Xyloccensin M and N, Two New B, D-*seco* Limonoids from *Xylocarpus granatum*

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Two new mexicanolide-type B, D-*seco* limonoids named xyloccensin M (1) and N (2) were isolated from the stem bark of *Xylocarpus granatum*. Their structures were elucidated with the help of modern spectroscopic techniques.

Key words: Xylocarpus granatum, B, D-*seco* Limonoid, Mexicanolide, Xyloccensin M and N

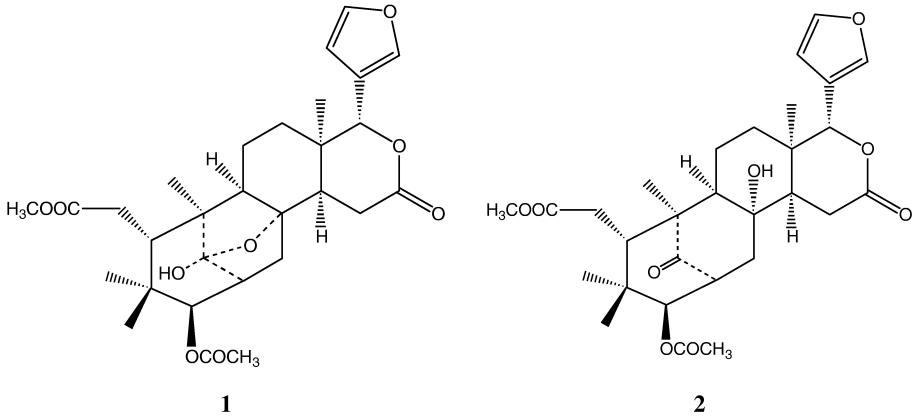
Introduction

The limonoids are modified triterpenes derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton found to date only in plants of the order Rutales. Limonoids show a broad spectrum of biological activities. Some like azadirachtin from the neem tree *Azadiracha indica* [1] and harrisonin from *Harrisonnia abyssinica* [2] show marked insect antifeedant and growth regulating activities, while the rubrins from *Trichilia rubra* are potent cell adhesion inhibitory agent [3]. Past investigations on the chemical constitutes of the seeds of two mangrove plants, *xylocarpus granatum and xylocarpus moluccensis*, have yielded eleven limonoids xyloccensins A – K [4 – 8]. Recently we have reported the isolation and structural elucidation of a novel heptacyclic A, B, D-*seco* limonoid, named xyloccensin L, which has an α 8, 30-epoxy ring and a rare 1, 29 oxygen bridge from the stem bark of *X. granatum* [9]. As part of our continuing search for bioactive natural products from tropical medicinal plants, we now describe the isolation and structural elucidation of two new mexicanolide-type B, D-*seco* limonoids named xyloccensin M (1) and N (2) from the same plant.

Results and Discussion

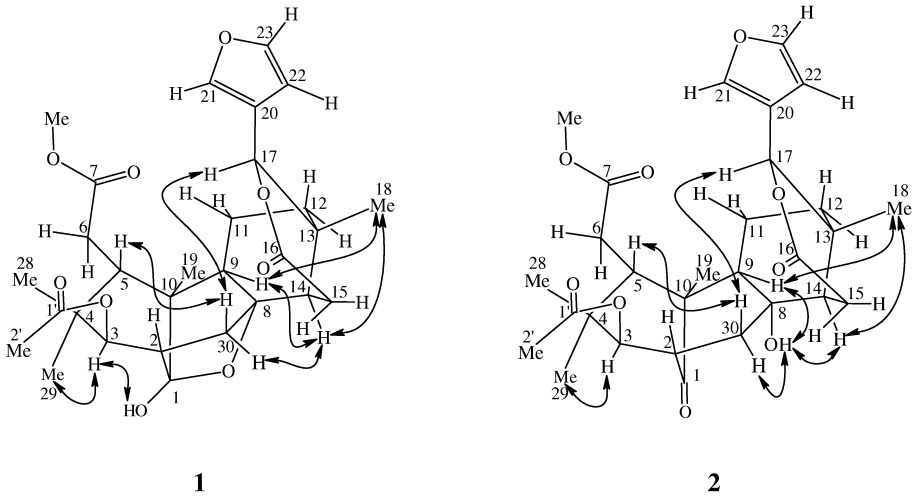
The ethanolic extract of the stem bark of *X. granatum* was subjected to sequential extraction with petroleum ether and ethyl acetate as described in the Experimental section. The resulting ethyl acetate extract was chromatographed on silica gel, octadecylsilyl silica gel, Sephadex LH-20 gel and followed by preparative reverse-phase C_{18} HPLC to yield xyloccensin M (**1**) and N (**2**) (Fig. 1).

