Xenibellols A and B, New Diterpenoids from the Formosan Soft Coral *Xenia umbellata*
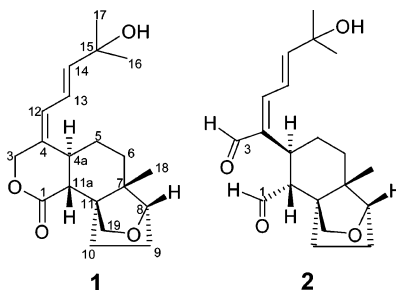
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ABSTRACT

Xenibellols A (1) and B (2), possessing an unprecedented diterpenoid skeleton, were isolated from the soft coral *Xenia umbellata*. The structures of xenibellols A and B were established by extensive analysis of spectroscopic data.

Soft corals of the genus Xenia are rich in diterpenoids.[1] As part of our search for novel bioactive substances from marine and terrestrial organisms,[2−4] the soft coral *Xenia umbellata* Lamarck was studied because CH_2Cl_2 extracts showed significant cytotoxicity to A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[5,6] Bioassay-guided fractionation resulted in the isolation of two novel cytotoxic diterpenoids (novel carbon skeleton), xenibellols A (**1**) and B (**2**).

The soft coral *X. umbellata* was collected at Green Island, Taiwan, in September, 2004, at a depth of 3−4 m. The bodies of the soft coral were freeze-dried to give 1.20 kg of a solid,

which was extracted with acetone−CH_2Cl_2 (5.0 L × 3). After removal of solvent in vacuo, the residue (66 g) was chromatographed over silica gel 60 using *n*-hexane and *n*-hexane−EtOAc mixtures of increasing polarity. Elution by *n*-hexane−EtOAc (1:9) afforded a fraction containing compounds **1** and **2**. Compounds **1** (12 mg, 0.018%) and **2** (4 mg, 0.006%) were further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm), eluting with MeOH−H_2O (65:35).

Compound **1** was isolated as a colorless oil, $[\alpha]^{25}_D$ +18° (*c* 0.1, $CHCl_3$). The IR spectrum of **1** exhibited absorptions due to hydroxyl (3420 cm^{-1}), lactone carbonyl (1732 cm^{-1}), and conjugated diene (1640 cm^{-1}) groups. The presence of the conjugated diene was also confirmed by the UV spectrum [λ_{max} 228 nm (ϵ 13 100)]. HRESIMS suggested a molecular formula of $C_{20}H_{28}O_4$ ([M + Na]$^+$, *m/z* 355.1886, Δ +0.0002 mmu).

The structure of **1** was completely assigned by a combination of one- and two-dimensional NMR methods. The carbon

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Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) Data^a of **1** and **2**

	1			**2**	
position	δ_H, mult (J in hertz)	δ_C (mult)	position	δ_H, mult (J in hertz)	δ_C (mult)
1		173.0 (qC)	1	9.45 s	203.3 (CH)
3	4.45 d (12.0), 4.98 d (12.0)	73.0 (CH$_2$)	3	9.37 s	195.2 (CH)
4		133.1 (qC)	4		141.5 (qC)
4a	2.95 m	35.1 (CH)	4a	3.11 m	31.0 (CH)
5	2.21 m, 1.64 m	28.5 (CH$_2$)	5	1.52 m	25.0 (CH$_2$)
6	1.56 m	29.5 (CH$_2$)	6	1.53 m	28.7 (CH$_2$)
7		48.6 (qC)	7		48.6 (qC)
8	3.84 s	82.9 (CH)	8	3.85 s	82.5 (CH)
9	1.88 m	28.0 (CH$_2$)	9	1.37 m, 1.82 m	28.4 (CH$_2$)
10	1.95 m	26.8 (CH$_2$)	10	1.47 m, 2.10 m	27.3 (CH$_2$)
11		47.6 (qC)	11		48.2 (qC)
11a	2.61 d (12.0)	42.2 (CH)	11a	3.45 d (12.0)	49.7 (CH)
12	6.13 d (11.4)	129.6 (CH)	12	6.88 m	152.1 (CH)
13	6.43 dd (15.0, 11.4)	121.0 (CH)	13	6.91 m	121.2 (CH)
14	5.90 d (15.0)	145.0 (CH)	14	6.39 dq (14.1, 3.6)	153.1 (CH)
15		71.0 (qC)	15		71.3 (qC)
16	1.37 s	30.1 (CH$_3$)	16	1.42 s	29.7 (CH$_3$)
17	1.37 s	30.1 (CH$_3$)	17	1.44 s	29.9 (CH$_3$)
18	1.19 s	15.8 (CH$_3$)	18	1.27 s	15.1 (CH$_3$)
19	3.50 dd (7.2, 2.7), 4.06 d (7.2)	74.9 (CH$_2$)	19	3.71 m	75.4 (CH$_2$)

