Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 7337–7344

TETRAHEDRON

Five novel norcembranoids from *Sinularia leptoclados* and *S. parva*

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Received 8 April 2003; revised 15 July 2003; accepted 23 July 2003

Abstract—Three new norcembrane-based diterpenoids, leptocladolides A (**1**), B (**4**) and C (**5**), along with five known metabolites **6**–**10**, have been isolated from the dichloromethane extract of a Taiwanese soft coral *Sinularia leptoclados*. Furthermore, a chemical investigation on the dichloromethane extract of *S. parva* has resulted in the isolation of two new related isomers, 1-*epi*-leptocladolide A (**2**) and 7*E*-leptocladolide A (**3**), in addition to **1** and **7**. The structures of new metabolites **1**–**5** were elucidated on the basis of extensive spectroscopic analyses and their relative stereochemistries were determined by NOESY experiments. The new metabolites **1** and **3** have been shown to exhibit significant cytotoxic activity against KB and Hepa59T/VGH cancer cell lines.

1. Introduction

Since the first discovery of the C-4 norcembrane-based diterpenoid **9** from a soft coral, *Sinularia lepoclados* Ehrenberg, by an Australian group,[1] several related norditerpenoids have been discovered during the investigations of other *Sinularia* species.[2–10] During the course of our investigation on the bioactive metabolites of Taiwanese soft corals,[11–15] four norditerpenoids, scabrolides A–D were isolated from *Sinularia scabra*.[16] Our recent investigation on the chemical constituents of *S. lepoclados* Ehrenberg has led to the isolation and identification of three new norditerpenoids; leptocladolides A (**1**), B (**4**), and C (**5**), along with five known metabolites **6**–**10** (Fig. 1). Also, *Sinularia parva* Tixier-Durivault which is investigated for the first time afforded leptocladolide A (**1**), and its related isomers 1-*epi*-leptocladolide A (**2**) and 7*E*-leptocladolide A (**3**), in addition to the known metabolite **7** (Fig. 1). The molecular structures, including the relative configuration of the new norditerpenoids, were elucidated by the extensive spectral analyses. The new metabolites **1**–**3** represent a new class of bicyclic norcembrane-based diterpenoids lacking C-5,8 ether linkages. Cytotoxicity of metabolites **1**–**10** against KB (human oral epidermoid carcinoma) and Hepa59T/VGH (human liver carcinoma) cancer cell lines also is reported.

2. Results and discussion

Organisms of *S. leptoclados* were homogenized with EtOAc and filtered. The organic layers were combined and evaporated under vacuum to afford a dark brownish viscous residue. The residue was triturated with *n*-hexane and subsequently with dichloromethane. The dichloromethane extract was subjected to a series of chromatographic purification, including HPLC, to afford metabolites **1**, **4** and **5**–**10**. The dichloromethane fraction from the ethanolic extract of *S. parva* was purified by chromatography to yield compounds **1**–**3**, and **7** (see Section 3).

Leptocladolide A (**1**) was obtained as a colorless oil. Its HRFABMS spectrum exhibited a pseudomolecular ion peak at *m/z* 377.1964, consistent with the molecular formula $C_{21}H_{28}O_6$ and eight degrees of unsaturation. The IR spectrum of **1** suggested the presence of hydroxy, ester carbonyl, and keto-carbonyl functionalities by absorptions at ν_{max} 3420, 1750, 1700, 1680 cm^{-1}, respectively. The FABMS exhibited peaks at *m/z* 359 $[M+H-H_2O]^+$ and 331 $[M+H-EtOH]^+$, revealing the presence of a hydroxy and an ethoxy group in **1**. The ^{13}C NMR spectrum of **1** measured in $CDCl_3$, showed signals of 21 carbon atoms, which were identified by the assistance of DEPT spectrum as three

Keywords: *Sinularia leptoclados*; *S. parva*; leptocladolide A–C; 1-*epi*-leptocladolide; 7*E*-leptocladolide; soft coral.
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doi:10.1016/S0040-4020(03)01138-4

