# Xyloccensins $X_1$ and $X_2$, Two New Mexicanolides from the Fruit of a Chinese Mangrove *Xylocarpus granatum*

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Z. Naturforsch. **61b,** 626 – 628 (2006); received November 2, 2005

Two new mexicanolides, named xyloccensins $X_1$ and $X_2$ (**1 – 2**), were isolated from the fruit of the Chinese mangrove *Xylocarpus granatum*. Their structures were elucidated on the basis of spectroscopical data, especially 2D NMR techniques including HSQC, HMBC, and NOESY.

*Key words:* Mexicanolide, *Xylocarpus granatum*

## Introduction

Mexicanolide, a tetranortriterpene with a characteristic bicyclo[3.3.$1^{2,10}$]nonane ring system, was isolated from *Khaya grandifoliola* and its structure was determined by single-crystal X-ray diffraction analysis [1]. To date, many mexicanolides showing antifeedant and antifungal activities have been reported from meliaceae plants [2 – 7]. Recently, we reported the isolation and identification of four novel mexicanolides and eight unique 8,9,30-phragmalin *ortho* esters, named xyloccensins L-W [8 – 12], from the stem bark and fruit of the Chinese mangrove *Xylocarpus granatum*. Further investigation of the fruit of the same plant resulted in the discovery of two new mexicanolides with $3\beta$, $8\alpha$-dihydroxylation and a $\Delta^{14,15}$ double bond, named xyloccensins $X_1$ and $X_2$ (**1 – 2**). Their structures were elucidated on the basis of spectroscopic data, especially 2D NMR techniques including HSQC, HMBC and NOESY.

## 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 followed by preparative reverse phase $C_{18}$ HPLC to yield xyloccensins $X_1$ (**1**) and $X_2$ (**2**) (Fig. 1).

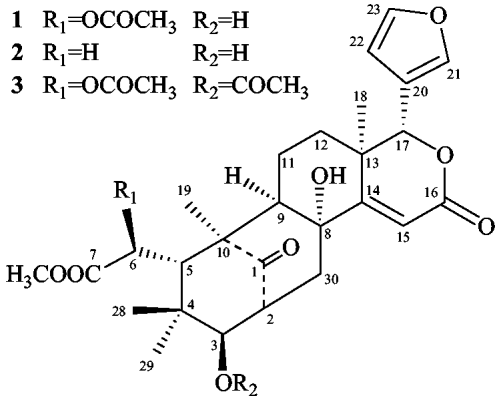


Fig. 1. Structures of compounds **1 – 3**.

Xyloccensin $X_1$ (**1**), a white powder, had a molecular formula of $C_{29}H_{36}O_{10}$ established by HR-TOFMS ($m/z$ 544.2304, calcd for [$M^+$] 544.2308). Consequently, **1** had an unsaturation index of 12. The $^1$H and $^{13}$C NMR data (Table 1) indicate that 7 of the 12 unsaturations come from three carbon-carbon double bonds and four carbonyls (as one ketone and three esters). Therefore, the other 5 units of unsaturations stem from five rings.

DEPT experiments revealed that **1** had sifx methyls (including an acetyl and a methoxy group), three methylenes, ten methines (four olefinic) and ten quaternary carbons (including four carbonyl groups). In addition, the NMR data showed the presence of a ketone ($\delta_C = 221.2$ s), a methoxycarbonyl ($\delta_H = 3.74$ s, $\delta_C = 53.5$ q, 172.6 s), an acetyl group ($\delta_H = 2.13$ s, $\delta_C = 21.0$ q, 171.4 s), and a $\beta$–furyl ring [$\delta_H = 6.57$ (br s), 7.53 (br.s) 7.68 (br.s); $\delta_C = 111.6$ d, 121.6 s,

Table 1. $^1$H (500 MHz) and $^{13}$C NMR (125 MHz) data for compounds **1 – 2** in methanol-$d_4$.

