Xylogranatins A—D, New Mexicanolides from the Fruit of a Chinese Mangrove *Xylocarpus granatum*

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Four new mexicanolides with a $\Delta^{14,15}$ double bond, named xylogranatins A and D (1—4), were isolated from the fruit of a Chinese mangrove *Xylocarpus granatum*, together with two known 8,9,30-phragmalin *ortho* esters, xyloccensins P and Q. Their structures were elucidated on the basis of spectroscopic data, especially 2D NMR techniques including HSQC, HMBC and NOESY.

Key words xylogranatin; mexicanolide; xylocarpus granatum

The mangrove *Xylocarpus granatum* is distinguished for producing antifeedant limonoids, especially mexicanolides and phragmalins. Previous investigations on the seeds of two meliaceae plants of mangrove, *X. granatum* and *X. moluccensis*, uncovered an obacunol, two phragmalins, three andirobins and 14 mexicanolides, including xyloccensins A—K.[1—5] Recently, we have reported the isolation and identification of 8 new mexicanolides and 11 novel phragmalins, named xyloccensins L—Z,[6—13] from the stem bark and fruit of a Chinese mangrove *Xylocarpus granatum*. Further investigation on the fruit of the same plant resulted in the discovery of the other four new mexicanolides with a $\Delta^{14,15}$ double bond, named xylogranatins A—D (**1**—**4**), together with two known 8,9,30-phragmalin *ortho* esters, xyloccensins P and Q. Their structures were elucidated on the basis of spectroscopic data, especially 2D NMR techniques including HSQC, HMBC and NOESY.

The structures of the known compounds xyloccensins P and Q were assigned by comparison with our published spectroscopic data.[8,9] And the structure arguments for the new compounds **1**—**4** are detailed below.

Results and Discussion

The ethanolic extract of the fruit of *X. granatum* was subjected to sequential extraction with *n*-hexane and ethyl acetate. The resulting ethyl acetate extract was chromatographed on silica gel and followed by preparative reverse-phase C_{18} HPLC to yield xylogranatins A—D (**1**—**4**) (Chart 1).

Xylogranatin A (**1**), a white powder, had a molecular formula of $C_{32}H_{40}O_9$ established by HR-ESI-MS spectrum (*m*/*z* 568.2670, Calcd for $[M^+]$ 568.2672). Consequently, **1** had an unsaturation index of 13. The IR (KBr) absorption bands at 3600—3200, 2975 and 1740—1715 cm^{-1} indicated the existence of hydroxyl, carbon–carbon double bond and several carbonyl groups. The ^1H- and ^{13}C-NMR data (Table 1) indicated that 8 units of the 13 unsaturations come from four carbon–carbon double bonds and four carbonyls (as one ketone and three esters). Therefore, the other 5 units of unsaturations come from five rings.

DEPT experiments revealed that **1** had 7 methyls (including a methoxy and a doublet methyl), 4 methylenes, 10 methines (five olefinic) and 11 quaternary carbons (including four carbonyls). In addition, the NMR data (Table 1) detected in $CDCl_3$ showed the presence of a ketone (δ_C 218.2 s), a methoxycarbonyl (δ_H 3.68 s, δ_C 52.0 q, 173.9 s), a tiglate group [δ_H 6.88 (q, J=7.0 Hz), 1.81 (d, J=7.0 Hz) and 1.83 s; δ_C 166.8 s, 128.0 s, 138.6 d, 12.2 q and 14.6 q] and a β-furyl ring [δ_H 6.45 br s, 7.39 br s 7.47 br s; δ_C 110.0 d, 119.8 s, 141.6 d, 142.9 d]. The above NMR data indicated that **1** was a mexicanolide having a tiglate group. Moreover, an α,β-unsaturated δ-lactone ring D, characterized by the following NMR data (δ_H 5.14 s, 6.09 s; δ_C 168.8 s, 115.7 d, 165.4 s, 79.8 d, 38.4 s) (Table 1), was confirmed by the HMBC correlations from H-15 (6.09 s) and H-17 (5.14 s) to C-13 (38.4 s), C-14 (168.8 s), C-16 (165.4 s), respectively (Fig. 1). Furthermore, the chemical shifts of C-8, C-9, (δ_C 72.1, 60.4) of ring C was quite the same as those (δ_C 73.7, 60.8) of xyloccensin N,[6] isolated from the stem bark of the same plant, suggested an α-hydroxyl substituted at C-8 as that in xyloc-

