Two New Limonoids with a 3-*O*-β-Tigloyl Group from the Seeds of the Chinese Mangrove *Xylocarpus granatum*

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A new mexicanolide and a new phragmalin, named 30α-hydroxyl xylogranatin A and xylogranatin E_2, respectively, have been isolated from the seeds of a Chinese mangrove *Xylocarpus granatum*. Their structures were identified on the basis of spectroscopic data. Each of them has a 3-*O*-β-tigloyl group. The first complete assignments of ^{1}H and ^{13}C NMR data for these limonoids were achieved by means of 2D NMR techniques, including ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra.

Key words: Mexicanolide, Phragmalin, 3-*O*-β-Tigloyl Group, *Xylocarpus granatum*, Complete NMR Assignments

Introduction

The mangrove *Xylocarpus granatum* (Meliaceae) is known to produce antifeedant limonoids, especially phragmalins and mexicanolides. Previous investigations on the seeds of two Meliaceae plants, the mangroves *X. granatum* and *X. moluccensis*, uncovered an obacunol, two phragmalins, three andirobins, and 14 mexicanolides, including xyloccensins A–K [1 – 5]. In the course of our search for potential lead structures from Chinese tropical mangrove plants, we have reported the isolation and identification of a mixture of butyrospermol fatty acid esters, eleven mexicanolides, and 13 phragmalins, named xyloccensins L–Z and xylogranatins A–E [6], from a Chinese mangrove *X. granatum*. Five phragmalins [7], among which four were the same as reported by us earlier [6d], have been obtained from the stem bark of the same plant. Two mexicanolides, named xyloccensins X and Y [8], the structures of which were different from those of the same names as we reported, have been identified in a mixture from the fruit of *X. moluccensis*. Recently, four unusual 9,10-*seco* limonoids, named also xylorganatins A–D [9], of which two had previously been isolated by us, have been obtained from the seeds of *X. granatum*. In the current paper, we present the isolation and characterization of a new mexicanolide and a new phragmalin, named 30α-hydroxyl xylogranatin A and xylogranatin E_2, respectively, from the seeds of the Chinese mangrove *Xylocarpus granatum*. Their structures were identified on the basis of spectroscopic data. Each of them contains a 3-*O*-β-tigloyl group. The first complete assignments of ^{1}H and ^{13}C NMR data for these limonoids were achieved by means of 2D NMR techniques, including ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra.

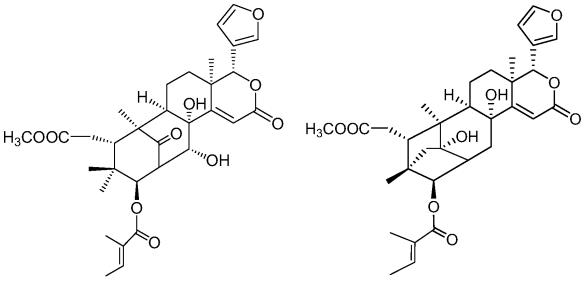


Fig. 1. Structures of compounds **1** – **2**.

Results and Discussion

The ethanolic extract of the seeds of *X. granatum* was subjected to sequential extraction with petroleum ether and ethyl acetate as described in the Experimental Section. The resulting ethyl acetate extract was chromatographed on silica gel, octadecylsilyl silica

