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Tetrahedron Letters 46 (2005) 4793–4796
Tetrahedron Letters

New xenicane diterpenoids from *Xenia florida*

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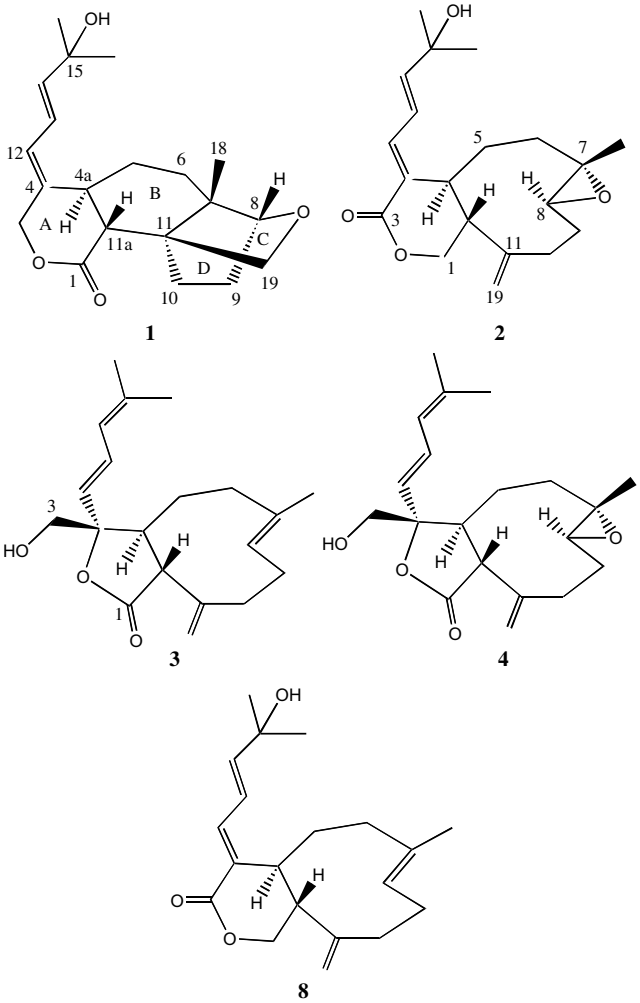
Received 20 April 2005; revised 6 May 2005; accepted 6 May 2005
Available online 25 May 2005

Abstract—Three new xenicane diterpenoids, designated xeniolactones A (**1**), B (**2**), and C (**3**), were isolated from *Xenia florida* collected in Taiwan. Compound **1** possesses a novel structure having a heterotricyclic skeleton in cyclononane system. The structures of **1**–**3** were elucidated on the basis of extensive spectroscopic analysis. The cytotoxicity of **1**–**3** was also evaluated against human cancer cell lines.

Xenicane diterpenoids are usually distributed in soft corals and gorgonians such as genus *Xenia*, *Capnella*, and *Acalycigorgia*.[1–5] These marine products possess very diverse chemical structures and interesting biological activities.[6,7] They were structurally divided into five types, xenicins, xeniolides, xeniaphyllanes, azamilides, and the nine-membered monocarbocyclic skeleton.[8] Southern coast of Taiwan has a rich source of marine invertebrates. Among them, specimens of *Xenia* are commonly encountered in Green Island and have several phenotypic color variants. In the search for bioactive constituents from Taiwanese marine soft corals,[9–11] a novel diterpene designated xeniolactone A (**1**) with an unusual bond between C-7 and C-11 in cyclononane system has been isolated from *Xenia florida*. In addition, two new xenicanes, xeniolactones B (**2**) and C (**3**) together with xeniolide H (**4**),[7] 9-deoxyxeniolide B (**5**),[12] florides C (**6**) and A (**7**),[13] were also isolated and characterized from the same collection. In this letter, we described the isolation, structural elucidation, biogenetic relationship, and biological activity of the three new marine metabolites.



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doi:10.1016/j.tetlet.2005.05.025

furnish xeniolactone A (**1**, 8 mg), B (**2**, 3 mg), and C (**3**, 20 mg), xeniolide H (**4**, 3 mg), 9-deoxyxeniolide B (**5**, 30 mg), florides C (**6**, 100 mg), and A (**7**, 11 mg).