| No | $\delta_H$1 (*J* in Hz) | $\delta_C$1 | $\delta_H$2 (*J* in Hz) | $\delta_C$2 |
|---|---|---|---|---|
| 1 | | 221.2 s | | 222.8 s |
| 2 | 3.05; m | 48.9 d | 3.06; m | 49.1 d |
| 3 | 3.61; d; 8.5 | 79.0 d | 3.77; d; 8.5 | 78.3 d |
| 4 | | 42.5 s | | 41.8 s |
| 5 | 3.62; br s | 45.5 d | 3.48; d; 8.0 | 42.6 d |
| 6 | 5.51; br s | 74.0 d | 2.39; d; 8.0<br>2.45; br s | 34.2 t |
| 7 | | 172.6 s | | 176.0 s |
| 8 | | 73.3 s | | 73.4 s |
| 9 | 1.87; d; 13.5 | 64.1 d | 1.87; d; 13.5 | 63.1 d |
| 10 | | 50.5 s | | 50.0 s |
| 11$\alpha$ | 1.83; dd; 13.0, 3.2 | 22.4 t | 1.79; dd; 13.0, 3.2 | 21.7 t |
| 11$\beta$ | 1.58; dd; 13.0, 3.2 | | 1.56; dd; 13.0, 3.2 | |
| 12$\alpha$ | 2.05; d; 14.5 | 34.3 t | 2.05; d; 14.5 | 34.4 t |
| 12$\beta$ | 1.30; dd; 14.5, 3.0 | | 1.27; dd; 14.5, 3.0 | |
| 13 | | 40.0 s | | 40.0 s |
| 14 | | 171.0 s | | 171.4 s |
| 15 | 6.27; s | 116.7 d | 6.28; s | 116.7 d |
| 16 | | 167.7 s | | 167.8 s |
| 17 | 5.32; s | 81.1 d | 5.35; s | 81.2 d |
| 18 | 1.26; s | 23.4 q | 1.28; s | 23.4 q |
| 19 | 1.09; s | 18.5 q | 1.10; s | 18.8 q |
| 20 | | 121.6 s | | 121.6 s |
| 21 | 7.68; br s | 143.7 d | 7.68; br s | 143.3 d |
| 22 | 6.57; br s | 111.6 d | 6.58; br s | 111.6 d |
| 23 | 7.53; br s | 144.4 d | 7.54; br s | 144.4 d |
| 28 | 1.01; s | 26.2 q | 0.81; s | 23.9 q |
| 29 | 0.93; s | 23.7 q | 0.75; s | 23.7 q |
| 30$\alpha$ | 3.27; d; 6.5 | 37.1 t | 3.31; d; 6.5 | 37.1 t |
| 30$\beta$ | 2.50; dd; 15.0, 5.0 | | 2.54; dd; 15.0, 5.0 | |
| 7-OMe | 3.74; s | 53.5 q | 3.69; s | 52.5 q |
| 6-Acetyl | | | | |
| 1’ | | 171.4 s | | |
| 2’ | 2.13; s | 21.0 q | | |

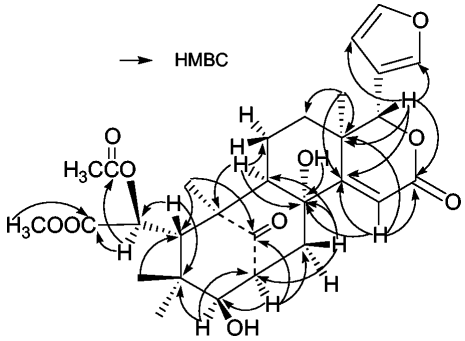


Fig. 2. HMBC correlations of compound **1**.

143.7 d, 144.4 d]. The above NMR data indicated that **1** was a mexicanolide. Moreover, an $\alpha,\beta$-unsaturated $\delta$–lactone ring D, characterized by the following NMR data ($\delta_H$ = 5.32 s, 6.27 s; $\delta_C$ = 171.0 s, 116.7 d, 167.7 s, 81.1 d, 40.0 s), was confirmed by the HMBC

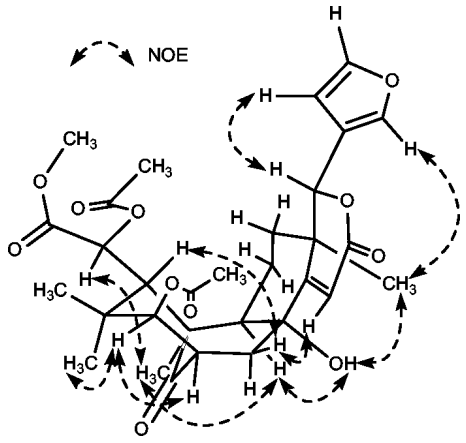


Fig. 3. Diagnostic NOE correlations of compound **3**.

correlations from H-15 (6.27 s) and H-17 (5.32 s) to C-13 (40.0 s), C-14 (171.0 s), C-16 (167.7 s), respectively. Furthermore, the chemical shifts of C-8, C-9, ($\delta_C$ = 73.3, 64.1) of ring C were quite the same as those ($\delta_C$ = 73.7, 60.8) of xyloccensin N[8], isolated from the stem bark of the same plant, suggesting a hydroxyl substituted at C-8 as in xyloccensin N. Additionally, the strong HMBC correlation from 6-H (5.51, brs) to the carbonyl carbon (171.4 s) of the acetyl group revealed that it was attached to C-6.