^{a} Spectra were recorded in CDCl$_3$ at 25 °C. Chemical shift values are given in parts per million relative to TMS. ^{13}C NMR multiplicities were obtained from a DEPT-135 experiment.

resonances at δ_C 133.1 (qC), 129.6 (CH), 121.0 (CH), and 145.0 (CH), in the ^{13}C NMR and DEPT spectra showed the presence of a diene (Table 1). Furthermore, the presence of four sp^3 oxygenated carbons was inferred from the carbon signals at δ_C 71.0 (qC), 73.0 (CH$_2$), 74.9 (CH$_2$), and 82.9 (CH). Four methylene groups were deduced from the DEPT signals at δ_C 26.8, 28.0, 28.5, and 29.5, two methine signals at δ_C 35.1 and 42.2, two quarternary carbon signals at δ_C 47.6 and 48.6, a lactone carbonyl at δ 173.0 (qC), and, finally, two methyl signals at δ_C 30.1 (CH$_3$).

The ^{1}H NMR spectrum confirmed the presence of a conjugated diene by the fact that three signals were observed at δ_H 5.90 (d, J = 15.0 Hz), 6.13 (d, J = 11.4 Hz), and 6.43 (dd, J = 15.0, 11.4 Hz). In addition, two oxygenated methylenes were observed at δ_H 4.98 (d, J = 12.0 Hz), 4.45 (d, J = 12.0 Hz), and δ_H 3.50 (d, J = 7.2 Hz), 4.06 (d, J = 7.2 Hz). One intense singlet signal is also observed at δ_H 1.37 (s, 6H), and this correspond to two methyl groups. In this manner, the seven degrees of unsaturation present in **1** were established.

The combined use of ^{1}H$-^1$H COSY and HMQC on **1** allowed us to distinguish three spin systems (see **a**–**c** in Figure 1) and two methyl groups linked to an oxygenated quaternary carbon. An HMBC experiment was used to assemble the skeletal fragments through quaternary carbons and heteroatoms. Thus, these substructures were connected through HMBC correlations between the protons H$_2$-3 (δ_H 4.45 and 4.98) and the carbons C-1 (δ_C 173.0), C-4 (δ_C 133.1), C-12 (δ_C 129.6), and C-4a (δ_C 35.1), between the protons H$_2$-19 (δ_H 3.50 and 4.06) and the carbons C-10 (δ_C 26.8), C-7 (δ_C 48.6), C-11 (δ_C 47.6), and C-8 (δ_C 82.9), between the methylene protons H$_2$-9 (δ_H 1.88) and carbons

C-11 and C-7, between the protons H-11a and the carbon C-1 and C-4, between the methyl protons Me-18 (δ_H 1.19)
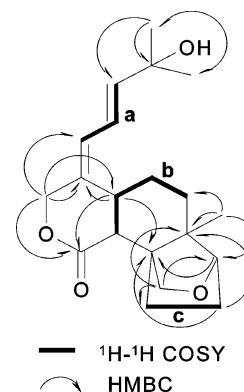
Figure 1. Key ^{1}H$-^1$H COSY and HMBC correlations of **1**.

and carbons C-6 (δ_C 29.5), C-7 (δ_C 48.6), and C-8 (δ_C 82.9), and between the methine proton H-4a (δ_H 2.95) and carbon C-4/C-11. These relationships are represented in Figure 1.

All these data allowed us to identify compound **1** as a new diterpenoid with a novel skeleton. With the gross structure of **1** in hand, the relative stereochemistry of compound **1** was deduced from NOESY correlations (Figure 2) and by comparison of its spectroscopic data to those of xenia diterpenes.[7–9] E geometry was assigned to the $\Delta^{4,12}$ double bond on the basis of the observation of a NOESY correlation between H-3α and H-12 and between H-13 and
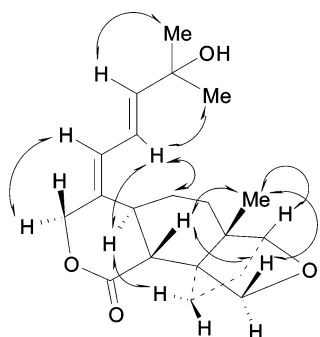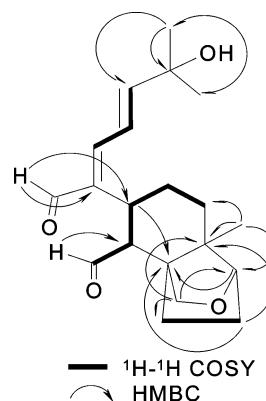
Figure 2. Key NOESY correlations of **1**.