Xeniolactone A (**1**), [α] +44 (*c* 0.8, CH_2Cl_2), was obtained as an amorphous powder and had a molecular formula $C_{20}H_{28}O_4$, as derived from its HRESIMS at *m/z* 355.1887 ($[M+Na]^+$, calcd 355.1884) indicating seven degrees of unsaturation. The presence of hydroxyl, lactonyl, and conjugated diene systems was evidenced by IR absorptions at 3426, 1736, and 1694 cm^{-1}. The ^1H NMR, ^{13}C NMR spectra (Table 1), and DEPT revealed that **1** contained a carbonyl ester (δ 172.9), four quarternary carbons including one olefinic carbon (δ 132.9) and one oxygenated carbon (δ 70.9), six methines including three olefinic carbons (δ_C 129.5, 120.9, 144.8) and protons (δ_H 6.09, 6.39, 5.87, H-12, H-13, H-14), and one oxygenated carbon (δ_C 82.8, C-8) and proton (δ_H 3.80, H-8), six methylenes including two oxygenated carbons (δ 72.8, 74.8, C-3, C-19) and protons (δ_H 4.41, 4.94; δ_H 4.17, 3.47) and three methyl groups (δ 29.9, two carbons, 15.7; δ_H 1.32, 1.08). The corresponding proton and carbon assignments were verified from HMQC experiment. This finding accounts for 3 of the 7 degrees of unsaturation, indicating four more rings in structure **1**. The COSY spectrum revealed five isolated spin systems, H-12/H-13/H-14, H-11a/H-4a/H-5/H-6, H-8/H-9/H-10, H-3a/H-3b, and H-19a/H-19b. Compound **1** possessing the usual ring A (lactone functionality)[6] was observed from its HMBC studies, which

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

No.	δ_H (mult, *J*, Hz)	δ_C	HMBC ^1H–^{13}C	COSY ^1H–^1H
1		172.9		
3α	4.41 (d, 12.4)	72.8	1, 4, 4a, 12	3β
3β	4.94 (d, 12.4)			3α
4		132.9		
4a	2.91 (m)	34.9		5α, 5β
5α	2.15 (m)	28.3	4a, 11a	4a
5β	1.59 (overlap)			4a
6α	1.58 (overlap)	29.4	4a, 5, 7, 18	5α, 5β
6β	1.50 (m)			5α, 5β
7		48.5		
8	3.80 (s)	82.8	10, 11, 19	
9α	1.82 (m)	27.9	10	9β, 10α, 10β
9β	1.83 (m)			9α, 10α, 10β
10α	1.90 (m)	26.6	7, 19	10β, 9α, 9β
10β	1.87 (m)			10α, 9α, 9β
11		47.9		
11a	2.57 (d, 12.0)	42	4, 4a, 5, 10, 11, 19	4a
12	6.09 (d, 11.2)	129.5	3. 4a	13
13	6.39 (dd, 11.2, 14.8)	120.9	4, 12	12, 14
14	5.87 (d, 15.2)	144.8	12, 15, 16, 17	13
15		70.9		
16, 17	1.32 (s)	29.9	14, 15	
18	1.08 (s)	15.7	6, 7, 8, 11	
19α	4.17 (d, 7.2)	74.8	8. 10. 11	19β 19α, 10α
19β	3.47 (dd, 3.6, 7.2)			

[a] Data were recorded in $CDCl_3$ on 400 MHz; chemical shifts (δ) and coupling constant are given in ppm and Hz, respectively.

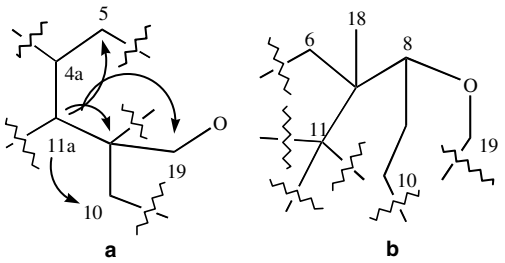


Figure 1. Structural fragments of **1**.