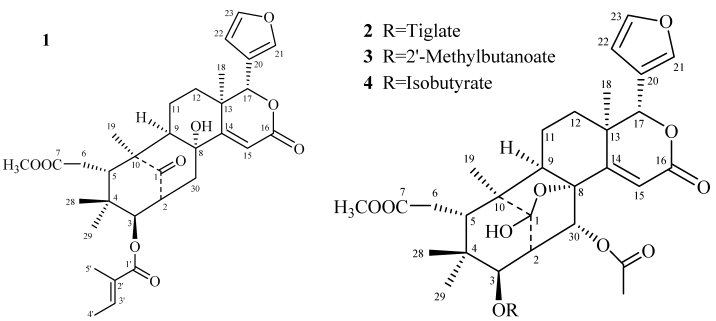

Chart 1. The Structures of Compounds **1**—**4**

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censin N. Additionally, the strong HMBC correlations between H_3-4′/C-2′, H_3-4′/C-3′, H_3-5′/C-1′, H_3-5′/C-2′, H-3′/C-1′, H-3/C-1′ (Fig. 1) confirmed the presence of the tiglate group and revealed that it was attached to the C-3.

The relative stereochemistry of **1** was established on the basis of the NOESY spectrum. The significant NOE interactions observed in **1** (Fig. 1) from H-3 to Me-29, but not from H-3 to H-5, or from H-3 to H-30β helped to establish this 3α-H and the corresponding 3β-tiglate group. When **1** detected in the solvent of DMSO-d_6, the proton of 8-OH showed a high peak at δ 5.21. The significant NOE correlations (Fig. 1) from this proton to H-9 and Me-18 indicated a *cis* orientation between these respective protons. Similarly, those (Fig. 1) from H-30β to H-15 and H-5 also indicated their mutual *cis* relationship. Based on the above results, the structure of **1**, named xylogranatin A, was elucidated as shown in Chart 1.

Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Data for Compounds **1** and **2** in $CDCl_3$

No	**1**		**2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		218.2 s		107.4 s
2	3.31; dd; 16.4, 7.5	44.8 d	2.98; dd; 9.0, 4.0	53.0 d
3	4.85; d; 8.5	78.2 d	5.10; d; 9.0	74.2 d
4		39.9 s		37.6 s
5	3.50; d, 8.5	41.4 d	2.70; d; 10.0	40.8 d
6	2.30; m 2.34; m	33.0 t	2.39; m 2.18; m	32.0 t
7		173.9 s		174.0 s
8		72.1 s		81.1 s
9	1.79; d; 13.0	60.4 d	2.15; m	51.4 d
10		48.3 s		43.0 s
11α 11β	1.64; dd; 13.0, 3.5 1.50; dd; 13.0, 3.5	20.6 t	2.36; m 1.78; m	14.8 t
12α 12β	1.95; d; 14.5 1.25; m	33.6 t	2.12; d; 13.5 1.38; dd; 13.5, 9.0	25.0 t
13		38.4 s		39.0 s
14		168.8 s		160.3 s
15	6.09; s	115.7 d	6.03; s	117.3 d
16		165.4 s		164.0 s
17	5.14; s	79.8 d	4.97; s	81.5 d
18	1.24; s	23.1 q	1.20; s	19.4 q
19	1.08; s	18.6 q	1.06; s	20.5 q
20		119.8 s		120.0 s
21	7.47; br s	141.6 d	7.48; br s	141.3 d
22	6.45; br s	110.0 d	6.41; br s	110.0 d
23	7.39; br s	142.9 d	7.40; br s	142.8 d
28	0.74; s	23.3 q	1.26; s	22.0 q
29	0.81; s	22.8 q	0.81; s	24.5 q
30α 30β	2.42; dd; 15.0, 10.5 3.04; dd; 15.0, 6.8	35.0 t	5.55; d; 4.0	76.5 d
7-OMe	3.68; s	52.0 q	3.68; s	51.9 q
3-Tiglate				
1′		166.8 s		167.0 s
2′		128.0 s		127.9 s
3′	6.88; q; 7.0	138.6 d	6.83; q; 7.0	138.6 d
4′	1.81; d; 7.0	12.2 q	1.78; d; 7.0	11.9 q
5′	1.83; s	14.6 q	1.79; s	14.5 q
30-OAc				
1′				170.2 s
2′			1.90, s	20.9 q

