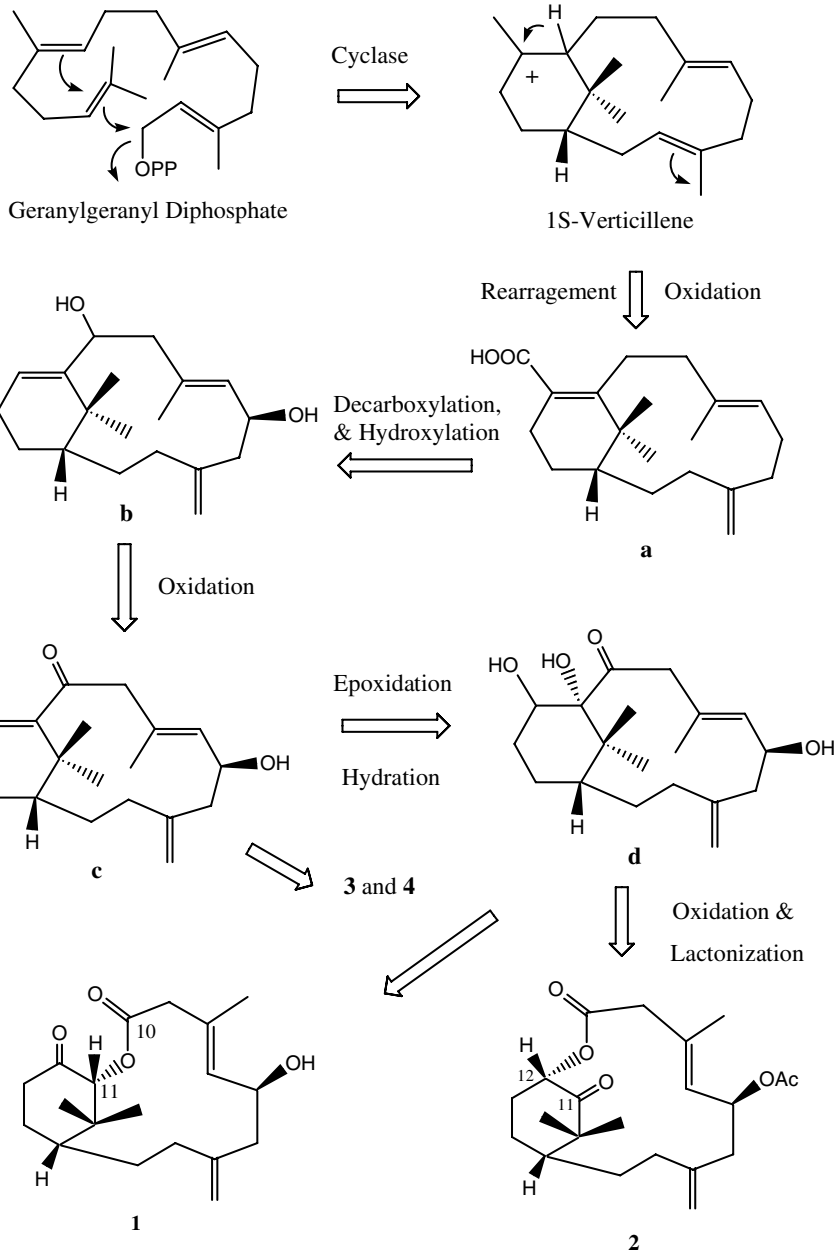
No.	δ_H (mult, *J*, Hz)	δ_C	HMBC ^1H–^{13}C	COSY ^1H–^1H
1	1.36 (m)	42.3	14, 15	2, 14
2	1.31 (m), 1.37 (m)	29.7		3
3	1.88 (m), 2.31 (m)	32.3		2
4		145.8		
5	2.09 (m), 2.32 (m)	42.2	4, 6	6, 7
6	4.15 (d, 14)	70.0		5
7	3.10 (s)	66.5	6	5
8		60.4		
9α	2.84 (d, 15)	53.1	8, 10	9β
9β	2.87 (d, 15)			9α
10		201.1		
11	6.33 (s)	148.9		
12		137.5	10	13
13	2.26 (m), 1.64 (m)	22.7		14, 12
14	1.38 (m), 0.95 (m)	24.0		1, 13
15		35.2		
16	1.30 (s)	32.3	1, 11, 15, 17	
17	1.19 (s)	24.8	1, 11, 15, 16	
18	4.92 (s), 4.84 (s)	113.9	3, 4, 5	
19	1.57 (s)	17.1	7, 8, 9	

[a] Chemical shifts (δ) in parts per million, *J* values in hertz are in parentheses. Assignment was made using HMQC and HMBC techniques.