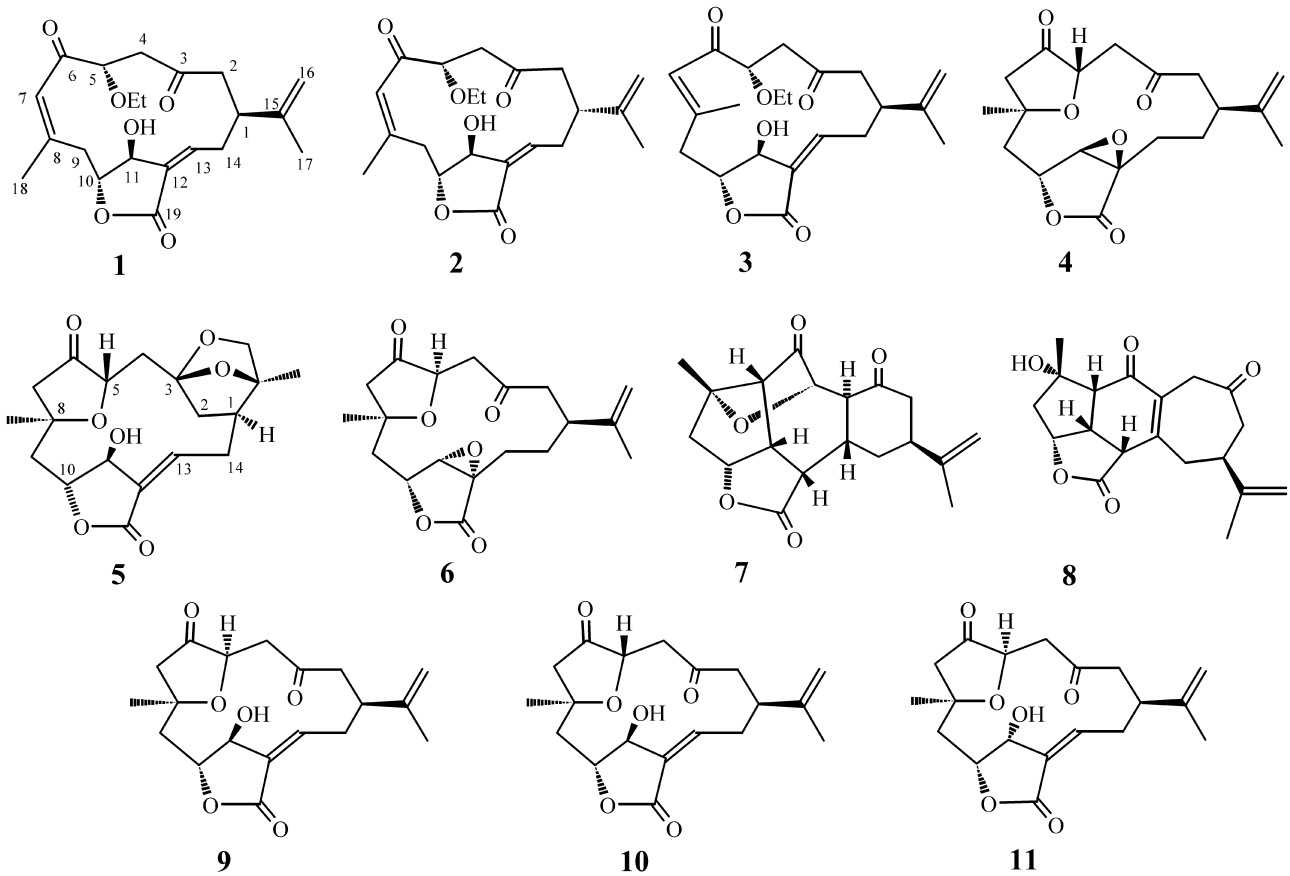

Figure 1. Structures of new metabolites**1**–**5** and known norcembranoids **6**–**10** of *Sinularia leptoclados* and *S. parva*.

methyls, five sp^3 methylenes (including one oxymethylene), one sp^2 methylene, four sp^3 methines (including three oxymethines), two vinylic methines, and six sp^2 quaternary carbons (Table 1). The signals appearing at δ 205.9, 199.1, and 168.2 were attributable to carbons of a normal ketone, an α,β-conjugated ketone, and an ester carbonyl, respectively. Furthermore, the six carbon signals appearing at δ_C 154.4 (s), 146.2 (d), 146.1 (s), 130.6 (s), 124.6 (d), and 111.2 (t) designate the presence of two trisubstituted and one 1,1-disubstituted carbon–carbon double bonds in **1**. From

Table 1. ^{13}C NMR spectral data of compounds **1**–**6** and **10**

C#	1[a]	2[a]	3[a]	4[b]	5[b]	6[b],[16]	10[c]
1	38.1 (d)[d]	41.3 (d)	39.1 (d)	39.8 (d)	44.4 (d)	40.8 (d)	41.9 (d)
2	45.1 (t)	46.5 (t)	43.2 (t)	49.9 (t)	38.6 (t)	48.3 (t)	48.2 (t)
3	205.9 (s)	207.1 (s)	205.9 (s)	208.4 (s)	108.7 (s)	207.6 (s)	208.2 (s)
4	43.0 (t)	43.5 (t)	46.4 (t)	43.9 (t)	35.2 (t)	44.8 (t)	44.5 (t)
5	82.3 (d)	81.5 (d)	81.2 (d)	78.1 (d)	77.3 (d)	75.0 (d)	77.5 (d)
6	199.1 (s)	200.5 (s)	199.1 (s)	211.9 (s)	214.6 (s)	213.8 (s)	212.9 (s)
7	124.6 (d)	124.2 (d)	125.1 (d)	51.0 (t)	51.6 (t)	49.4 (t)	51.9 (t)
8	154.4 (s)	155.1 (s)	152.8 (s)	79.5 (s)	79.4 (s)	79.1 (s)	79.4 (s)
9	35.5 (t)	35.4 (t)	43.0 (t)	40.5 (t)	42.0 (t)	42.4 (t)	42.3 (t)
10	80.9 (d)	82.1 (d)	83.2 (d)	78.2 (d)	83.1 (d)	75.9 (d)	83.8 (d)
11	75.8 (d)	74.6 (d)	72.3 (d)	63.1 (d)	76.7 (d)	62.7 (d)	75.6 (d)
12	130.6 (s)	130.3 (s)	131.3 (s)	62.1 (s)	130.6 (s)	60.7 (s)	131.8 (s)
13	146.2 (d)	146.7 (d)	147.4 (d)	21.2 (t)	146.0 (d)	21.3 (t)	145.3 (d)
14	27.2 (t)	30.9 (t)	27.7 (t)	25.5 (t)	27.5 (t)	26.4 (t)	31.7 (t)
15	146.1 (s)	146.2 (s)	145.2 (s)	145.3 (s)	84.8 (s)	145.8 (s)	148.0 (s)
16	111.2 (t)	110.7 (t)	111.2 (t)	113.1 (t)	75.4 (t)	112.7 (t)	110.3 (t)
17	22.5 (q)	21.6 (q)	22.5 (q)	18.7 (q)	14.8 (q)	18.7 (q)	21.1 (q)
18	25.5 (q)	25.6 (q)	23.2 (q)	28.5 (q)	30.5 (q)	25.6 (q)	29.6 (q)
19	168.2 (s)	168.0 (s)	168.3 (s)	172.3 (s)	169.2 (s)	174.0 (s)	169.6 (s)
OEt	66.3 (t)	65.9 (t)	66.2 (t)				
	15.3 (q)	15.3 (q)	15.2 (q)				

[a] Spectra recorded at 125 MHz in $CDCl_3$ at 25°C.
[b] Spectra recorded at 100 MHz in $CDCl_3$ at 25°C.
[c] Spectra recorded at 75 MHz in $CDCl_3$ at 25°C.
[d] Multiplicity deduced by DEPT and indicated by usual symbols. The values are in ppm downfield from TMS.

the above findings, **1** was thus suggested to be a bicyclic norditerpenoid possessing three olefinic bonds, one ester and two keto-carbonyl groups, one hydroxy group, and an ethoxy group. By comparison of the ^{13}C NMR spectral data of **1** with those of **10**[9] (Table 1), a known metabolite also isolated in the present study, it was suggested that **1** has the same norcembrane carbon skeleton as that of **10** but with an additional trisubstituted double bond (δ_C 124.6, d and 154.4, s). The location of this double bond was established at C-7 and C-8 due to the downfield shift of H_3-18 (δ_H 2.00, 3H, s) and the appearance of an additional vinylic proton at δ_H 6.45 (1H, s, H-7) in the ^1H NMR spectrum of **1** (Table 2). This was further supported by the $^1H/^{13}C$ long-range correlations observed in the HMBC spectrum (Fig. 2) between H_3-18 and both C-8 (δ_C 154.4, s) and C-7 (δ_C 124.6, d), and between H-7 and the carbonyl carbon, C-6 (δ_C 199.1, s). The position of the ethoxy group at C-5 was also established through $^1H/^{13}C$ long-range correlation observed between H-5 (δ_H 4.16, 1H, dd, J=10.0, 3.0 Hz) and both the methylene carbon (δ_C 66.3, t) of the ethoxy group and C-6 carbonyl carbon. On the basis of the above observations, and by the assistance of a series of 2D NMR ($^1H-^1H$ COSY, HMQC and HMBC) experiments, it was possible to establish the planar structure of **1**, as illustrated in Figure 2.