Xylogranatin B (**2**) was isolated as a white powder. Its molecular formula was established as $C_{34}H_{42}O_{11}$ by HR-ESI-MS spectrum (*m/z* 626.2730, Calcd for [M^+] 626.2727). The ^{1}H- and ^{13}C-NMR spectral data (Table 1) of **2** were similar to those of **1**, except for the absence of a ketone carbon (δ 218.2 in **1**) and the presence of an acetal carbon (δ 107.4 s) and one more acetoxy group (δ_H 1.90 s; δ_C 20.9, 170.2) (Table 1). In particular, the presence of this acetal carbon signal and an oxygenated quaternary carbon at δ 81.1 (C-8) strongly suggested that **2** had the same ring structure as that of xyloccensin M.[6] Moreover, the HMBC correlation (Fig. 2) from H-30 (δ_H 5.55, d, J=4.0 Hz) to the carbonyl carbon (δ_C 170.2) of the acetoxy group indicated that it was attached to the C-30 (δ_C 76.5). Additionally, the significant NOE interactions (Fig. 2) observed from H-30 to H-5, H-15 and H-17, but not from H-30 to H-3, helped to establish this 30β-H and the corresponding 30α-acetoxy group. Thus the structure of **2**, named xylogranatin B, was characterized as shown in Chart 1.

Xylogranatin C (**3**), a white powder, had a molecular formula of $C_{34}H_{44}O_{11}$ established by HR-ESI-MS spectrum (*m/z* 628.2881, Calcd for [M^+] 628.2884). The ^{1}H- and ^{13}C-NMR spectral data (Table 2) of **3** were similar to those of **2**, except for the absence of a tiglate group. Instead, a 2′-methylbutanoate group appeared. It was characterized by the following NMR data [δ_H 2.30 m, 1.65 m, 1.38 m, 0.89 (t, J=7.0 Hz), 1.15 (d, J=7.0 Hz); δ_C 177.3 s, 42.4 d, 27.1 t, 12.1 q, 17.2 q] (Table 2) and confirmed by the HMBC correlations from H_3-4′ (0.89, t, J=7.0 Hz) and H_3-5′ (1.15, d, J=7.0 Hz) to C-1′ (177.3 s), C-2′ (42.4 d), C-3′ (27.1 t), respectively. Moreover, the HMBC correlation from H-3 (δ_H 5.03, d, J=9.0 Hz) to the carbonyl carbon (δ_C 177.3) of the 2′-methylbutanoate group indicated that it was attached to the C-3 (δ_C 76.0). Furthermore, the significant NOE interactions observed from H-3 to H_3-29, but not from H-3 to H-5, H_3-28, H-30, helped to establish this 3α-H and the corre-

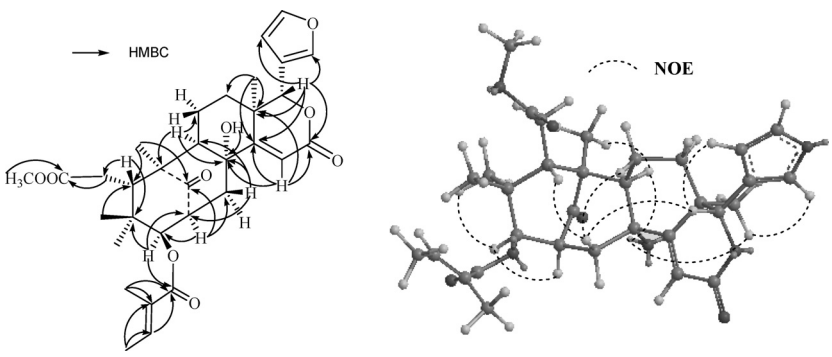

Fig. 1. Selected HMBC and NOE Correlations of Compound **1**

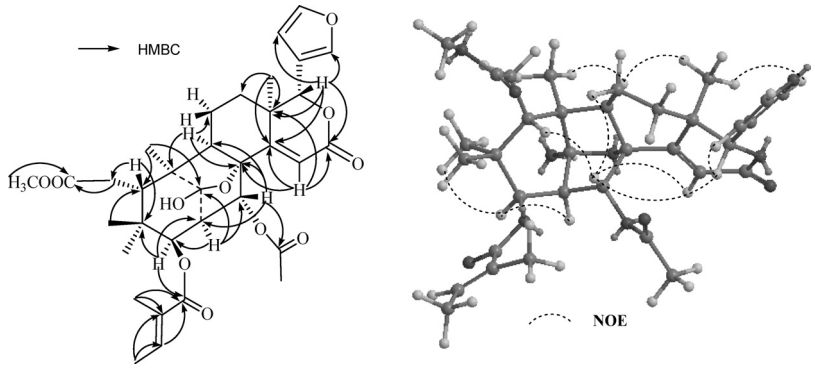

Fig. 2. Selected HMBC and NOE Correlations of Compound **2**

Table 2. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Data for Compounds **3** and **4** in Methanol-d_4