The electrospary ionization (ESI)-MS (positive ion mode) of **1** showed pseudo-molecular peaks at m/z 553 $[M+Na]^+$ and 569 $[M+K]^+$, which proposed the molecular weight as 530. The HRESI-MS measurements indicated that the elemental composition was $C_{29}H_{38}O_9$, in agreement with the 1D NMR data (Table 1). Consequently, **1** had an unsaturation index of eleven, which included two carbon-carbon double bonds, three ester functions and six rings. The ^1H and ^{13}C NMR spectral data (Table 1) of **1** were similar to those of xyloccensin J [7] except for the absence of a 2′-methylpropanoyloxy group in C-30 and a hydroxyl one in C-2. In particular, the presence of an acetal carbon signal at δ 108.5 and an oxygenated quaternary carbon at δ 80.4 strongly suggested that **1** had the same ring structure as xyloccensin J [7]. An acetyl group was established by the typical chemical shifts (δ_H 1.99; δ_C 170.5, 20.8) (Table 1). And the HMBC correlation from 3-H (δ_H 4.92, d, J = 8.7 Hz) to C-1′ (δ_C 170.5) indicated that it attached to the C-3 (δ_C 76.3). Additionally, the significant NOE interactions (Fig. 2) observed from H-3 to Me-29, but not from H-3 to H-5, or from H-3 to H-30α helped to establish this 3β-acetoxy group. Furthermore, NOE correlations (Fig. 2) from H-17 to H-30β and from H-30β



1 **2**

Fig. 1. Structures of xyloccensin M and N.



1 **2**

Fig. 2. Diagnostic NOE correlations in xyloccensin M and N.

to H-5 indicated a *cis* orientation between these respective protons. Similarly, those (Fig. 2) from H-14 to Me-18 and H-9 also indicated their mutual *cis* relationship. Thus **1** was characterized as 2α-deoxy-30α-des-(2′-methylpropanoyloxy)-xyloccensin J, named xyloccensin M.

Compound **2** was isolated as amorphous powder. Its molecular formula was established as $C_{29}H_{38}O_9$, which was the same as **1**, by HRESI-MS and NMR data (Table 2). The IR (3650-3200, 1740, 1708, 1635, 875 cm^{-1}) data indicated the presence of carbon-carbon double bond, hydroxyl, keto and ester carbonyl groups. From the ^{1}H and ^{13}C NMR spectral data (Table 2), it was evident that six of eleven unsaturation index were present as double bonds: two carbon-carbon double bonds (as furan ring) and four C=O (as one keto and three esters). A β-furyl moiety and one methoxycarbonyl group were also apparent from the spectra. The ^{1}H and ^{13}C NMR spectral data (Table 2) of **2** were similar to those of khayanone [10] except for the absence of a hydroxyl in C-6 and a keto in C-3, as well as the presence of additional an acetoxy group (δ_H 2.08; δ_C 170.1, 20.5) (Table 2). The HMBC correlation from 3-H (δ_H 4.73, d, J = 8.7 Hz) to C-1′ (δ_C 170.1) indicated that it attached to the C-3 (δ_C 79.2). And the significant NOE interactions observed from H-3 to Me-29, but not from H-3 to H-5, or from H-3 to H-30α helped to establish this 3β-acetoxy group, which was the same as **1**. Additionally, the chemical shifts of C-8, C-9, C-14 (δ_C 73.7, 60.8, 51.9) of ring C was almost the same as those (δ_C 73.8, 61.8, 51.7) of khayanone [10], suggested a α hydroxyl (δ_H 3.05, brs.) substi-

Table 1. ^{1}H (HMQC) and ^{13}C NMR data, HMBC and ^{1}H-^{1}H COSY correlations of xyloccensin M (**1**) (500 and 125 MHz, acetone-d_6).