Acetylation of **1** with acetic anhydride in pyridine afforded 3-acetyl xyloccensin $X_1$ (**3**). This confirmed that C-3 of **1** was substituted by a hydroxyl group. Significant NOE interactions observed in **3** (Fig. 3) from H-3 to Me-29, but not from H-3 to H-5, or from H-3 to H-30$\beta$ helped to establish this 3$\alpha$-H and the corresponding 3$\beta$–acetoxy group, which disclosed the presence of a 3$\beta$-OH in **1**. When the $^1$H NMR spectrum of **3** was taken in the solvent of DMSO-$d_6$, the proton of 8-OH showed a peak at $\delta$ = 5.24. The NOE correlations (Fig. 3) from this proton to H-9 and Me-18 indicated a *cis* orientation of these respective protons. Similarly, those (Fig. 3) from H-30$\beta$ to H-15 and H-5 also indicated their mutual *cis* relationship. The stereochemistry of C-6 remained to be determined. Simulating the three-dimensional structure of **1** and **3** by Cambridgesoft Chem3D software Ultra version 8.0 uncovered that the orientation of H-5 and H-6 was nearly vertical. This result was not only in agreement with that two broad single peaks of H-5 and H-6, observed in the $^1$H NMR spectra of **1** and **3**, have weak $^1$H-$^1$H COSY correlations, but also were the same as those in xyloccensin O[10], exhibiting the same substituted group at C-6 as **1** and **3**. Therefore, the orientation of 6-acetoxy

group was determined as $\beta$ and the relative structures of **3** and **1** were assigned as shown in Fig. 1.

Xyloccensin $X_2$ (**2**) was isolated as a white powder. Its molecular formula was established as $C_{27}H_{34}O_8$ by HR-TOFMS ($m/z$ 486.2258, calcd. for $[M^+]$ 486.2254). The NMR data of **2** were almost the same as those of **1**, except for the absence of the 6-acetoxy group. The $^1$H-$^1$H COSY correlations from H-5 to $H_2$-6 confirmed this result. Therefore, compound **2** was identified as 6-deacetoxy xyloccensin $X_1$.

## Experimental Section

*General procedure*

NMR spectra were recorded in methanol-$d_4$ and DMSO-$d_6$ 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 IR spectra recorded on a Perkin-Elmer FT-IR 1760X spectrophotometer. TOF-MS spectra were measured on a Bruker APEX II spectrometer in positive or negative ion mode. Optical rotations were recorded on a POLAPTRONIC HNQW5 automatic high-resolution polarimeter (Schmidt & Haensch Co. Ltd.) and Preparative HPLC was carried out on ODS columns ($250 \times 10$ 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 $\mu$m) (Unicorn) were used.

*Plant material*

The fruit of *Xylocarpus granatum* was collected in October 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) is kept in the Herbarium of South China Sea Institute of Oceanology.

*Extraction and isolation*

The dried fruit (4.8 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 (105 g) was chromatographed on silica gel column and eluted using chloroform-methanol gradient (100:0 to 2:1) to yield 80 fractions. Fractions 26 to 31 (1.2 g) were combined and further purification with preparative HPLC (YMC-Pack ODS-5-A, $250 \times 20$ mm i. d., methanol–water 45 : 55 to 50 : 50) yielded xyloccensin $X_1$ (**1**, 15 mg) and $X_2$ (**2**, 12 mg).

*Xyloccensin $X_1$ (**1**)*

A white powder, $[\alpha]_D^{25} + 45$ (*c* 1.2, MeCN). – UV (MeCN) $\lambda_{max}$ 210 nm; – IR (KBr): $\tilde{\nu} = 3600 - 3210$, 2985, 1740 – 1715 cm$^{-1}$. – $^1$H (500 MHz, methanol-$d_4$) and $^{13}$C NMR (125 MHz, methanol-$d_4$): see Table 1. – MS (HR-TOF): $m/z = 544.2304$ $[M]^+$. ($C_{29}H_{36}O_{10}$ requires 544.2308).

*Xyloccensin $X_2$ (**2**)*

A white powder, $[\alpha]_D^{25} + 25$ (*c* 1.5, MeCN). – UV (MeCN) $\lambda_{max}$ 210 nm; – IR (KBr): $\tilde{\nu} = 3600 - 3200$, 2985, 1740 – 1715 cm$^{-1}$. – $^1$H (500 MHz, methanol-$d_4$) and $^{13}$C NMR (125 MHz, methanol-$d_4$): see Table 1. – MS (HR-TOF): $m/z = 486.2258$ $[M]^+$. ($C_{27}H_{34}O_8$ requires 486.2254).

*Acknowledgement*

Support for this work from the Natural Science Foundation of Guangdong Province (04100729) is gratefully acknowledged.

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