No	**3**		**4**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		109.0 s		109.0 s
2	2.92; dd; 9.0, 4.0	54.5 d	2.92; dd; 9.0, 4.0	54.3 d
3	5.03; d; 9.0	76.0 d	5.03; d; 9.0	75.8 d
4		38.7 s		38.9 s
5	2.67; d; 8.2	42.0 d	2.67; d; 10.0	42.0 d
6	2.37; m 2.34; m	32.9 t	2.39; m 2.18; m	32.9 t
7		175.9 s		175.9 s
8		82.2 s		82.2 s
9	2.16; m	52.4 d	2.15; m	52.8 d
10		44.2 s		44.2 s
11α	2.36; m	16.0 t	2.36; m	15.9 t
11β	1.93; m		1.93; m	
12α	2.10; d; 13.5	26.4 t	2.12; d; 13.5	26.2 t
12β	1.68; dd; 13.5, 9.0		1.38; dd; 13.5, 9.0	
13		40.3 s		40.0 s
14		162.6 s		163.2 s
15	5.93; s	118.3 d	5.96; s	118.0 d
16		165.8 s		165.8 s
17	5.03; s	83.0 d	5.03; s	83.0 d
18	1.25; s	19.9 q	1.26; s	19.9 q
19	1.06; s	21.0 q	1.06; s	20.9 q
20		121.4 s		121.5 s
21	7.60; br s	142.8 d	7.60; br s	142.8 d
22	6.51; br s	110.9 d	6.51; br s	111.0 d
23	7.56; br s	144.6 d	7.56; br s	144.5 d
28	1.29; s	22.4 q	1.29; s	22.5 q
29	0.80; s	25.1 q	0.80; s	25.2 q
30	5.62; d; 4.0	77.6 d	5.63; d; 4.0	78.2 d
7-OMe	3.69; s	52.9 q	3.69; s	52.4 q
R	2′-methylbutanoate		Isobutyrate	
1′		177.3 s		177.4 s
2′	2.30, m	42.4 d	2.51; m	35.5 d
3′	1.65; m 1.38; m	27.1 t	1.17; d; 7.0	19.5 q
4′	0.89; t; 7.0	12.1 q	1.14; d; 7.0	19.2 q
5′	1.15; d; 7.0	17.2 q		
30-OAc				
1′		171.5 s		172.0 s
2′	1.98; s	21.0 q	2.01, s	21.4 q

sponding 3β-2′-methylbutanoate group. Therefore, the structure of **3**, named xylogranatin C, was identified 3β-detigloyoxy-2′-methylbutanoyloxy xylogranatin B.

Xylogranatin D (**4**) was isolated as a white powder. Its molecular formula was established as $C_{33}H_{42}O_{11}$ by HR-ESI-MS spectrum (m/z 614.2730, Calcd for [M$^+$] 614.2727). The ^{1}H- and ^{13}C-NMR spectral data (Table 2) of **4** were similar to those of **2**, except for the absence of a tiglate group. Instead, an isobutyrate group appeared. It was characterized by the following NMR data [δ_H 2.51 m, 1.17 (d, J=7.0 Hz), 1.14 (d, J=7.0 Hz); δ_C 177.4 s, 35.5 d, 19.5 q, 19.2 q] (Table 2) and confirmed by the HMBC correlations from H_3-3′ (1.17, d, J=7.0 Hz) and H_3-4′ (1.14, d, J=7.0 Hz) to C-1′ (177.4 s) and C-2′ (35.5 d), respectively. Moreover, the HMBC correlation from H-3 (δ_H 5.03, d, J=9.0 Hz) to the carbonyl carbon (δ_C 177.4) of the isobutyrate group indicated that it was attached to the C-3 (δ_C 75.8). Furthermore, the significant NOE interactions observed from H-3 to H_3-29, but not from H-3 to H-5, H_3-28, H-30, helped to establish this 3α-H and the corresponding 3β-isobutyrate group. Consequently, the structure of **4**, named xylogranatin D, was characterized as 3β-detigloyoxy-isobutanoyloxy xylogranatin B.