H-4a/H-5. The *E* geometry of the Δ^{13} double bond was established by the large coupling constant observed between H-13 and H-14 (J = 15.0 Hz). The coupling constant (J = 12.0 Hz) between H-4a and H-11a suggested a trans ring junction, which implied that H-4a was α-oriented.[7−9] NOE correlations from Me-18 to H-8/H-11a/H-19β and from H-4a to H-10 were observed. This suggests that H-11a, H-19β, H-8, and Me-18 are on the β face of the molecule, while H-4a, H$_2$-10, and H$_2$-9 are on the opposite (α) face of the molecule.

Compound **2** was isolated as a colorless oil, $[\alpha]^{25}_D$ +15° (*c* 0.1, CHCl$_3$). The IR spectrum of **2** exhibited absorptions due to hydroxyl (3480 cm^{-1}) and α,β,γ,δ-unsaturated aldehyde (1710, 1660 cm^{-1}) groups. The presence of the α,β,γ,δ-unsaturated aldehyde was also confirmed by the UV spectrum [λ_{max} 233 nm (ϵ 11 200)]. HRESIMS suggested a molecular formula of C$_{20}$H$_{28}$O$_4$ ([M + Na]$^+$, *m/z* 355.1887, +0.0003 mmu).

The NMR data of **2** were analogous to those of **1** except that the δ-lactone was replaced by two aldehyde groups. The carbon resonances at δ_C 195.2 (qC), 141.5 (qC), 152.1 (CH), 121.2 (CH), and 153.1 (CH) in the ^{13}C NMR and DEPT spectra of **2** showed the presence of an α,β,γ,δ-unsaturated aldehyde, and the ^{1}H NMR spectrum of **2** confirmed the presence of an α,β,γ,δ-unsaturated aldehyde by the fact that signals were observed at δ_H 6.91, 6.88, 6.39, and 9.37.[10] HMBC correlations between the protons H-3 and the carbons C-4/C-12/C-4a confirmed the location the α,β,γ,δ-unsaturated aldehyde (Figure 3). COSY correlation between H-1 and H-11a and HMBC correlation between H-1 and C-11a showed the presence of the other aldehyde at C-11a (Figure 3).

The relative stereochemistry of compound **2** was deduced from NOESY correlations (Figure 4) and by comparison of its spectroscopic data to those of xenia diterpenes.[7−10] *E* geometry was assigned to the $\Delta^{4,12}$ and $\Delta^{13,14}$ double bonds

Figure 3. Key ^{1}H−^{1}H COSY and HMBC correlations of **2**.

by comparing the ^{1}H and ^{13}C NMR data with those of xenia diterpenes containing similar side chains.[7−10] The NOE
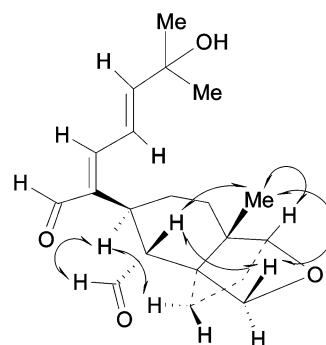
Figure 4. Key NOESY correlations of **2**.

correlations from Me-18 to H-8/H-11a/H-19β and NOE correlations from H-4a to H-1/H-10 were observed. This suggests that H-11a, H-19β, H-8, and Me-18 are on the β face of the molecule, while H-4a, H$_2$-10, and H$_2$-9 are on the opposite (α) face of the molecule.

Xenibellols A (**1**) and B (**2**) exhibited cytotoxicity against P-388 cell with ED$_{50}$ of 3.6 and 2.8 μg/mL, respectively. Biogenetically, xenibellol A (**1**) may be a double cyclization ([2 + 2 + 2] reaction) product of the hydroxy analogue of xeniolide-G.[9] The dialdehyde of xenibellol B (**2**) was suggested as the precursor of the lactone ring of xenibellol A (**1**).[10]

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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