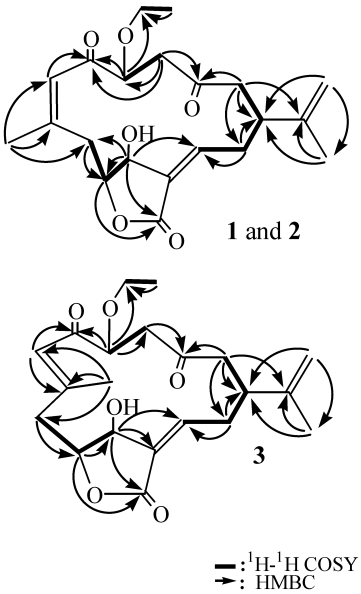

Figure 2. $^1H-^1H$ COSY and HMBC correlations for **1**–**3** and **5**.

The relative stereochemistry of the four chiral centers at C-1, C-5, C-10, and C-11 in **1** was determined on the basis of the NOE correlations observed in a NOESY spectrum (Fig. 3), in addition to the chemical shifts and coupling constants of the concerned protons. The *Z*-geometry of the 7,8-double bond was established by the NOE interaction between H-7 (δ_H 6.45, s) and H_3-18 (δ_H 2.00, s). One proton attaching at C-14 and resonating at δ_H 3.74 (ddd, J=15.5, 12.0, 4.0 Hz) was found to show NOE interactions with H-1 (δ_H 2.85, br d, J=4.0 Hz) and was assigned arbitrary as H-14α. Thus, the isopropenyl group located at C-1 should be β-oriented. The other proton attaching at C-14, H-14β (δ_H 2.25, dt, J=15.5, 4.0 Hz), showed NOE interactions with the olefinic proton H-13 (δ_H 6.51, dd, J=12.0, 4.0 Hz), confirming the upward orientation of H-13. The significant NOE interactions shown between oxymethine H-11 and olefinic H-13 revealed the parallel orientation of $C_{11}-H$ and $C_{13}-H$, and hence, the S^* configuration at C-11 and the *cis* orientation of the 12,13-double bond, according to a molecular model represented in Figure 3. One H-9 (δ_H 3.53, dd, J=13.0, 9.5 Hz) showed strong NOE correlation with H-11 and was assigned as H-9α. Therefore, the significant NOE interaction observed between the other H-9 resonating at δ_H 2.48 (dd, J=13.0, 8.5 Hz) and H-10 depicted the β-orientation of H-10, and hence the R^* configuration at C-10. The NOE interactions disclosed

Table 2. ^1H NMR spectral data of compounds **1**–**5**

	1[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
H-1	2.85 br d (4.0)[c]	2.77 br t (7.0)	2.97 dd (9.0, 4.5)	2.76 m	2.37 dt (8.0, 4.0)
H-2α	2.49 d (14.0)	2.52 dd (15.5, 7.0)	2.07 dd (17.0, 9.0)	2.58 dd (11.2, 2.8)	2.12 dd (14.0, 3.2)
H-2β	2.86 d (14.0)	2.30 dd (15.5, 7.0)	2.27 dd (17.0, 4.5)	2.27 t (11.2)	1.94 dd (14.0, 8.0)
H-4α	2.85 dd (13.5, 10.0)	2.93 dd (16.0, 9.5)	3.06 dd (16.0, 3.0)	2.72 dd (14.2, 10.4)	1.84 dd (14.4, 11.6)
H-4β	2.56 dd (13.5, 3.0)	2.80 dd (16.0, 2.5)	2.94 dd (16.0, 7.0)	2.63 dd (14.4, 2.4)	2.27 d (14.4, 1.5)
H-5	4.16 dd (10.0, 3.0)	4.03 dd (9.5, 2.5)	4.05 dd (7.0, 2.5)	4.46 dd (10.4, 2.0)	3.99 dd (11.5, 1.5)
H-7α	6.45 s	6.49 s	6.39 s	2.45 d (18.0)	2.39 d (18.4)
H-7β				2.58 d (18.0)	2.49 d (18.4)
H-9α	3.53 dd (13.0, 9.5)	2.77 m	2.54 dd (14.0, 3.0)	2.14 dd (15.8, 2.6)	2.39 dd (14.2, 8.0)
H-9β	2.48 dd (13.0, 8.5)	3.46 m	2.86 dd (14.0, 4.5)	2.38 dd (15.6, 4.4)	1.95 d (14.2)
H-10	4.69 dd (9.5, 8.5)	4.77 t (7.5)	4.73 br dd (4.5, 3.0)	4.72 dd (4.2, 3.0)	4.63 d (8.0)
H-11	4.51 s	4.50 s	4.63 s	4.14 s	4.47 s
H-13α				2.32 ddd (16.0, 10.8, 2.0)	
H-13β	6.51 dd (12.0, 4.0)	6.51 dd (11.5, 6.0)	6.47 dd (12.0, 4.5)	1.88 ddd (16.0, 7.8, 2.2)	6.70 dd (12.4, 6.0)
H-14α	3.74 ddd (15.5, 12.0, 4.0)	3.55 m	3.53 ddd (14.0, 12.0, 4.5)	1.60 m	3.56 ddd (14.0, 12.4, 4.0)
H-14β	2.25 dt (15.5, 4.0)	2.18 dt (13.5, 7.0)	2.22 dt (14.0, 4.5)	1.25 ddd (14.4, 10.8, 2.8)	2.27 dd (14.0, 6.0)
H-16	4.94 s	4.85 s	4.87 s	4.91 s	3.61 d (6.8) [α]
	4.75 s	4.72 s	4.53 s	4.80 s	3.39 d (6.8) [β]
17-Me	1.83 3H, s	1.81 3H, s	1.81 3H, s	1.68 3H, s	1.59 3H, s
18-Me	2.00 3H, s	2.06 3H, s	2.27 3H, s	1.46 3H, s	1.62 3H, s
5-OEt	3.64 ddd (16.0, 7.0, 2.0)	3.55 dq (15.0, 7.0)	3.63 dq (15.0, 7.0)		
	3.59 ddd (16.0, 7.0, 2.0)	3.48 dq (15.0, 7.0)	3.55 dq (15.0, 7.0)		
	1.23 3H, t (7.0)	1.20 3H, t (6.5)	1.21 3H, t (7.0)		

[a] Spectra recorded at 500 MHz in $CDCl_3$ at 25°C.
[b] Spectra recorded at 400 MHz in $CDCl_3$ at 25°C.
[c] The J values are in Hz in parentheses.

