Xylomexicanins A and B, New $\Delta^{14,15}$-Mexicanolides from Seeds of the Chinese Mangrove *Xylocarpus granatum*

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Two new mexicanolide-type limonoids, named xylomexicanin A (**1**) and xylomexicanin B (**2**), were isolated from seeds of the Chinese mangrove *Xylocarpus granatum*. Their structures were elucidated on the basis of spectroscopic methods. Compound **1** exhibited antiproliferative activity against human breast carcinoma cells (KT), while **2** did not show inhibitory effects on eleven human tumour cell lines tested.

Key words: Xylocarpus granatum, Limonoids, Antiproliferative Activity

Introduction

Xylocarpus granatum Koenig (Meliaceae), a marine mangrove plant, is used as a folk medicine in Southeast Asia for the treatment of diarrhea, cholera, and fever diseases such as malaria and also as an antifeedant (Champagne *et al.*, 1992). Since the first limonoid, gedunin, was reported from this plant (Taylor, 1965), the unique structural patterns of limonoids have attracted wide attention. Hitherto, more than 50 limonoid derivatives have been isolated from *X. granatum*, and they have been classified into phragmalin-, mexicanolide-, obacunol-, and andirobin-types (Ng and Fallis, 1979; Wu *et al.*, 2005, 2006; Yin *et al.*, 2006, 2007; Cui *et al.*, 2007). Within the framework of our investigation on the seeds of *X. granatum*, we report herein the isolation, structural elucidation, and antiproliferative activities of two new mexicanolide-type limonoids.

Results and Discussion

Compound **1** was isolated as colourless crystals, and its molecular formula was established from the HR-EI mass spectrum to be $C_{31}H_{38}O_{10}$ (unsaturation value of 13). The UV absorption maximum at 208 nm and the IR absorption bands at 3404 and 1722 cm^{-1} (in KBr) suggested the presence of hydroxy and ester functionalities. The ^1H and ^{13}C NMR data (Table I) indicated that nine units of the 13 unsaturations come from four carbon-carbon double bonds and five carbonyl groups. Therefore, the other four unsaturation units indicated four rings.

The ^{13}C NMR and DEPT spectra revealed that **1** has five carbonyl groups, three sp^2 quaternary carbon atoms, three sp^3 quaternary carbon atoms, five sp^2 methine, five sp^3 methine, three sp^3 methylene and seven methyl groups (including a methoxy group). Analysis of the ^1H, ^{13}C, and 2D

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Table I. ^{1}H and ^{13}C NMR data of **1** and **2** (500 MHz for ^{1}H, 125 MHz for ^{13}C in $CDCl_3$).

	1		**2**	
Position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		198.8		217.4
2		128.6	3.31 dd; 7.0, 17.5	44.6
3	6.99 s	161.8	4.91 d; 8.0	77.6
4		36.8		39.8
5	2.27 m	45.1	3.48 d; 8.5	41.4
6a	2.47 d; 14.5	34.6	2.34 m	33.0
6b	2.28 m		2.29 m	
7		173.4		173.9
8		80.1		72.7
9		208.7	1.83 d; 13.0	60.7
10	2.27 m	42.8		48.4
11α	2.53 m	33.0	1.67 m	20.7
11β	3.05 dd; 19.5, 6.5		1.51 dd; 4.5, 13.0	
12α	1.64 dd; 14.0, 7.0	25.6	1.99 dt; 3.0, 14.5	33.6
12β	2.62 ddd; 14.0, 14.0, 7.0		1.25 m	
13		38.3		38.6
14		163.6		168.3
15	6.16 s	118.5	6.13 s	116.0
16		163.3		164.8
17	5.34 s	80.1	5.18 s	79.8
18	0.98 s	18.6	1.29 s	23.1
19	1.04 d; 5.5	11.4	1.14 s	18.5
20		119.6		119.8
21	7.56 br s	141.4	7.51 br s	141.7
22	6.47 br s	109.8	6.50 br s	110.4
23	7.45 br s	143.2	7.44 br s	143.1
28	1.19 s	27.8	0.77 s	23.2
29	1.13 s	20.4	0.86 s	22.9
30α		66.9	2.37 dd; 10.0, 15.0	35.9
30β	6.48 s		3.18 dd; 6.5, 15.0	
8-OH	3.87 s			
7-OMe	3.69 s	52.0	3.72 s	52.1
1'		175.9		175.4
2'	2.56 m	34.1	2.48 m	41.3
3'	1.18 d; 7.0	19.2	1.48 m	26.6
			1.73 m	
4'	1.16 d; 7.0	18.5	0.97 t; 7.0	11.7
5'			1.20 d; 6.5	16.8