Carbon No.	^{1}H NMR δH; mult.; J(Hz)	^{13} C NMR δC; mult.	HMBC correlations	^{1}H-^{1} H COSY correlations
1		108.5; s		
2	2.74; m	46.2; d	1, 3, 4, 30	3, 30α, 30β
3	4.92; d; 8.7	76.3; d	1′, 2, 4, 5, 29, 30	2
4		38.4; s		
5	2.84; dd; 10.0; 2.5	40.9; d	3, 4, 6, 7, 10, 19	6a, 6b
6a	2.48; d; 10.0	32.9; t	4, 5, 7, 10	5
6b	2.45; d; 2.5		4, 5, 7, 10	5
7		174.8; s		
8		80.4; s		
9	1.50; dd; 13.2; 2.0	64.0; d	5, 8, 10, 11	11α, 11β,
10		44.8; s		
11α	1.72; brdt; 13.2; 3.0	19.7; t	9, 13	9, 11β, 12α, 12β
11β	1.80; m		8, 9, 13	9, 12α, 11α, 12β
12α	1.36; m	36.2; t	9, 11, 17, 18	11α, 11β, 12β
12β	1.74; m		9, 11, 13, 17	11α, 11β, 12α
13		36.3; s		
14	2.14; dd; 12.5; 6.0	46.2 d	8, 9, 13, 15, 16, 17, 18	15α, 15β
15α	2.63; dd; 19.0; 6.0	28.5; t	8, 13, 14, 16	14, 15β
15β	2.82; dd; 19.0; 12.5		8, 13, 14, 16	14, 15α
16		169.8; s		
17	5.46; s	78.3; d	12, 13, 14, 16, 18, 20, 21, 22	
18	1.05; s	22.7; q	12, 13, 14, 17	
19	1.01; s	21.2; q	1, 5, 9, 10	
20		122.9; s		
21	7.61; brd; 1.0	141.8; d	20, 22, 23	22
22	6.47; brd; 1.0	110.9; d	20, 21, 23	23
23	7.59; t; 1.5	144.0; d	20, 21, 22	21, 22
28	1.23; s	22.7; q	3, 4, 5, 29	
29	0.74; s	24.7; q	3, 4, 5, 28	
30α	1.80; d; 14.0	29.6; t	2, 8, 9, 14	2, 30β
30β	2.35; dd; 14.0; 9.6		1, 2, 3, 4,	2, 30α
7-OMe	3.66; s	51.8; q	7	
1-OH	5.99; s		1, 2, 10	
3-acetyl				
1′		170.5; s		
2′	1.99; s	20.8; q	1′	

Table 2. ^{1}H (HMQC) and ^{13}C NMR data, HMBC and ^{1}H-^{1}H COSY correlations of xyloccensin N (**2**) (500 and 125 MHz, acetone-d_6).

Carbon No.	^{1}H NMR δH; mult.; J(Hz)	^{13} C NMR δC; mult.	HMBC correlations	^{1}H-^{1} H COSY correlations
1		218.0; s		
2	3.16; m	46.2; d	1, 3, 4, 30	3, 30α, 30β
3	4.73; d; 8.7	79.2; d	1′, 2, 4, 5, 29, 30	2
4		39.8; s		
5	3.74; dd; 10.0; 2.5	43.9; d	3, 4, 6, 7, 10, 19	6a, 6b
6a	2.40; d; 10.0	33.1; t	4, 5, 7, 10	5
6b	2.44; d; 2.5		4, 5, 7, 10	5
7		174.6; s		
8		73.7; s		
9	1.80; dd; 13.2; 5.0	60.8; d	5, 8, 10, 11	11α, 11β,
10		49.1; s		
11α	1.20; brdt; 13.0; 3.0	19.9; t	9, 12, 13	9, 11β, 12α, 12β
11β	1.82; m		8, 9, 12	9, 12α, 11α, 12β
12α	1.38; m	34.5; t	9, 11, 13, 17	11α, 11β, 12β
12β	1.63; m		9, 11, 17, 18	11α, 11β, 12α
13		36.2; s		
14	2.00; dd; 7.6; 2.0	51.9 d	8, 13, 15, 16, 17, 18, 30	15α, 15β
15α	2.32; dd; 19.0; 2.0	32.8; t	8, 13, 14, 16	14, 15β
15β	2.56; dd; 19.0; 7.6		8, 13, 14, 16	14, 15α
16		170.0; s		
17	5.82; s	78.1; d	12, 13, 14, 16, 18, 20, 21, 22	
18	1.03; s	23.5; q	12, 13, 14, 17	
19	1.08; s	19.0; q	1, 5, 9, 10	
20		122.9; s		
21	7.83; dd; 1.5; 1.0	142.2; d	20, 22, 23	22, 23
22	6.60; dd; 1.5; 1.0	110.8; d	20, 21, 23	21, 23
23	7.59; t; 1.5	143.9; d	20, 21, 22	21, 22
28	0.87; s	23.1; q	3, 4, 5, 29	
29	0.78; s	24.0; q	3, 4, 5, 28	
30α	2.54; d; 14.6	29.1; t	2, 8, 9, 14	2, 30β
30β	2.86; dd; 14.6; 9.6		1, 2, 3, 4,	2, 30α
7-OMe	3.73; s	52.3; q	7	
8α-OH	3.05; brs		8, 9, 14, 30	
3-acetyl				
1′		170.1; s		
2′	2.08; s	20.5; q	1′	