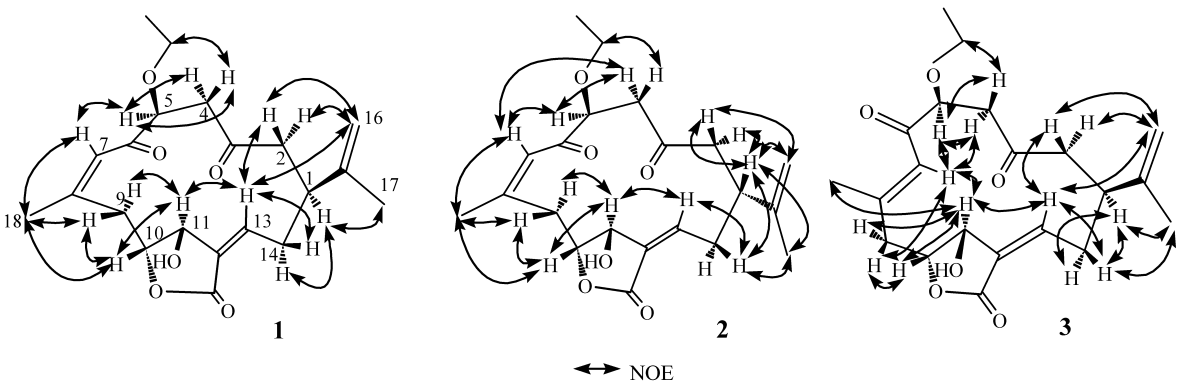

Figure 3. Key NOESY correlations o f **1**, **2**, and **3**.

between both H-10 and H-9β and H_3-18, H_3-18 and H-7, and H-7 and H-5, revealed that H-5 is positioned on the upward face of the fourteen-membered ring as shown in Figure 3 and is *syn* oriented relative to H-10. On the basis of the above findings and other key NOE interactions observed (see Fig. 3), the structure of leptocladolide A (**1**), was unambiguously established as shown in formula **1**.

The new metabolite, 1-*epi*-leptocladolide (**2**), also isolated from *S. parva*, was obtained as colorless oil. On the basis of its HRFABMS (*m/z* 377.1966, $[M+H]^+$), along with the ^1H and ^{13}C NMR spectral data, the molecular formula of **2** was established as $C_{21}H_{28}O_6$. As in the case of **1**, **2** also revealed the presence of a hydroxy group (EIMS *m/z* 358 $[M-H_2O]^+$, IR ν_{max} 3414 cm^{-1}) and an ethoxy group (EIMS *m/z* 330 $[M-EtOH]^+$). Furthermore, it was found that the ^1H and ^{13}C NMR spectral data of **2** were very similar to those of leptocladolide A (**1**) (Tables 1 and 2), suggesting that **2** could be an epimer of **1**. By the assistance of 2D NMR spectra, including COSY, HMQC, and HMBC, **2** was shown to possess the same molecular framework as that of **1** (Fig. 2). However, the significant downfield shifts for C-1 ($\Delta\delta_C$ +3.2 ppm), C-2 ($\Delta\delta_C$ +1.4 ppm) and C-14 ($\Delta\delta_C$ +3.7 ppm) in comparison with those of **1** (Table 1), suggesting that **2** might be the C-1 epimer of **1**. By careful investigation on the NOESY spectrum of **2** (Fig. 3), it was found that H-1 showed significant NOE interactions with H_2-2 and H-14β (δ_H 2.18, 1H, dt, *J*=13.5, 7.0 Hz), revealing the β-orientation of H-1 and thus the *R** configuration at C-1. Further analyses on other NOE interactions revealed that **2** possessed the same relative configurations at C-5, C-10, and C-11, as those of **1** (Fig. 3). Therefore, **2** was found to be the C-1 epimer of **1** and the structure of this new metabolite could be established as described by formula **2**.

The third related new norditerpenoid isolated from *S. parva*, 7*E*-leptocladolide A (**3**), has the same molecular formula $C_{21}H_{28}O_6$ and was considered to be an isomer of **1** and **2** on the basis of HREIMS and NMR spectral data (Tables 1 and 2). Also, spectral data revealed the presence of a hydroxy group (IR ν_{max} 3429 cm^{-1}, EIMS *m/z* 358 $[M-H_2O]^+$) and an ethoxy group (IR EIMS *m/z* 330 $[M-EtOH]^+$) in **3**. In general, the ^1H and ^{13}C NMR data of **3** were found to be similar to those of **1** and **2**. Nevertheless, δ_C of C-1 and C-14 of **3** were more or less close to those of **1** in comparison with those of **2** (Table 1), suggesting that the relative configuration at C-1 is possibly the same as that of **1**. Moreover, the upfield shifts observed for C-8 ($\Delta\delta_C$ −2.3−1.6 ppm) and C-18 ($\Delta\delta_C$ −2.4−2.3 ppm), and the downfield shift of C-9 ($\Delta\delta_C$ +7.6−7.5 ppm) in comparison with those of **1** and **2** (Table 1), suggested that **3** could possess different geometry for the 7,8-carbon, carbon double bond, in contrast to those of **1** and **2**. According to the NOESY spectrum of **3** (Fig. 3), H-7 did not show NOE response with H_3-18, confirming the *E*-geometry of 7,8-double bond. Furthermore, it was found that the relative stereochemistry of C-1 in **3** is similar to that of **1** on the basis of NOE correlations observed between H-1 (δ_H 2.97, 1H, dd, *J*=9.0, 4.5 Hz) and H_2-14, and between olefinic H-16 (δ_H 4.53, s) and those of olefinic H-13 (δ_H 6.47, dd, *J*=12.0, 4.5 Hz) and H_2-2. The significant interaction exhibited between oxymethine H-11 and olefinic proton H-13 again revealed the *S** configuration at C-11, the same as that in **1**. By consideration of the above findings, along with other NOE responses observed as shown in Figure 3, the structure of compound **3** was unambiguously established as 7*E*-leptocladolide A.