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

Carbon No.	^{1}H NMR (δ_H, mult., J in Hz)		^{13}C NMR (δ_C, mult.)	
	1	**2**	**1**	**2**
1			213.1, s	80.0, s
2	3.20, dd, 8.0, 4.0	2.74, dd, 10.5, 6.5	57.5, d	40.4, d
3	4.96, d, 8.0	4.71, d, 10.5	78.4, d	80.2, d
4			40.1, s	45.7, s
5	3.05, d, 7.5	2.73, d, 10.5	43.1, d	37.6, d
6a	2.32, m	2.29, brs	32.9, t	34.4, t
6b	2.36, m	2.35, 10.5		
7			173.6, s	173.7, s
8			75.7, s	73.9, s
9	1.88, d, 12.0	1.73, d, 10.0	61.8, d	47.8, d
10			49.7, s	46.0, s
11α	1.69, dd, 13.5, 3.0	2.14, m	20.7, t	26.4, t
11β	1.11, dd, 13.5, 3.0	1.85, m		
12α	1.99, d, 14.0	1.88, m	32.9, t	33.4, t
12β	1.30, m	1.32, m		
13		39.2, s	38.4, s	
14		165.4, s	169.4, s	
15	6.55, s	6.17, s	118.4, d	116.1, d
16		165.2, s	165.2, s	
17	5.20, s	5.16, s	79.4, d	80.2, d
18	1.30, s	1.32, s	22.2, q	23.4, q
19	1.16, s	1.07, s	17.3, q	20.7, q
20			119.9, s	120.0, s
21	7.50, br s	7.51, br s	141.9, d	141.6, d
22	6.51, br s	6.50, br s	110.7, d	110.4, d
23	7.44, br s	7.43, br s	143.0, d	143.1, d
28	0.87, s	0.88, s	23.2, q	15.1, q
29	0.84, s	1.45, d, 10.0 2.07, d, 10.0	23.0, q	42.8, t
30	5.32, br s	2.35, m	72.4, d	34.4, t
7-OMe	3.72, s	3.68, s	52.2, q	51.8, q
30-OH	3.33, br s			
Tigloyl-1′			167.8, s	168.1, s
2′			128.2, s	128.4, s
3′	6.99, q, 7.0	7.05, q, 7.0	139.7, d	138.8, d
4′	1.89, d, 7.0	1.86, d, 7.0	12.2, q	12.1, q
5′	1.91, s	1.90, s	14.8, q	14.7, q

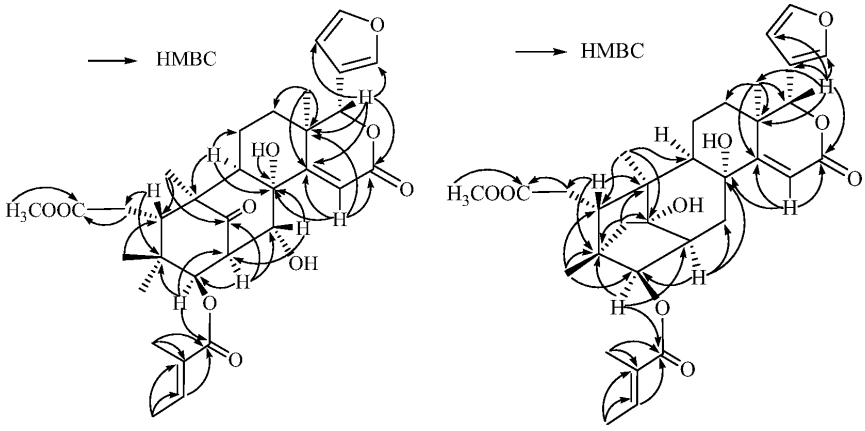
gel, and Sephadex LH-20 gel, followed by preparative reverse-phase C_{18} HPLC to yield 30α-hydroxyl xylogranatin A (**1**) and xylogranatin E_2 (**2**) (Fig. 1).

The electrospary ionization ESI-MS (positive ion mode) of **1** showed *pseudo*-molecular peaks at *m/z* = 585 $[M + H]^+$ and 607 $[M + Na]^+$, which suggested that **1** had a molecular weight of 584. The molecular formula was determined as $C_{32}H_{40}O_{10}$ (unsaturation values of 13) from the HRESI-MS spectrum (*m/z* = 607.2530, calcd. 607.2519 for $[M + Na]^+$). The UV maximum at 216 nm and IR (KBr) absorption bands at 3580 – 3220, 2976 and 1740 – 1710 cm^{-1} indicated the existence of hydroxyl groups, carbon–carbon double bonds, and several carbonyl groups in **1**. The ^{1}H and ^{13}C NMR data (Table 1) showed the presence of a ketone (δ_C = 213.1), an oxygenated quaternary carbon (δ_C = 75.7), three oxygenated methines (δ_C = 72.4, 78.4, 79.4), a methoxycarbonyl group (δ_H = 3.72, s, δ_C = 52.2, q, 173.6, s), a tigloyl group [δ_H = 1.89 (d, J = 7.0 Hz), 1.91, s, 6.99 (q, J = 7.0 Hz), δ_C = 12.2, q, 14.8, q, 128.2, s, 139.7, d, 167.8, s] and a β-furyl ring (δ_H = 6.51, br s, 7.44, br s, 7.50, br s, δ_C = 110.7, d, 119.9, s, 141.9, d, 143.0, d).