NMR data disclosed the presence of a keto carbonyl group (δ_C 208.7), an α,β-unsaturated keto carbonyl group (δ_H 6.99, s; δ_C 161.8, 128.6 and 198.8), a hydroxy group (δ_H 3.87, s), a methoxycarbonyl group (δ_H 3.69, s; δ_C 52.0, 173.4), a characteristic β-furyl ring (δ_H 7.56, 7.45, 6.47, each br s; δ_C 143.2, 141.4, 119.6, 109.8), and an isobutyryloxy group [δ_H 2.56 m, 1.18 (d, J = 7.0 Hz), 1.16 (d, J = 7.0 Hz); δ_C 175.9, 34.1, 19.2, 18.5]. These observations indicated that **1** is a mexicanolide-type limonoid. Moreover, an α,β-unsaturated δ-lactone ring (D-ring), characterized by the NMR data (δ_H 5.34, s, 6.16, s; δ_C 163.6, 118.5, 163.3, 80.1, 38.3), was confirmed by the HMBC correlations from H-15 (δ_H 6.16, s) and H-17 (δ_H 5.34, s) to C-13 (δ_C 38.3), C-14 (δ_C 163.6), and C-16 (δ_C 163.3), respectively. The HMBC cross-peaks from H-17 to C-20 (δ_C 119.6), C-21 (δ_C 141.4), and C-22 (δ_C 109.8) indicated that the furyl ring is connected to C-17. A methyl singlet at δ_H 0.98 (Me-18) and the methylene protons of C-12 showed HMBC correlations to C-13 of the lactone ring. These correlations and the observation of an H_2-11-H_2-12 spin system in the ^{1}H-^{1}H COSY indicated the connections of this proton spin system and Me-18 with C-13. Furthermore, the chemical shift of C-8 (δ_C 80.1)

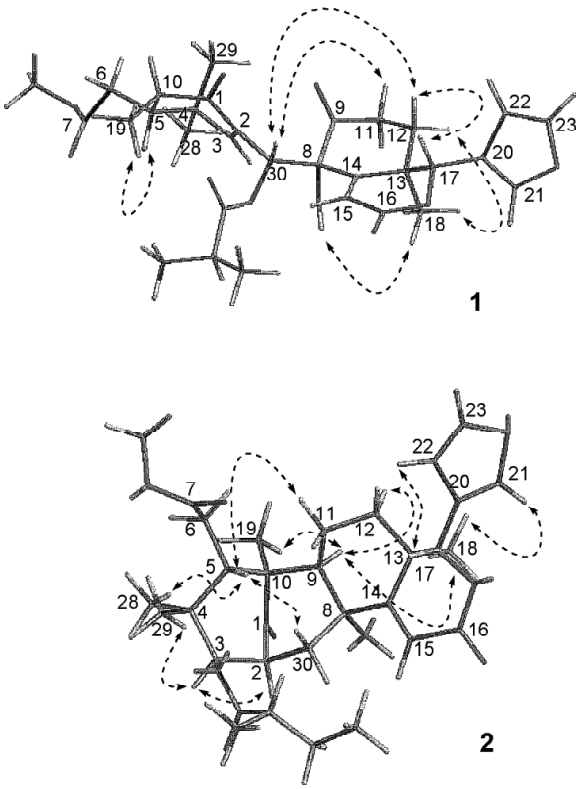

Fig. 1. Selected NOESY correlations of compound **1** and compound **2**. Molecular modeling calculations were performed using the MM2 force field implemented in the Chem3D program V5.0 (Cambridge-Soft, Cambridge, MA, USA). A conformational search was carried out by minimizing the energy using standard MM2 constants based on the structure elucidated by the NOESY data.