The new norditerpenoid metabolite leptocladolide B (**4**) was isolated from *S. leptoclados* as a white solid. Its EIMS (*m/z* 384 $[M]^+$) and ^{13}C NMR spectral data, including those of DEPT (Table 1), established a molecular formula $C_{19}H_{24}O_6$ and eight degrees of unsaturation. The ^{13}C NMR data of **4** showed signals of 19 carbon atoms, including those of an 1,1-disubstituted double bond (δ_C 113.1, t and 145.3, s), one ester and two ketone carbonyl groups (δ_C 172.3, s, 208.4, s, and 211.9, s, respectively). Interpretation of ^1H and ^{13}C NMR spectral data (Tables 1 and 2), by the assistance of 2D NMR spectra, revealed that **4** possesses a trisubstituted oxotetrahydrofuran moiety [^1H NMR δ_H 4.46 (1H, dd, *J*=10.4, 2.0 Hz); ^{13}C NMR δ_C 211.9 (s), 79.5 (s), and 78.1 (d)], a γ-lactone group with a trisubstituted epoxide [^1H NMR δ_H 4.14 (1H, s) and 4.72 (1H, dd, *J*=4.4, 3.0 Hz); ^{13}C NMR δ_C 172.3 (s), 78.2 (d), 63.1 (d), and 62.1 (s)]. These observations revealed that the structure of **4** should be similar to that of scabrolide D (**6**), previously isolated from *S. scabra*[16] (Table 1). Therefore, compound **4** was suggested to be a stereoisomer of **6**. Careful investigation on NOESY spectrum of **4**, in comparison with that of **6**, could be useful for determination of the relative stereochemistry of **4** (Fig. 5). Assuming the α-orientation of H_3-18, it was found that H_3-18 exhibited NOE correlations with H-7α (δ_H 2.45, d, *J*=18.0 Hz), H-9α (δ_H 2.14, dd, *J*=15.8, 2.6 Hz), and H-4α (δ_H 2.72, dd, *J*=14.2, 10.4 Hz), but not with H-4β (δ_H 2.63, dd, *J*=14.4, 2.4 Hz) or H-5 (δ_H

4.46, dd, J=10.4, 2.0 Hz). However, significant NOE interactions between H-5 and H-4β were observed, indicating the β-orientation of H-5. Thus, C-5 is S^* configured. Moreover, the downfield shifts observed at C-5 ($\Delta\delta_C$ +2.9 ppm) and C-18 ($\Delta\delta_C$ +2.9 ppm) and the upfield shift at C-6 ($\Delta\delta_C$ −1.9 ppm) relative to those of **6** were considered as additional evidences for the 5S^* configuration[9,10,16] in **4**, in contrast to **6**[16] and other 5R^*-related norcembranoids.[3,8–10] H-1 was found to exhibit NOE response with H_3-18, suggesting the β-orientation of the isopropenyl group at C-1. The methyl protons, H_3-16, showed NOE responses with H-2β (δ_H 2.27, t, J=11.2 Hz) and H-14β (δ_H 1.25, ddd, J=14.4, 10.8, 2.8 Hz), but not with H-14α (δ_H 1.60, m), which in turn correlated with H-11 (δ_H 4.14, s), and revealing the α-orientation of H-11. H-11 did not show NOE response with H-10, and revealing the β-orientation of H-10. Thus, the relative structure of **4** was fully established.

A novel norcembranoid leptocladolide C (**5**), isolated from *S. leptoclados*, was obtained as a white solid. Its HREIMS (m/z 364.1519, M^+) and the ^{1}H, and ^{13}C NMR spectral data, suggested a molecular formula of $C_{19}H_{24}O_7$, consistent with eight degrees of unsaturation. A hydroxy group was suggested to be present in **5** (EIMS, m/z 346 $[M-H_2O]^+$ and IR ν_{max} 3424 cm^{-1}). The ^{13}C NMR spectrum displayed nineteen carbon signals which were assigned into two methyls, six methylenes including an oxygenated one (δ_C 75.4, t), five methines including three oxygenated and one vinylic (δ_C 83.1, d, 77.3, d, 76.7, d and 146.0, d, respectively), and six quaternary carbons including one oxygenated (δ_C 79.4, s) and one dioxygenated (δ_C 108.7, s). The carbon signals appearing at δ_C 214.6 (s), 169.2 (s), 146.0 (d), and 130.6 (s) indicated the presence of a normal ketone, and an α,β-conjugated ester in **5**. Therefore, metabolite **5** is a pentacyclic norcembranoid. The ^{1}H NMR of **5** (Table 2) showed two 3H singlets at δ_H 1.62 and 1.59 which were attributed to two tertiary methyl groups, three 1H signals at δ_H 4.63 (d, J=8.0 Hz), 4.47 (s), and 3.99 (dd, J=11.5, 1.5 Hz) ascribable to three oxymethine groups, two 1H doublets at δ_H 3.61 (d, J=6.8 Hz) and 3.39 (d, J=6.8 Hz) of an oxymethylene group, and the signal of a vinylic proton of a trisubstituted double bond (δ_H 6.70, dd, J=12.4, 6.0 Hz). Comparison of the ^{13}C NMR spectral data of **5** with those of sinuleptolide **10**[9] (Table 1) revealed that **5** should possess the same partial structure extending from C-4 to C-14 as **10**, while the signals of C-3 carbonyl carbon (δ 208.2, s) and 1,1-disubstituted double bond of the isopropenyl group (δ_C 148.0, s and 110.3, t) of **10** disappeared and were replaced by carbon signals resonating at δ_C 108.7 (s), 84.8 (s), and 75.4 (t), respectively. These results suggested that C-3 carbonyl has been transformed into a ketal with two oxygen atoms further connected with C-15 and C-16, respectively, to furnish tetrahydrofuran and tetrahydropyran rings. After investigating the ^{1}H–H COSY spectrum of **5**, it was possible to establish the sequential proton sets extending from H_2-4 to H-5, H-9 to H-11, and H-13 to H_2-2 through H-1 (Fig. 4). In the HMBC spectrum of **5**, it was found that H_3-17 (δ 1.59, 3H, s) exhibited long-range ^{1}H/^{13}C correlations with C-16 (δ_C 75.4, t), C-1 (δ_C 44.4, d), and C-15 (δ_C 84.8, s), indicating that this ring-junctured methyl group should be located at C-15. According to the above

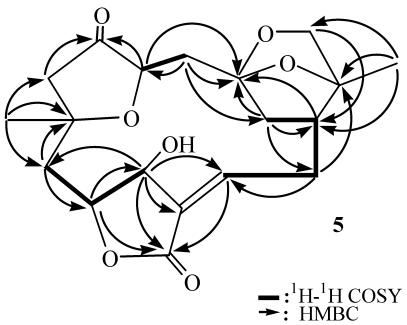

Figure 4. ^{1}H–^{1}H COSY and HMBC correlations for **5**.