Experimental

General Procedures NMR spectra were recorded in $CDCl_3$ or methanol-d_4 using a Bruker AV-500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) with tetramethylsilane as the internal standard. UV spectra were obtained on a Beckman DU-640 UV spectrophotometer and electrospary ionization (ESI)-MS spectra measured on a Bruker APEX II spectrometer in positive ion mode. Optical rotation data were recorded on a Polaptronic HNQW5 automatic high-resolution polarimeter (Schmidt & Haensch Co. Ltd.). Preparative HPLC was carried out on ODS columns (250×10 mm i.d., YMC) with a Waters 996 photodiode array detector. For CC, silica gel (200—300 mesh) (Qingdao Mar. Chem. Ind. Co. Ltd.) and octadecylsilyl silica gel (80—100 μm) (Unicorn) were used.

Plant Material The fruit of *Xylocarpus granatum* was collected in June 2004 from Hainan island, southern China. The identification of the plant was performed by Prof. Yongshui Lin, Laboratory of Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher sample (NO. GKLMMM-002-2-2) is kept in the Herbarium of South China Sea Institute of Oceanology.

Extraction and Isolation The dried fruit (4.0 kg) of *X. granatum* was extracted with hot 95% ethanol for three times. The extract was concentrated under reduced pressure, followed by suspension in water. After defatting with petroleum ether, the aqueous layer was further extracted with ethyl acetate. The ethyl acetate extract (60 g) was chromatographed on silica gel column and eluted using chloroform–methanol system (100 : 0—2 : 1) to yield 120 fractions. Fractions 50 to 78 (4.2 g) were combined and further purification with preparative HPLC (YMC-Pack ODS-5-A, 250×20 mm i.d., acetonitrile–water 35 : 65 to 40 : 60) yielded xylogranatins A (**1**, 40 mg), B (**2**, 22 mg), C (**3**, 8 mg), D (**4**, 5 mg).

Xylogranatin A (**1**): A white powder; $[\alpha]_D^{25}$ −25° (c=1.0, acetonitrile); UV (MeCN) λ_{max} 215.9 nm; IR ν_{max} (KBr) 3600—3200, 2975, 1740—1715, 1635, 870 cm^{-1}; ^{1}H- and ^{13}C-NMR (see Table 1); HR-ESI-MS m/z 568.2670 [Calcd for $C_{32}H_{40}O_9$ [M$^+$], 568.2672].

Xylogranatin B (**2**): A white powder; $[\alpha]_D^{25}$ −85° (c=1.2, acetonitrile); UV (MeCN) λ_{max} 215.9 nm; IR ν_{max} (KBr) 3450, 3140, 2955, 2870, 1730,

1635, 870 cm^{-1}; ^1H- and ^{13}C-NMR (see Table 1); HR-ESI-MS *m*/*z* 626.2730 [Calcd for $C_{34}H_{42}O_{11}$ $[M^+]$, 626.2727].

Xylogranatin C (**3**): A white powder; $[\alpha]_D^{25}$ −105° (*c*=0.8, acetonitrile); UV (MeCN) λ_{max} 215.9 nm; IR ν_{max} (KBr) 3446, 3141, 2951, 2873, 1733, 875 cm^{-1}; ^1H- and ^{13}C-NMR (see Table 2); HR-ESI-MS *m*/*z* 628.2881 [Calcd for $C_{34}H_{44}O_{11}$ $[M^+]$, 628.2884].

Xylogranatin D (**4**): A white powder; $[\alpha]_D^{25}$ −92° (*c*=0.5, acetonitrile); UV (MeCN) λ_{max} 215.9 nm; IR ν_{max} (KBr) 3448, 3139, 2950, 2871, 1731, 873 cm^{-1}; ^1H- and ^{13}C-NMR (see Table 2); HR-ESI-MS *m*/*z* 614.2730 [Calcd for $C_{33}H_{42}O_{11}$ $[M^+]$, 614.2727].

Acknowledgments Support for this work from the Important Project of Chinese Academy of Sciences (KZCX3-SW-216) and the Natural Science Foundation of Guangdong Province (04100729) is gratefully acknowledged.

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