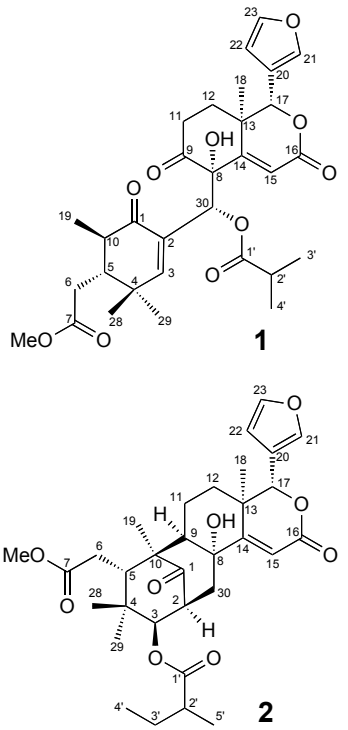

Fig. 2. Chemical structures of compounds **1** and **2**.

and one exchangeable proton at δ_H 3.87, which showed HMBC correlations with C-8, -14, -30 (δ_C 66.9) and a keto carbonyl carbon atom at δ_C 208.7 (C-9), indicated that a free hydroxy group is positioned at C-8. The observation of long-range correlations from H_2-11 and H_2-12 to C-9 and no HMBC correlation from H_2-11 to any carbon atom of ring A supported the idea that compound **1** is a 9,10-secomexicanolide (Yin *et al.*, 2006).

Two COSY-related geminal methyl groups at δ_H 1.19 and 1.13 are typical of Me-28 and Me-29 attached to C-4 (δ_C 36.8) of ring A in mexicanolides. Using the methylene protons of C-6 as the starting point, which showed HMBC correlations to the characteristic methoxycarbonyl C-7 (δ_C 173.4), and to C-4 and C-10 (δ_C 42.8), the spin system of H-6a-H-6b-H-5-H-10-Me-19 was easily determined by ^{1}H-^{1}H COSY. The H-3 olefinic proton signal at δ_H 6.99 (1H, s) was confirmed by the HMBC correlations to C-1, -2, -4, -5, -28 and -30. The chemical shift of C-30 at δ 66.9 indicated that it was an oxygenated carbon atom, and the HMBC correlations from H-30 (δ_H 6.48, s) to C-1' (δ_C 175.9) confirmed that the isobutyryloxy group is attached to C-30.

The relative configuration of **1** was determined through NOESY analysis. The 3D structure in Fig. 1 was deduced by molecular modeling calculations. The NOESY correlations of H-30/H-11β (δ_H 3.05, dd, J = 19.5, 6.5 Hz), H-30/H-12β (δ_H 2.62, ddd, J = 14.0, 14.0, 7.0 Hz), and H-17/H-12β suggested that H-30 and H-17 are in β-orientation.

The orientation of 8-OH remained to be determined. Fortunately, its active proton appeared as a high and narrow peak at δ_H 3.87. Its strong NOESY correlation with Me-18 disclosed its α-configuration. Moreover, the NOESY correlation of H-5 (δ_H 2.27, m)/Me-19 (δ_H 1.04, d, J = 5.5 Hz) indicated that both protons are on the same side. These evidences suggested that **1** shares the same backbone configuration with xylogranatin C, a 9,10-seco-mexicanolide isolated from the same fraction (Yin *et al.*, 2006). Therefore, the structure of **1** was elucidated as shown in Fig. 2; the compound was named xylomexicanin A.

Compound **2** was obtained as an amorphous powder. Its molecular formula was determined as $C_{32}H_{42}O_9$ (unsaturation value of 12) by its HR-EI mass spectrum. The UV maximum at 215 nm and the IR (KBr) absorption bands at 3432, 2967 and 1723 cm^{-1} indicated the existence of hydroxy, carbon-carbon double bond and carbonyl groups. The ^1H and ^{13}C NMR data (Table I) indicated that 7 units of the 12 unsaturations come from three carbon-carbon double bonds and four carbonyl groups (one ketone and three esters). Therefore, the other 5 unsaturation units come from five rings.