observations and by careful analysis of HMBC spectrum with the aid of HMQC spectral data, the framework of **5** could be established as shown in Figure 4. The relative stereochemistry of **5** was deduced by careful study of the NOESY spectrum. As in the case of **4**, it was found that the α-oriented H_3-18 did not exhibit NOE response with H-5, indicating the S^* configuration of C-5. This was further confirmed by the diagnostic chemical shifts of C-5, C-6, and C-18 which are similar to those of 5S^*-related norcembranoids as in cases of **4** and **10**. One proton of H_2-2 resonating at δ_H 1.94 (1H, dd, J=14.0, 8.0 Hz) showed significant NOE interaction with H-5, and was assigned as H-2β. The other proton, H-2α (δ_H 2.12, dd, J=14.0, 3.2 Hz), exhibited NOE correlation with H-14α (δ_H 3.56, ddd, J=14.0, 12.4, 4.0 Hz), which further correlated with H-1, and revealing the α-orientation of H-1. The protons of the ring-junctured methyl, H_3-17, exhibited marked NOE correlation with H-1, implying the α-orientation of the methyl substituent at C-15. One oxymethylene proton, H-16α, showed significant correlation with H-1, suggesting that the oxymethylene moiety of the

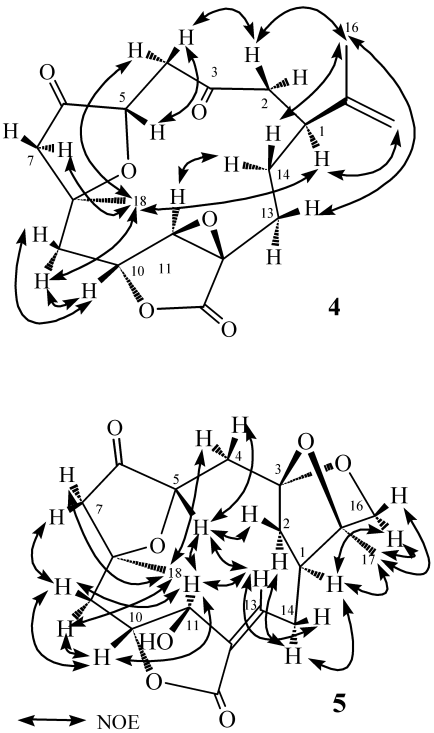

Figure 5. Key NOESY correlations for **4** and **5**.

tetrahydropyran attached at C-3 should be α-oriented. Furthermore, H-11 exhibited significant correlation with H-13 and H-10, revealing the β-orientation of H-10 and α-orientation of H-11, as those in **1**–**3**. From the above findings and other correlations observed (Fig. 5), the structure of leptocladolide C was unambiguously established as in formula **5**.

It is worthwhile to mention that the new metabolites **1**–**3** represent a new class of bicyclic norcembrane-based diterpenoids lacking 5,8-ether linkages. Also, **5** was found to contain a novel 2,7-dioxa-bicyclo[2,2,1]heptane structural unit, which has not been found previously in diterpenoids, to the best of our knowledge.

Other known compounds **6**–**10**, which were also isolated from *S. leptoclados*, were found to be identical with the previously reported scabrolide D (**6**) and scabrolide A (**8**)[16] isolated from *S. scabra*, ineleganolide (**7**)[8] from *S. inelegans*, norcembranoid **9** from *S. leptoclados*,[1] *S. polydactyla*[17] and *S. scabra*,[16] and sinuleptolide **10** isolated from an unidentified *Sinularia* species[9] by comparison of the physical (mp and $[\alpha]_D$) and spectral (MS, ^1H and ^{13}C NMR) data. Although **9** was the first 5*R**,11*S**-norcembranoid discovered,[1,3,4,9,17] as confirmed by single-crystal X-ray analyses,[1,17] it was misleadingly configured occasionally as an 5*R**,11*R**-epimer (**11**).[1,17] This contradiction prompted us to reinvestigate the ^1H and ^{13}C NMR spectral data of **9** in $CDCl_3$ and pyridine-d_5. It was found that the spectral data in $CDCl_3$ are in full agreement with those published by Bowden et al.[1] and Duh et al.,[17] who represented **9** incorrectly as an 5*R**,11*R**-epimer. Moreover, the NMR spectral data of **9** in pyridine-d_5 were found to be superimposable to those published by Shoji et al.,[9] where it was presented as the 5*R**,11*S**-epimer. Thus, the structure of **11**, shown in Refs. 1,16,17, should be revised as **9**. Furthermore, the NMR spectra of **10**, isolated in the present study and previously published as **9** by our group,[16] were remeasured in pyridine-d_5 and gave data (see Section 3) which are nicely fitted with those of sinuleptolide (5*S**,11*S**-epimer, **10**).[9] Thus, the relative configuration of this metabolite should also be revised as that of **10**. On the basis of all above findings, we can conclude that metabolite **11** has not been discovered yet.

Our previous study revealed strong cytotoxic activity for the tricyclic norcembranoids **9** and **10** against the growth of KB and Hepa59T/VGH cancer cells (ED_{50} 2.3–2.6 μg/mL).[16] This result prompted us to extend our study on biologically activity of the related norcembranoids. The cytotoxicity of metabolites **1**–**8** was thus evaluated on the same cell lines. It was found that the new metabolites **4** and **5** and the known compounds (**6**–**8**) were inactive (ED_{50} >20 μg/mL) against the growth of both cells, while the three new bicyclic norcembranoids (**1**–**3**) exhibited variable activity. Leptocladolide A (**1**) exhibited significant cytotoxicity against KB and Hepa59T/VGH cells lines with ED_{50} of 5.9 and 2.6 μg/mL, respectively, while 7*E*-leptocladolide A (**3**) was found to be more cytotoxic against Hepa59T/VGH cells (ED_{50} 3.2 μg/mL) relative to KB cells (ED_{50} 12.0 μg/mL). The related C-1 epimeric metabolite **2** showed only weak cytotoxic activity against both cell lines (ED_{50} 15.1 and 14.5 μg/mL, respectively).

3. Experimental

3.1. General experimental procedures

Melting points were determined using a Fisher–Johns melting point apparatus. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. EIMS was obtained with a VG Quattro GC/MS spectrometer. HRMS spectra were recorded on a Finnigan MAT-95XL mass spectrometer. The NMR spectra were recorded on a Bruker AVANCE DPX300 FT NMR at 300 MHz for ^1H and 75 MHz for ^{13}C or on Bruker AMX-400 FT NMR at 400 MHz for ^1H and 100 MHz for ^{13}C or on a Varian Unity INOVA 500 FT NMR at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard, unless otherwise indicated. Si gel (Merck, 230–400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

3.2. Organism

S. leptoclados and *S. parva* were collected by hand via SCUBA at the coast of Kenting, Taiwan in February, 2000 and in December, 2001, respectively, at a depth of 15–20 m, and stored in a freezer until extraction. A voucher samples was deposited at the Department of Marine Resources, National Sun Yat-Sen University (specimens no. SC01 for *S. leptoclados* and SC 36 for *S. parva*).