The above listed NMR spectral data coupled with four tertiary methyls in the nucleus of **1** suggested that it was a mexicanolide. The ^{1}H and ^{13}C NMR data (Table 1) of **1** were similar to those of xylogranatin A [6j] except for the presence of an additional hydroxyl group (δ_H = 3.33, br s). It was suggested to be attached at C-30 of **1**, deduced from its ^{1}H–^{1}H COSY correlation to H-30 (δ_H = 5.32, br s) and the HMBC cross-peak to C-30 (δ_C = 72.4, d). Moreover, its relative configuration was determined to be the α form by the NOE spectrum. NOE correlations (Figs. 2 and 3) from H-30 to H-17 and H-5 indicated the β form for H-30 and a corresponding 30α-OH. Thus, **1** was characterized as 30α-hydroxyl xylogranatin A.

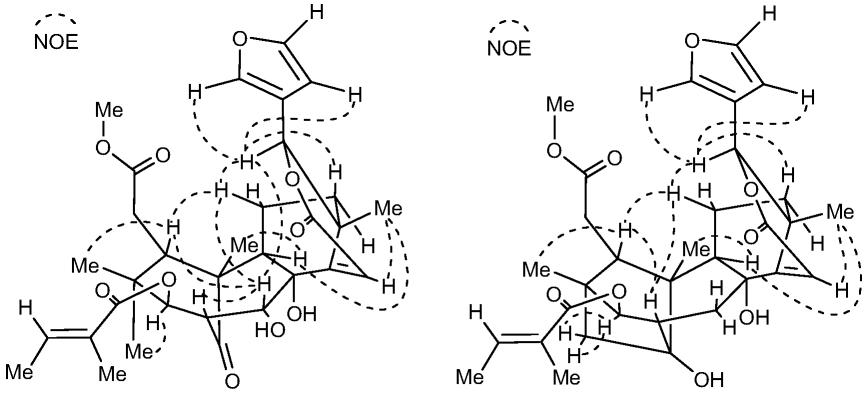
Xylogranatin E_2 (**2**) was isolated as a white powder. Its molecular formula was established as $C_{32}H_{40}O_9$ by HRESI-MS (*m/z* = 591.2580, calcd. 591.2570 for $[M + Na]^+$). The ^{1}H and ^{13}C NMR data (Table 1) showed the presence of two oxygenated quaternary carbons (δ_C = 73.9, 80.0), two oxygenated methines (δ_C = 80.2, 80.2), a methoxycarbonyl group (δ_H = 3.68, s, δ_C = 51.8, q, 173.7, s), a tigloyl group [δ_H = 1.86 (d, J = 7.0 Hz), 1.90, s, 7.05 (q, J = 7.0 Hz), δ_C = 12.1, q, 14.7, q, 128.4, s, 138.8, d, 168.1, s] and a β-furyl ring (δ_H = 6.50, br s, 7.43, br s, 7.51, br s, δ_C = 110.4, d, 120.0, s, 141.6, d, 143.1, d).

These NMR spectral data coupled with three tertiary methyls in the nucleus of **2** suggested that it was a phragmalin. The ^{1}H and ^{13}C NMR data (Table 1) of **2** were similar to those of xylogranatin E [6l], except for the absence of an oxygen bridge between C-1 and C-29. The ^{1}H and ^{13}C NMR data of C-29 [δ_H = 1.45 (d, 10.0 Hz), 2.07 (d, 10.0 Hz), 42.8, t] indicated that it was situated between C-1 and C-4 as that in normal phragmalins. HMBC cross-peaks from H_2-29 to C-1 and C-4 corroborated this connection. The relative stereochemistry of xylogranatin E_2 was established as the same as that of xylogranatin E on the basis of NOE correlations as shown in Fig. 3. The significant NOE interaction from H-3 to H_2-29, but not from H-3 to H-5, helped to establish this 3α-H and the corre-



1 **2**

Fig. 2. Selected HMBC correlations for compounds **1** and **2**.