revealed correlations of H-3α (δ 4.41, d, *J* = 12 Hz) with C-1 (δ 172.9), C-4a (δ 34.9), C-4 (δ 132.9) and C-12 (δ 129.5), and correlations of H-11a (δ 2.57 d, *J* = 12 Hz) with C-4 and C-4a. HMBC correlations from H-11a to C-5 (δ 28.3), C-10 (δ 26.6), C-11 (δ 47.9), and C-19 (δ 74.8) established the partial structure **a** (Fig. 1). HMBC correlations from H-18 to C-6 (δ 29.4), C-7 (δ 48.5), C-8 (δ 82.8) and C-11, and correlations between H-8/C-10, C-11, and C-19 established the partial structure **b**. On the other hand, H-6 was found to correlate with C-4a, C-5, C-7, and C-18. The connectivity between C-7 and C-11 was further implied by the correlation of H-10 with C-7. The above observation established ring B. Assembling of partial structures **a** and **b** constitutes rings C and D, which contains a tetrahydrofuran moiety. The relative stereochemistry of xeniolactone A (**1**) was determined by analyses of the coupling constants and of the NOESY correlations. A coupling constant of 12 Hz between H-4a and H-11a implied that they are *trans*-disposition. Another *trans*-orientation appeared between C-13 and C-14 with a coupling constant of 15 Hz. Assuming that **1** has the same absolute configuration at C-4a as other naturally occurring xenicanes,[14] NOESY experiment was performed to ascertain the relative stereochemistry around the other chiral centers. The absence of correlation between H-4a and H-11a suggested a *trans*-disposition. The presence of mutual correlations between H-11a, H-3β, H-5β, and Me-18 agreed with all β-configuration. The correlations among Me-18, H-6β, H-8, and H-19β suggested that H-8 should be placed on the β-face of the molecule. On the other hand, the mutual correlations among H-4a, H-5α, H-13, and H-10α indicated that H-4a and the ethylene moiety (C-9 and C-10) were all α-orientation. The detailed NOESY correlation is illustrated in Figure 2. It is worth noting that the presence of a planar zigzag orientation between H-10α and H-19β was consistent with the long-range ^4J value (3.6 Hz) in this strained system.[15] The zero or very small *J* values between H-8 and H-9α/H-9β also agreed with their dihedral angles.[16] In fact, H-8 is located in the middle of H-9α and H-9β from a view of Fisher projection. Thus, the relative stereochemistry of **1** was unambiguously established.

Compound **2** possesses the molecular formula $C_{20}H_{28}O_4$, as deduced from the HREIMS,[17] indicating seven degrees of unsaturation. Similar to those of **1**, the IR spectrum of **2** showed the presence of lactone and hydroxy functionalities (ν_{max} 1720 and 3423 cm^{-1}). However, the ^{13}C NMR spectrum revealed the presence of an exomethylene (δ 150.4, s and 116.6, t)

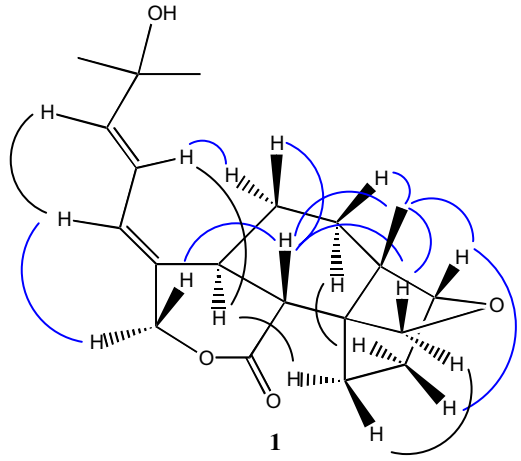


Figure 2. Key NOESY correlation and relative stereochemistry of **1**.

and an epoxy (δ 64.3, d and 60.8, s) group. Moreover, by the aid of HMBC and ^{1}H–^{1}H COSY spectra, the signals appearing at δ 172.7, s, 153.2, d, 139.0, d, 133.5, s, 121.1, d, δ 50.4, d, and 42.8, d were correspondent to a conjugated lactone group and two ring-junctured methines, respectively. Comparison of NMR data of **2** with those of 9-deoxyxeniolide A (**8**)[12] established **2** to be the 7,8-epoxy–xeneolide. The NOESY interactions (Fig. 3) observed between H-8 and H-4a and 7-CH_3 and H-11a designated the relative configurations 4a*S**,7*S**,8*S**, 11*R**.