3.3. Extraction and separation

The sliced bodies of *S. leptoclados* (724 g, wet wt) were homogenized with EtOAc and filtered. The organic layers were combined and concentrated under vacuum to afford a dark brown viscous residue (23.4 g). The residue was triturated with *n*-hexane first to afford *n*-hexane soluble layer, and then with dichloromethane. The dichloromethane soluble layer was evaporated under reduced pressure to afford a residue (4.1 g) which was subjected to column chromatography on silica gel, using *n*-hexane, *n*-hexane and EtOAc mixture of increasing polarity, and finally pure EtOAc, to yield 35 fractions. Fractions 21 and 24 eluted with *n*-hexane–EtOAc (2:1), were further purified on silica gel using *n*-hexane–EtOAc (gradient, 2:1–1:1) to yield **4** (1.9 mg) and **6** (4.1 mg) from fraction 21, and **7** (5.7 mg) from fraction 24, respectively. Fractions 26 and 27 eluted with *n*-hexane–EtOAc (1:1), were purified separately by normal phase HPLC using *n*-hexane–EtOAc (1:1) to afford **1** (1.3 mg) from fraction 26 and **8** (3.4) from fraction 27, respectively. Fraction 28 eluted with *n*-hexane–EtOAc (1:1), was further chromatographed over silica gel using *n*-hexane–acetone (5:1) to furnish **10** (344.2 mg) and a mixture, which was further purified by normal phase HPLC using *n*-hexane–EtOAc (9:11) to afford **9** (304.5 mg) and **5** (11.2 mg), respectively.

The tissues of *S. parva* (200 g, wet wt) were exhaustively extracted with EtOH. The alcoholic extract was concentrated under vacuum to afford a dark brown residue. The residue was triturated with *n*-hexane and then triturated further with dichloromethane. The dichloromethane was

concentrated to afford a residue (0.82 g), which primarily fractionated over silica gel, using the same solvent systems as described above to yield 15 fractions. Fractions 11 and 12 eluted with *n*-hexane–EtOAc (1:1) was further isolated and purified by normal phase HPLC, using *n*-hexane–EtOAc (1:1) to yield **2** (1.2 mg) and **7** (2.3 mg) from fraction 11, and **1** (0.8 mg), and **3** (1.1 mg) from fraction 12.

3.3.1. Leptocladolide A (1). Colorless oil, $[\alpha]_D^{25}=-33.3°$ (*c* 0.24, $CHCl_3$); IR (neat) ν_{max} 3420, 2975, 2361, 2361, 1750, 1700, 1680, 1614, 1445, 1381, 1175, 1098 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively; FABMS *m/z* 377 (0.8, $[M+H]^+$), 359 (0.5, $[M+H-H_2O]^+$), 331 (0.6, $[M+H-EtOH]^+$), 307 (7.9, $[M+H-C_2H_5-C_3H_5]^+$, 289 (6.3, $[M-EtOH-C_3H_5]^+$ or $[M+H-C_2H_5-C_3H_5-H_2O]^+$), 154 (100.0); HRFABMS *m/z* 377.1964 (calcd for $C_{21}H_{29}O_6$, 377.1965).

3.3.2. 1-*epi*-Leptocladolide A (2). Colorless oil, $[\alpha]_D^{29}=-55.0°$ (*c* 0.40, $CHCl_3$); IR (neat) ν_{max} 3414, 2922, 2361, 1751, 1726, 1711, 1611, 1381, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively; EIMS (70 eV) *m/z* 376 (1.4, $[M]^+$), 358 (1.3, $[M-H_2O]^+$), 330 (4.2, $[M-EtOH]^+$), 313 (2.1, $[M+H-EtOH-H_2O]^+$, 167 (52.5), 109 (46.9); FABMS *m/z* 377 (0.9, $[M+H]^+$), 359 (0.5, $[M+H-H_2O]^+$), 331 (0.9, $[M+H-EtOH]^+$), 307 (0.9, $[M+H-C_2H_5-C_3H_5]^+$, 289 (1.3, $[M-EtOH-C_3H_5]^+$ or $[M+H-C_2H_5-C_3H_5-H_2O]^+$), 154 (55); HRFABMS *m/z* 377.1966 (calcd for $C_{21}H_{29}O_6$, 377.1965).

3.3.3. 7*E*-Leptocladolide A (3). Colorless oil, $[\alpha]_D^{29}=-63.5°$ (*c* 0.52, $CHCl_3$); IR (neat) ν_{max} 3429, 2974, 2924, 2361, 1750, 1707, 1690, 1614, 1379, 1092 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively; EIMS (30 eV) *m/z* 376 (0.2, $[M]^+$), 358 (0.3, $[M-H_2O]^+$), 330 (0.6, $[M-EtOH]^+$), 313 (0.8, $[M+H-EtOH-H_2O]^+$, 193 (8.6), 167 (39.4); HREIMS *m/z* 376.1871 (calcd for $C_{21}H_{28}O_6$, 376.1880).

3.3.4. Leptocladolide B (4). White solid, mp 172–173°; $[\alpha]_D^{25}=+10.0°$ (*c* 0.24, $CHCl_3$); IR (neat) ν_{max} 2930, 1775, 1761, 1705, 1385, 1090 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz),), see Tables 1 and 2, respectively; EIMS (70 eV) *m/z* 348 (8.0, $[M]^+$), 298 (4.2), 149 (23.8), 134 (53.5), 109 (34.6).

3.3.5. Leptocladolide C (5). White solid, mp 215–216°; $[\alpha]_D^{25}=+83.0°$ (*c* 0.30, $CHCl_3$); IR (neat) ν_{max} 3424, 3017, 2924, 1757, 1674, 1585, 1443, 1379, 1186, 1096 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2, respectively; EIMS (70 eV) *m/z* 365 (22.0, $[M+H]^+$), 364 (100.0, $[M]^+$), 347 (24.0, $[M+H-H_2O]^+$), 346 (4.7, $[M-H_2O]^+$), 157 (45), 109 (27); HREIMS *m/z* 364.1519 (calcd for $C_{19}H_{24}O_7$, 364.1522).