H-16/C-1,C-11,C-15 and H-17/C-1,C-11,C-15 and H-18/C-4,C-3,C-5 allowed positioning the C-16, C-17 and C-18, respectively.

The molecular formula of **4** was deduced from ESIMS and DEPT NMR as $C_{19}H_{26}O_2$, indicating seven degrees of unsaturation.[11] The UV and IR bands, and NMR spectral data of **4** were closely similar to those of **3** suggesting a norditerpene skeleton with the function of α,β-unsaturated ketone as **3**. The presence of an additional α,β-unsaturated ketone was demonstrated by a proton signal at δ_H 6.26 (H-7) along with an additional carbonyl signal at δ_C 198.3 (C-6) and lack of hydroxynated carbons. The COSY spectrum showed the correlation of H-12/H-13/H-14/H-1/H-2/H-3. Detailed inspection of the HMBC spectrum of **4** revealed that both the H-5 protons at δ_H 3.17 and 3.01 (d, $J = 11$ Hz) as well as the H-7 proton showed correlations with carbonyl carbon at δ_C 198.3. Moreover, the methyl protons at C-19 correlated with C-7 (δ 131.2), C-8 (δ 147.4) and C-9 (δ 55.1) confirming a double bond between C-7 and C-8. Thus the structure of **4** was established for cespihypotin D.

A plausible biogenetic pathway of **1**–**4** was proposed as shown in Scheme 1 based on the biosynthesis of taxane diterpenes and recently published norditerpenoids.[1,6] The precursor geranylgeranyl diphosphate is transformed to an intermediate, 1*S*-verticillene by the enzyme cyclase. Subsequent steps involving rearrangement and oxidation yield intermediate **a**, then decarboxylation and hydroxylation produce intermediates **b** and **c**, which may lead to cespihypotins C (**3**) and D (**4**). Cespihypotins A (**1**) and B (**2**) might be derived from norditerpenes



Scheme 1. Plausible biogenetic pathway of **1**–**4**.

d via epoxidation, hydration, oxidation and lactonization, which involves attack of the C-12 hydroxy or C-11 hydroxy on the carbonyl at C-10 and subsequent bond cleavage between C-10 and C-11.

This letter describes the first isolation of four new norditerpenoids including the novel structures of **1** and **2** from *C. hypotentaculata*, which is a species closely related to *C. taeniata* in the family of Xeniidae. The occurrence of norditerpenoids **1**–**4** is of significance in the chemotaxonomy of the genus *Cespitularia*.

Acknowledgement

This work was supported by a grant from the National Science Council of the Republic of China (Grant No. NSC-94-2323-B-110-001) awarded to Y.-C.S.