1 **2**

Fig. 3. Significant NOE correlations for compounds **1** and **2**.

sponding 3-*O*-β-tigloyl group. Similarly, NOE interactions between H-2 / H-5, H-2 / H-11β, H-5 / H-11β, H-11β / H-17, H_3-18 / H-15, H-9 / H_3-18, H-9 / H_3-19 (Fig. 3) indicated a *cis* orientation between these respective protons. Based on the above results, the structure of xylogranatin E_2, was elucidated as shown in Fig. 1.

Experimental Section

Plant material

The seeds of *Xylocarpus granatum* were collected in January 2006 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-002-6) is maintained in the Herbarium of the South China Sea Institute of Oceanology.

Extraction and isolation

The dried seeds (6.0 kg) of *X. granatum* were crushed and extracted three times with 95 % ethanol at r. t. After removal of the solvent by evaporation, the residue was suspended in water and defatted with petroleum ether. Then the aqueous layer was further extracted with ethyl acetate and concentrated to give a brown gum, which was subjected to silica gel chromatography (chloroform/methanol 100 : 0 to 2 : 1). The fractions eluted with chloroform/methanol (25 : 1 to 15 : 1) were combined and purified by preparative HPLC (YMC-Pack ODS-5-A, 250 × 20 mm i. d., acetonitrile/water 30 : 70 to 45 : 55) to yield **1** (10 mg) and **2** (15 mg).

30α-Hydroxyl xylogranatin A: Amorphous powder. – UV (MeCN): λ_{max} = 216 nm. – $[\alpha]_D^{20}$ = −55 (*c* = 0.8, acetonitrile). – IR (KBr): ν = 3580 – 3220, 2976, 1740 – 1710, 1634, 870 cm^{-1}. – ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 1. – HRESI-MS: *m/z* = 607.2530 (calcd. 607.2519 for $C_{32}H_{40}NaO_{10}$, $[M + Na]^+$).

Xylogranatin E_2: Amorphous powder. – UV (MeCN): λ_{max} = 217 nm. – $[\alpha]_D^{20} = -35$ (c = 0.7, acetonitrile). – IR (KBr): ν = 3600 – 3200, 2980 and 1740 – 1710, 1636, 875 cm^{-1}. – ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 1. – HRESI-MS: m/z = 591.2580 (calcd. 591.2570 for $C_{32}H_{40}NaO_9$, $[M + Na]^+$).

Spectra

UV spectra were obtained on a Beckman DU-640 UV spectrophotometer and IR spectra on a Perkin-Elmer FT-IR 1760X spectrophotometer. Electrospray ionization ESI-MS spectra were measured on a Bruker APEX II spectrometer in positive ion mode. Optical rotations were recorded on a POLAPTRONIC HNQW5 automatic high-resolution polarimeter (Schmidt & Haensch Co. Ltd.). NMR experiments were recorded on a Bruker AV-500 spectrometer operating at 500 and 125 MHz for ^1H and ^{13}C, respectively, and equipped with an inverse-detection 5 mm probe (TBI probe, ^1H 90° pulse width = 6.1 μs, ^{13}C 90° pulse width = 12.3 μs) and operating at r. t. with tetramethylsilane as internal standard. About 10 mg samples were dissolved in $CDCl_3$ (0.5 mL) to record the NMR spectra.

1D spectra were acquired using 64 K data points and spectral widths of 10000 Hz and 27500 Hz for ^1H and ^{13}C, respectively. 32 K data points were used for the processing with an exponential function for all 1D spectra.

Standard pulse sequences were used for 2D spectra. Spectral widths of 5000 Hz and 25000 Hz were used for ^1H and ^{13}C, respectively. Relaxation delays of 2.0 s were used for all 2D NMR experiments. The 2D spectra used 1024 × 512 (^1H–^1H COSY), 2048 × 512 (HSQC), and 4096 × 512 (HMBC) data point matrices, which were zero filled to 1024 × 1024, 2048 × 1024 and 4096 × 1024, respectively. Non-shifted qsine-bell window functions were used along the F_1 and F_2 axes for all 2D spectra. The HMBC experiments used an 80 ms delay time to obtain ^1H and ^{13}C long-range correlations. Z-PFGs were used to obtain HSQC, HMBC spectra. Data processing was carried out with Bruker XWINNER 3.50 programs.

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