Compound **3** also showed a similar IR spectrum to those of **1** and **2**. Its MS and NMR data suggested the molecular formula $C_{20}H_{28}O_3$ and seven degrees of unsaturation.[18] The ^{13}C NMR data revealed the presence of a lactone carbonyl (δ 177.4, s) and four olefinic double bonds (δ 146.0, s, 138.2, s, 134.5, s, 127.3, d, 127.1, d, 126.1, d, 124.0, d, 120.5, t). The ^{1}H NMR data also assigned three olefinic methyls (δ 1.80, 1.78, and 1.58, each 3H, s), one exomethylene (δ 5.14 and 5.06, each 1H, s) and two angular methines (δ 3.47, 1H, d, J = 11.7 Hz and 2.57, 1H, m) characteristic for xeniolides. Comparison of ^{13}C NMR data of **3** with that of xeniolide H (**4**)[7] indicated the presence of a trisubstituted carbon–carbon double bond (δ 134.5, s and 126.1, d) instead of 7,8-epoxy group (δ 64.4, d and 59.2, s) in **4**.[7] The above observations, together with correlations observed in the COSY and HMBC spectra, further established the structure of **3**.

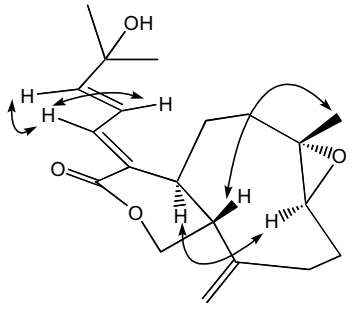
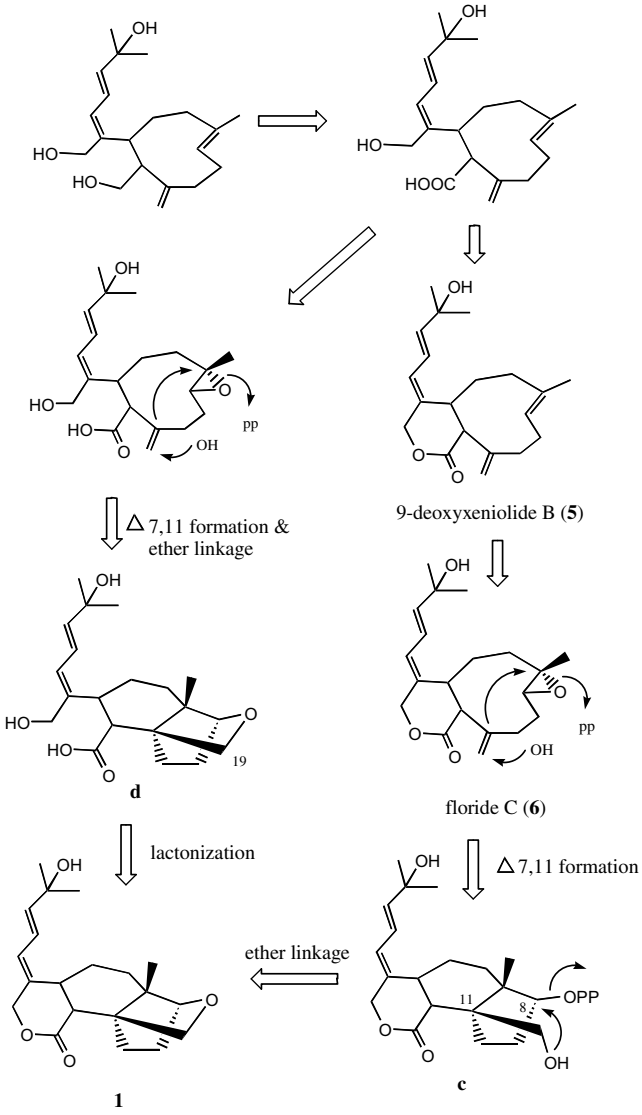


Figure 3. Key NOESY correlation of **2**.



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

A plausible biogenetic pathway of **1** was proposed as shown in Scheme 1 based on recently published structures such as florides A and C[13] and 9-deoxyxeniolide B.[12] Xeniolactone A (**1**) might be derived from floride C (**6**) through two steps. First step involves attack of a hydroxy ion at C-19, followed by 7,11 bond formation, and epoxide ring opening. The second step deals with ether-linkage formation and elimination of the leaving group OPP from the intermediate **c**. An alternative pathway for **1** may be produced from the intermediate **d**, which transforms to **1** via above-mentioned steps and the final lactonization.

This letter reports the first isolation of the new diterpenoids from *X. florida*, which belongs to the family Xeniidae. Two human cancer cell lines were chosen to test compounds **1**–**3** in vitro cytotoxic potentialities. Compound **1** exhibited mild cytotoxicity against human colon adenocarcinoma (WiDr) and medullocarcinoma (Daoy) tumor cells at 13.6 and 15.3 μg/ml, respectively, while compounds **2** and **3** were inactive (>20 μg/ml).