3.3.6. Scabrolide D (6). White solid, mp 83–84°; $[\alpha]_D^{25}=-58.3°$ (*c* 0.24, $CHCl_3$); IR (neat) ν_{max} 2932, 1761, 1751, 1666, 1381, 1090 cm^{-1}; MS; ^1H and ^{13}C NMR spectral data were found to be in full agreement with those reported previously.[16]

3.3.7. Ineleganolide (7). White solid, mp 187–189°; $[\alpha]_D^{25}=+48.5°$ (*c* 0.68, $CHCl_3$) [lit.,[8] +26.4° (*c* 0.05, $CHCl_3$)]; IR (neat) ν_{max} 2966, 1757, 1707, 1645, 1377, 1321, 1217, 1170, 1067, 1024 cm^{-1}; EIMS (70 eV) *m/z* 330 (21.4, $[M]^+$), 215 (7.6), 164 (15.9), 135 (23.6), 121 (24.4), 105 (23.8); IR, MS, ^1H and ^{13}C NMR spectral data were found to be in full agreement with those reported previously.[8]

3.3.8. Scabrolide A (8). White solid, mp 92–93°; $[\alpha]_D^{29}=-104.0°$ (*c* 0.48, $CHCl_3$); IR (neat) ν_{max} 3462, 2920, 2361, 2338, 1757, 1699, 1651, 1373, 1088 cm^{-1}; MS; ^1H and ^{13}C NMR spectral data were found to be in full agreement with those reported previously.[16]

3.3.9. Norcembranoid 9. Colorless needles, mp 226–227°; $[\alpha]_D^{25}=-119.0°$ (*c* 0.16, $CHCl_3$); IR (neat) ν_{max} 3684, 3029, 2942, 1757, 1715, 1674, 1207, 1180, 1099 cm^{-1}; ^1H NMR (Pyridine-d_5, 300 MHz), δ 6.62 (1H, dd, *J*=11.1, 3.7 Hz, H-13), 4.96 (1H, s, H-11), 4.95 (1H, d, *J*=8.0 Hz, H-10), 4.78 (1H, s, H-16), 4.77 (1H, s, H-16), 4.55 (1H, dd, *J*=9.6, 2.7 Hz, H-5), 4.19 (1H, ddd, *J*=15.6, 11.1, 6.3 Hz, H-14), 3.04 (1H, m, H-1), 2.84 (1H, dd, *J*=15.9, 2.8 Hz, H-4), 2.82 (1H, m, H-2), 2.80 (1H, m, H-4), 2.52 (1H, d, *J*=16.5 Hz, H-7), 2.46 (1H, d, *J*=16.5 Hz, H-7), 2.46 (1H, m, H-2), 2.44 (1H, m, H-9), 2.20 (1H, br d, *J*=11.0 Hz, H-14), 2.18 (1H, d, *J*=13.8 Hz, H-9), 1.65 (3H, s, H_3-17), 1.41 (3H, s, H_3-18); ^{13}C NMR (pyridine-d_5, 75 MHz), δ 215.0 (C-6), 205.6 (C-3), 169.5 (C-19), 147.6 (C-15), 142.7 (C-13), 134.2 (C-12), 110.3 (C-16), 84.3 (C-10), 79.3 (C-8), 75.6 (2C, C-5, C-11), 51.5 (C-7), 45.6 (C-2), 43.8 (C-4), 42.8 (C-9), 39.4 (C-1), 27.9 (C-14), 25.9 (C-18), 21.7 (C-17); IR, MS; ^1H and ^{13}C NMR spectral data were found to be in full agreement with those reported previously.[1,17]

3.3.10. Sinuleptolide (10). White powder, mp 193–194°; $[\alpha]_D^{25}=+62.5°$ (*c* 0.08, $CHCl_3$); IR (neat) ν_{max} 3688, 3023, 2940, 2361, 1757, 1713, 1672, 1217, 1180, 1097 cm^{-1}; MS; ^1H NMR ($CDCl_3$, 300 MHz), δ 6.52 (1H, dd, *J*=10.8, 6.1 Hz, H-13), 4.86 (1H, s, H-16), 4.80 (1H, s, H-16), 4.65 (1H, d, *J*=6.6 Hz, H-10), 4.61 (1H, s, H-11), 4.39 (1H, dd, *J*=9.1, 3.7 Hz, H-5), 3.71 (1H, ddd, *J*=13.5, 10.5, 4.5 Hz, H-14), 2.82 (1H, m, H-1), 2.67 (1H, d, *J*=13.8 Hz, H-2), 2.63–2.54 (2H, m, H_2-4), 2.54 (2H, m, H-2, H-7), 2.41 (1H, d, *J*=17.0 Hz, H-7), 2.39 (1H, dd, *J*=15.0, 4.0 Hz, H-9), 2.16 (1H, dt, *J*=13.5, 7.0 Hz, H-14), 2.05 (1H, d, *J*=15.0 Hz, H-9), 1.83 (3H, s, H_3-17), 1.49 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; ^1H NMR (Pyridine-d_5, 300 MHz), δ 6.65 (1H, dd, *J*=11.1, 4.5 Hz, H-13), 4.99 (1H, d, *J*=7.6 Hz, H-10), 4.92 (1H, s, H-11), 4.81 (1H, s, H-16), 4.77 (1H, s, H-16), 4.67 (1H, dd, *J*=11.1, 1.5 Hz, H-5), 4.13 (1H, ddd, *J*=13.5, 10.1, 3.6 Hz, H-14), 2.98 (1H, m, H-1), 2.90 (1H, dd, *J*=13.8, 2.0 Hz, H-2), 2.89 (1H, t, *J*=14.8 Hz, H-4), 2.59 (1H, d, *J*=18.0 Hz, H-7), 2.57 (1H, m, H-2), 2.48 (1H, d, *J*=18.0 Hz, H-7), 2.34 (1H, dd, *J*=15.5, 7.5 Hz, H-9), 2.15 (1H, d, *J*=15.5 Hz, H-9), 2.20 (1H, m, H-14), 1.70 (3H, s, H_3-17), 1.59 (3H, s, H_3-18); ^{13}C NMR (Pyridine-d_5, 75 MHz), δ 213.9 (C-6), 206.7 (C-3), 169.8 (C-19), 147.8 (C-15), 142.7 (C-13), 133.5 (C-12), 110.4 (C-16), 84.7 (C-10), 79.8 (C-8), 77.3 (C-5), 76.0 (C-11), 51.4 (C-7), 46.1 (C-2), 44.6 (C-4), 41.6 (C-9), 40.0 (C-1), 29.5 (C-14), 29.5 (C-18), 21.6 (C-17); EIMS (70 eV) *m/z* 348 (8.0, $[M]^+$), 298 (4.2), 149 (23.8), 134 (53.5), 109

(34.6); IR, MS spectral data were found to be in full agreement with those reported previously.[9]

3.4. Cytotoxicity testing

Hepa59T/VGH and KB cells were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **3**–**8** were performed using MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[18,19]

Acknowledgements

This work was supported by a grant from the National Science Council of the Republic of China (Contract No. NSC-90-2323-B-110-003) awarded to J.-H.S.

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