The ^{13}C NMR spectrum displayed seven methyl (including a methoxy), five methylene, ten methine groups (including four olefinic) and ten quaternary carbon atoms (including four carbonyls). In addition, the NMR data exhibited a keto carbonyl (δ_C 217.4), a methoxycarbonyl (δ_H 3.72, s; δ_C 52.1, 173.9), and a β-furyl ring (δ_H 7.51, 7.44, 6.50, each br s; δ_C 143.1, 141.7, 119.8, 110.4). These NMR data and the 2D NMR studies including ^1H-^1H COSY, HMQC, and HMBC experiments indicated that compound **2** has a normal mexicanolide skeleton with a $\Delta^{14,15}$ double bond. Based on comparison of the NMR spectral data of **2** with those of xylogranatin A, isolated from the fruit of *X. granatum* (Wu *et al.*, 2006), the signals were almost identical, with the exception of the resonances of a 2'-methylbutanoyl group in **2** [δ_H 2.48 m, 1.73 m, 1.48 m, 0.97 (t, J = 7.0 Hz), 1.20 (d, J = 6.5 Hz); δ_C 175.4, 41.3, 26.6, 11.7, 16.8] instead of a tiglate group in xylogranatin A. The 2'-methylbutanoyl group was indicated to be attached at C-3 by the chemical shift of H-3 (δ_H 4.91, d, J = 8.0 Hz) and the long-range correlation from H-3 to C-1' (δ_C 175.4) in the HMBC spectrum to be attached at C-3. The chemical shift of C-8 (δ_C 72.7) implied that the remaining free hydroxy group is located at C-8.

The relative configuration of **2** was assigned to be the same as that of xylogranatin A by comparing their NMR data and NOESY spectra (Fig. 1). The significant NOESY correlations from H-3 to Me-29 (δ_H 0.86, s), but not from H-3 to H-5, or from H-3 to H-30β implied that H-3 is α-orientate. The significant NOESY correlations of H-9 (δ_H 1.83, d, J = 13.0 Hz)/Me-18 (δ_H 1.29, s) indicated their α-orientation. Similarly, the NOESY correlations of H-30β (δ_H 3.18, dd, J = 6.5, 15.0 Hz)/H-5 (δ_H 3.48, d, J = 8.5 Hz) also implied their mutual *cis* relationship. In known mexicanolides, 8-OH was recognized as being exclusively in the α-orientation. On the basis of the above results, the structure of **2**, named xylomexicanin B, was elucidated as shown in Fig. 2.

The *in vitro* antiproliferative activities of compounds **1** and **2** against eleven human tumour cell lines (HeLa, HEC-1, SHIN3, HOC-21, HAC-2, HLE, U251-SP, T-98, MM1-CB, HMV-1 and KT) were evaluated. Compound **1** showed significant antiproliferative activity against the KT cell line with an IC_{50} value of 4.59 μM, while cisplatin, as a positive control, exhibited activity toward the KT cell line with an IC_{50} value of 7.43 μM. Compound **2** did not show inhibitory effects on the survival activity up to 30.00 μM in any cell lines tested (Table II).

Experimental

General

IR: Nicolet Magna-IR 550. MS: Bruker APEX II spectrometer. Optical rotations: Perkin-Elmer 243B digital polarimeter. NMR: Varian Ino-

Table II. Antiproliferative activities of compounds **1** and **2**.

Cell line	IC_{50} [μM]		
	1	**2**	Cisplatin
HeLa	>30.00	>30.00	20.31
HEC-1	>30.00	>30.00	>30.00
SHIN3	>30.00	>30.00	13.01
HOC-21	>30.00	>30.00	>30.00
HAC-2	>30.00	>30.00	>30.00
KT	4.59	>30.00	7.43
HLE	>30.00	>30.00	>30.00
U251-SP	>30.00	>30.00	21.26
T-98	>30.00	>30.00	25.52
MM1-CB	>30.00	>30.00	25.83
HMV-1	>30.00	>30.00	27.10

va-500. Chromatography: Silica gel 200–300 mesh (Qingdao Marine Chemical Factory, China). Preparative HPLC: Waters Delta Prep 3000 pump, UV 2487 detector, Whatman partisil 10 ODS-2 (9.4 × 250 mm) column.