Cytotoxicity assay: The bioassay used against WiDr (colon adenocarcinoma) and Daoy (medullocarcinoma) tumor cells was based on a MTT assay method. The procedure of assay was carried out as previously described.[19]

Acknowledgements

This work was supported by a grant from the National Science Council of the Republic of China (Grant No. NSC-93-2320-B-110-003) awarded to Y.C.S.

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17. Amorphous powder, $[\alpha]_D^{25}$ +68 (*c* 0.3, CH_2Cl_2); IR (neat) ν_{max} 3423, 1720 cm^{-1}; UV λ_{max} (MeOH) 235 nm; ^1H NMR (CD_3OD, 400 MHz): δ 4.19 (1H, dd, J = 5.2, 11.2 Hz, H-1), 3.74 (1H, d, J = 11.2 Hz, H-1), 3.25 (1H, m, H-4a), 1.62 (1H, m, H-5), 1.69 (1H, m, H-5), 1.30 (1H, m, H-6), 2.17 (1H, dt, J = 13.6, 3.6 Hz, H-6), 3.11 (1H, dd, J = 10.8, 4.4 Hz, H-8), 1.49 (1H, m, H-9), 2.25 (1H, m, H-9), 2.44 (1H, m, H-10), 2.33 (1H, m, H-10), 2.55 (1H, m, H-11a), 6.97 (1H, d, J = 11.6 Hz, H-12), 6.56 (1H, dd, J = 11.6, 15.6 Hz, H-13), 6.34 (1H, d, J = 15.6 Hz, H-14), 1.31 (6H, s, H-16, H-17), 1.35 (3H, s, H-18), 5.18 (1H, s, H-19), 5.06 (1H, s, H-19); ^{13}C NMR (CD_3OD, 100 MHz): δ 72.3 (t, C-1), 172.7 (s, C-3), 133.5 (s, C-4), 42.8 (d, C-4a), 37.4 (t, C-5), 40.5 (t, C-6), 60.8 (s, C-7), 64.3 (d, C-8), 26.6 (t, C-9), 32.1 (t, C-10), 150.4 (s, C-11), 50.4 (d, C-11a), 139.0 (d, C-12), 121.1 (d, C-13), 153.2 (d, C-14), 71.5 (s, C-15), 29.7 (s, C-16, C-17), 17.0 (q, C-18), 116.6 (t, C-19); HRESIMS *m/z* 355.1885 (calcd for $C_{20}H_{28}O_4$, 355.1884).
18. Amorphous powder, $[\alpha]_D^{25}$ −60 (*c* 0.1, CH_2Cl_2); IR (neat) ν_{max} 3420, 1768, 1652 cm^{-1}; UV λ_{max} (MeOH) 228 nm; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (1H, d, J = 12.6 Hz, H-3), 3.76 (1H, d, J = 12.6 Hz, H-3), 2.57 (1H, m, H-4a), 1.92 (2H, m, H-5), 1.91 (1H, m, H-6), 2.23 (1H, m, H-6), 5.30 (1H, br t, J = 5.9 Hz, H-8), 2.09 (1H, dd, J = 12.6, 8.1 Hz, H-9), 2.41 (1H, m, H-9), 1.82 (1H, m, H-10), 2.43 (1H, m, H-10), 3.47 (1H, d, J = 11.7 Hz, H-11a), 5.54 (1H, d, J = 15.3 Hz, H-12), 6.58 (1H, dd, J = 15.3, 10.5 Hz, H-13), 5.81 (1H, d, J = 10.5 Hz, H-14), 1.78 (3H, s, H-16), 1.80 (3H, s, H-17), 1.58 (3H, s, H-18), 5.06 (1H, s, H-19), 5.14 (1H, s, H-19); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 1.774 (s, C-1), 66.0 (t, C-3), 88.4 (s, C-4), 48.9 (d, C-4a), 27.4 (t, C-5), 39.8 (t, C-6), 134.5 (s, C-7), 126.1 (d, C-8), 28.7 (t, C-9), 32.7 (t, C-10), 146.0 (s, C-11), 59.5 (d, C-11a), 127.1 (d, C-12), 127.3 (d, C-13), 124.0 (d, C-14), 138.2 (s, C-15), 18.5 (q, C-16), 26.1 (q, C-17), 17.6 (q, C-18), 120.5 (t, C-19); HRESIMS *m/z* 339.2938 (calcd for $C_{20}H_{28}O_3$, 339.2935).
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