tuted at C-8 as that in khayanone. And this was confirmed by the strong HMBC correlation from the proton of this hydroxyl to C-8. Furthermore, the significant NOE interactions observed from 8α-OH to 9-H, 14H and 30αH helped to establish this α configuration (Fig. 2). Consequently, **2** was assigned as 6-deoxy-3-deoxo-3β-acetoxy-khayanone, named xyloccensin N.

Xyloccensin M and N was a pair of isomers of mexicanolides. As viewed from the biosynthetic pathway, xyloccensin N was the possible biosynthetic intermediate of xyloccensin M. And it represented to our knowl-

edge that this was the first time to get a pair of isomers of mexicanolides from the same plant simultaneously.

Experimental Section

General

NMR spectra were recorded in acetone-d_6 using a Varian INOVA-500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) with tetramethylsilane as internal standard. Electrospary ionization (ESI)-MS spectra were measured on a Bruker APEX II spectrometer in positive ion mode. Optical rotations were measured with an AA-10R digital polarimeter. 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.), octadecylsilyl silica gel (80 – 100 μm) (Unicorn) and Sephadex LH-20 gel (Pharmacia) were used. The spray reagent used for TLC was 5% H_2SO_4 and 5% phosphomolybdic acid in 95% ethanol.

Plant material

Xylocarpus granatum was collected in July 2001 from Sanya of Hainan Province, 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) is kept in the Herbarium of South China Sea Institute of Oceanology.

Extraction and isolation

The dried stem bark (2.3 kg) of *X. granatum* was extracted with hot 95% and 50% ethanol three times respectively. After removal of the solvent by evaporation, the residue was suspended in water and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate. The resulting ethyl acetate extract (80 g) was subjected to silica gel CC using chloroform-methanol system (100 : 0 ∼ 2 : 1) to yield 120 fractions. Fractions 6 to 11 (5 g) were combined and subjected to CC on silica gel, octadecylsilyl silica gel and Sephadex LH-20 gel, followed by preparative reverse-phase C_{18} HPLC using acetonitrile-water system (35:65) to yield xyloccensin M (10 mg) and N (6 mg).

xyloccensin M (1)

Amorphous powder, $[\alpha]_D^{25}$ −88 ° (c 0.8, acetone). – IR (KBr) $\tilde{\nu}$ = 3450, 3141, 1730, 1635, 870 cm^{-1}. – ^{1}H NMR and ^{13}C NMR (acetone-d_6): See Table 1. – HR-ESI-MS, *m/z*: 553.2419 [M+Na]$^+$. ($C_{29}H_{38}O_9Na$ requires 553.2413)

xyloccensin N (2)

Amorphous powder, $[\alpha]_D^{25}$ +10 ° (c 0.5, acetone). – IR (KBr) $\tilde{\nu}$ = 3650 – 3200, 1740, 1708, 1635, 875 cm^{-1}. – ^{1}H NMR and ^{13}C NMR (acetone-d_6): See Table 1; – HR-ESI-MS, *m/z*: 553.2410 [M+Na]$^+$. ($C_{29}H_{38}O_9Na$ requires 553.2413)

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