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6. Shen, Y. C.; Lin, Y. S.; Kuo, Y. H.; Cheng, Y. B. *Tetrahedron Lett.* **2005**, *46*, 4893–4897.
7. Amorphous powder, $[\alpha]_D^{25}$ −43.4 (*c* 0.3, acetone); IR (neat) ν_{max} 3447, 2927, 1738, 1460, 1391, 1243, 1024, 902 cm^{-1}; UV λ_{max} (MeOH) 206 nm; EIMS *m/z* 320 ($[M]^+$); ESIMS *m/z* 343 $[M+Na]^+$, 321 $[M+H]^+$. HRESIMS *m/z* 343.1887 ($C_{19}H_{28}O_4Na$, calcd 343.1885).
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9. Cespihypotin B (**2**): $[\alpha]_D^{25}$ −97 (*c* 0.1, EtOAc); IR (neat) ν_{max} 3421, 2930, 1736, 1456, 1370, 1240, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.85 (m, H-1), 1.61 (2H, m, H-2), 1.90 (1H, m, H-3), 1.80 (1H, m, H-3), 2.36 (2H, m, H-5), 5.48 (1H, td, $J = 9.6$, 2.4 Hz, H-6), 5.34 (1H, d, $J = 9.6$ Hz, H-7), 2.88 (1H, d, $J = 13.5$ Hz, H-9α), 3.20 (1H, d, $J = 13.5$ Hz, H-9β), 5.07 (1H, m, H-12), 1.86 (1H, m, H-13), 2.45 (1H, m, H-13), 1.60 (1H, m, H-14), 1.90 (1H, m, H-14), 1.23 (3H, s, H-16), 1.15 (3H, s, H-17), 4.90 (1H, s, H-18), 4.87 (1H, s, H-18), 1.78 (3H, s, H-19), 2.03 (3H, s, OAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 43.1 (d, C-1), 28.6 (t, C-2), 33.6 (s, C-3), 145.5 (s, C-4), 43.1 (t, C-5), 71.4 (d, C-6), 133.1 (d, C-7), 130.1 (s, C-8), 46.4 (t, C-9), 169.6 (s, C-10), 211.3 (s, C-11), 72.2 (d. C-12), 26.2 (t, C-13), 20.1 (t, C-14), 47.8 (s, C-15), 27.7 (q, C-16) 23.2 (q, C-17), 112.5 (t, C-18), 17.3 (q, C-19), 170.2 (s, *C*OCH_3), 21.4 (q, CO*C*H_3); ESIMS *m/z* 385 $[M+Na]^+$; HRESIMS *m/z* 385.1990 ($C_{21}H_{30}O_5Na$, calcd 385.1991).
10. Cespihypotin C (**3**): amorphous powder, $[\alpha]_D^{25}$ 3.4 (*c* 0.15, acetone); UV λ_{max} (MeOH) 234 nm; IR (neat) ν_{max} 3420, 1701, 1654, 1275, 751 cm^{-1}; EIMS *m/z* 304 ($[M]^+$); ESIMS *m/z* 327 $[M+Na]^+$; HRESIMS *m/z* 327.1938 ($C_{19}H_{28}O_3Na$, calcd 327.1936).
11. Cespihypotin D (**4**): $[\alpha]_D^{25}$ 46.7 (*c* 0.15, acetone); UV λ_{max} (MeOH) 238 nm; IR (neat) ν_{max} 3421, 2924, 1681, 1610, 1435, 1266, 900, 738 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.66 (m, H-1), 1.99 (1H, m, H-2), 1.86 (1H, m, H-2), 2.61 (1H, m, H-3), 2.19 (1H, m, H-3), 3.17 (1H, d, $J = 11$ Hz, H-5), 3.01 (1H, d, $J = 11$ Hz, H-5), 6.26 (1H, s, H-7), 3.80 (1H, d, $J = 10.5$ Hz, H-9α), 3.01 (1H, d, $J = 10.5$ Hz, H-9β), 6.33 (1H, s, H-12), 2.35 (1H, m, H-13), 2.19 (1H, m, H-13), 1.98 (1H, m, H-14), 1.72 (1H, m, H-14), 1.25 (3H, s, H-16), 0.84 (3H, s, H-17), 4.90 (1H, s, H-18), 4.77 (1H, s, H-18), 1.99 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 43.6 (d, C-1), 29.7 (t, C-2), 32.6 (s, C-3), 143.8 (s, C-4), 54.0 (t, C-5), 198.3 (s, C-6), 131.2 (d, C-7), 147.4 (s, C-8), 55.1 (t, C-9), 199.8 (s, C-10), 147.5 (s, C-11), 134.8 (d. C-12), 23.6 (t, C-13), 29.3 (t, C-14), 35.9 (s, C-15), 23.5 (q, C-16), 32.4 (q, C-17), 112.2 (t, C-18), 18.8 (q, C-19); ESIMS *m/z* 309 $[M+Na]^+$.