Plant material

Seeds of *X. granatum* were collected in March 2006 at Hainan Island, Southern China, dried at ambient temperature, and identified by Dr. Wen-Qing Wang, School of Life Sciences, Xiamen University, China. A voucher specimen (No. HEBNMC-2006–1) has been deposited in the herbarium of School of Pharmaceutical Sciences, Hebei Medical University, China.

Extraction and isolation

Dried seeds (3.0 kg) of *X. granatum* were extracted with 95% ethanol at room temperature. After evaporation of the solvent under reduced pressure, the residue was suspended in water and extracted with petroleum ether and dichloromethane, successively. The dichloromethane extract (65.8 g) was chromatographed on silica gel and eluted using a petroleum ether/acetone system (95:5 to 2:3) to yield 200 fractions. Fractions 90–115 (8.4 g) were combined and subjected to preparative HPLC (methanol/water, 60:40) to yield compound **1** (10.0 mg). Column chromatography of the combined fractions 149–162 (2.6 g), using a petroleum ether/acetone gradient (10:1 to 7:3) as mobile phase, afforded 125 subfractions. Subfractions 85–94 (0.15 g) were further purified by preparative HPLC (methanol/water, 65:35 to 100:0) to afford compound **2** (4.8 mg).

Xylomexicanin A (**1**): Colourless crystals; $[\alpha]_D^{25}$ –28° (*c* 0.21, MeOH). – HR-EI-MS: calcd. for $C_{31}H_{38}O_{10}$, *m/z* (M^+) = 570.2465; found, 570.2472. – UV (MeOH): λ_{max} = 208 nm. – IR (KBr): ν_{max} = 3404, 2984, 2963, 1722, 1671, 1369, 1272, 1245, 1164, 1151, 1015 cm^{-1}.

Xylomexicanin B (**2**): Amorphous powder; $[\alpha]_D^{25}$ –21° (*c* 0.12, MeOH). – HR-EI-MS: calcd. for $C_{32}H_{42}O_9$, *m/z* (M^+) = 570.2829; found, 570.2833. – UV (MeOH): λ_{max} = 215 nm. – IR (KBr): ν_{max} = 3432, 2967, 1723, 1599, 1383, 1026 cm^{-1}.

Biological evaluation

Human tumour cell lines used were as follows: HeLa (cervical cancer cell line), HEC-1 (endometrial adenocarcinoma cell line), SHIN3 (ovarian clear-cell cystadenocarcinoma cell line), HOC-21 (ovarian clear-cell cystadenocarcinoma cell line), HAC-2 (ovarian clear-cell carcinoma cell line), HLE (hepatoma cell line), U251-SP (glioma cell line), T-98 (glioma cell line), MM1-CB (melanoma cell line), HMV-1 (melanoma cell line), and KT (breast carcinoma cell line) (Suzuki and Fuse, 1981; Suzuki *et al.*, 1995). Cells were cultured in Eagle's minimal essential medium (EMEM) (GIBCO/BRL, Grand Island, NY, USA), containing 10% (v/v) calf serum (Intergen, Purchase, NY, USA) and antibiotics (100 μg/ml of streptomycin and 100 units/ml of penicillin G) (Meiji Seika, Tokyo, Japan), at 37 °C in a humidified atmosphere containing 5% CO_2. Cell survival was estimated by the MTT assay as described elsewhere (Zhai *et al.*, 2005). Briefly, logarithmically proliferating cells were plated into 96-well plates ($1 \cdot 10^4$ cells/well) with the medium containing the test compounds at the indicated doses, followed by culture for 2 d. Then, the activity of mitochondrial succinic dehydrogenase was measured by further incubation of the cells with 0.5 mg/ml MTT (Sigma) for 4 h, followed by estimation of the absorbance at 570 nm with a reference wavelength of 655 nm. Cell viability was calculated from the absorbance as percentage of the survived cells.

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