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Xyloccensins O and P, Unique 8,9,30-Phragmalin Ortho Esters from *Xylocarpus granatum*

Jun Wu,*,† Qiang Xiao,‡ Jianshe Huang,† Zhihui Xiao,† Shuhua Qi,† Qingxin Li,† and Si Zhang†

Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, P.R. China, and Institute of Organic Chemistry, Jiangxi Science & Technology Normal University, Nanchang, 330013, P.R. China

wwujun2003@yahoo.com

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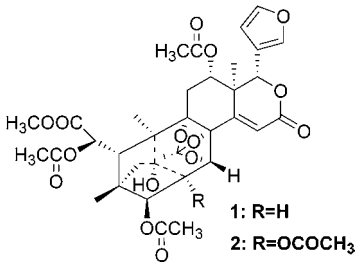
ABSTRACT



Two unique 8,9,30-phragmalin ortho esters, xyloccensins O (1) and P (2), were isolated from the mangrove plant *Xylocarpus granatum*. They are a new type of ortho ester of phragmalin. The structures were determined by spectroscopic and single-crystal X-ray diffraction techniques. The biogenetic pathway to these new phragmalins was also proposed.

Limonoids, which have been found to date only in plants of the order Rutales, are triterpene derivatives from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton. They show a broad range of biological activities. For example, azadirachtin from the neem tree *Azadiracha indica*[1] and harrisonin from *Harrisonnia abyssinica*[2] have remarkable insect antifeedant and growth regulating activities. The rubrins from *Trichilia rubra*[3] are potential cell adhesion inhibitory agents. Limonoids are classified by the type of four rings in the intact triterpene nucleus, which are usually oxidized and designated as A, B, C, and D. Phragmalins, such as xyloccensin E isolated from *Xylocarpus moluccensis* and khayanolides A−C from *Khaya senegalensis*,[4] have characteristic tricyclo[3.3.1^{2,10}.1^{1,4}]decane or tricyclo[4.2.1^{10,30}.1^{1,4}]decane ring systems. To date, 37 phragmalins have been reported from Meliaceae plants, and 26 of them are phragmalin ortho esters.[4b,5] They can be classified according to the position of the orthoacetate groups as 1,8,9- and 8,9,14-phragmalin ortho esters.

Previous investigations on the seeds of two mangrove plants, *X. granatum* and *X. moluccensis*, have found 11 limonoids, xyloccensins A−K.[6] Recently, we have reported

* To whom correspondence should be addressed. Tel: (86-20) 8902-3100. Fax: (86-20) 8445-1672.

† Chinese Academy of Sciences.

‡ Jiangxi Science & Technology Normal University.

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the isolation and identification of three novel mexicanolides, named xyloccensin L, M, and N,[7] from the stem bark of *X. granatum*. In the current paper, we presented the isolation and characterization of two highly oxidized octacyclic B- and D-*seco*-limonoids—xyloccensins O (**1**) and P (**2**) (Figure 1). The compounds belong to a completely new type of phragmalins, named 8,9,30-phragmalin ortho esters.

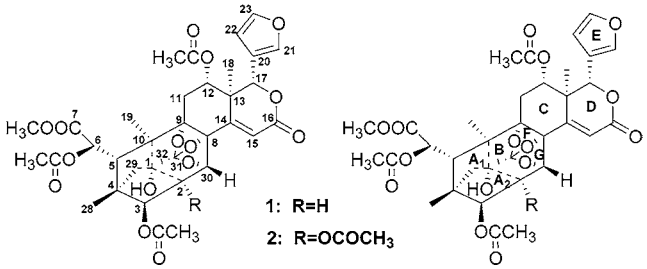

Figure 1. Structures of compounds **1** and **2**.

The dried stem bark (2.3 kg)[8] of *X. granatum* was extracted with hot 95% ethanol. 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 (80 g) was chromatographed on a silica gel column. Further purification with preparative HPLC (YMC-Pack ODS-5-A, 250 × 20 mm i.d., acetonitrile−water 35:65) yielded xyloccensins O (**1**, 160 mg) and P (**2**, 100 mg).

Xyloccensin O (**1**)[9] was isolated as colorless crystals. The molecular formula was determined to be $C_{35}H_{40}O_{15}$ (unsaturation value of 16) from the HRESIMS spectrum (*m*/*z* 701.2447, calcd for $[M + H]^+$ 701.2445). The UV maximum at 214 nm and the IR (KBr) absorption bands at 3600−3200, 2985, 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 eight units of the 16 unsaturations come from three carbon−carbon double bonds and five carbonyls. Therefore, the other eight units of unsaturations come from eight cycles. DEPT experiments revealed that compound **1** had eight tertiary methyls (including three acetyls and one methoxy), two methylenes, 11 methines (four olefinic), and 14 quaternary carbons. In addition, the NMR data showed the presence of a hydroxyl (δ_H 3.46 s), a methoxy (δ_H 3.80 s, δ_C 53.6 q), an orthoacetate (δ_H 1.68 s, δ_C 16.6 q, 120.4 s), and three acetyl groups (δ_H 1.52 s, δ_C 19.8 q, 170.2 s; δ_H 1.95 s, δ_C 21.8 q, 170.5 s; δ_H 2.21 s, δ_C 21.0 q, 169.3 s), together with a β-furyl ring [δ_H 6.64 (dd, J = 2.0, 1.0 Hz), 7.54 (br s) 7.62 (br s); δ_C 111.1 d, 122.5 s, 142.8 d, 144.3 d].

The 2D NMR studies including $^1H-^1H$ COSY, HMQC, and HMBC experiments elucidated that compound **1** was a unique phragmalin. The H-6 methine proton at δ 6.24 (br s) adjacent to an ester carbonyl was coupled with a broad singlet H-5 at δ 2.61. The presence of this fragment and the characteristic low-field H-17 singlet at δ 5.92 strongly

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR Spectral Data for Xyloccensins O (1) and P (2) in Acetone-d_6

no.	δ_C **1**	δ_H **1** (J in Hz)	δ_C **2**	δ_H **2** (J in Hz)
1	82.6 s		84.6 s	
2	45.6 d	3.09 dd; 12.0; 4.0	84.3 s	
3	78.4 d	5.33 d; 12.0	86.2 d	5.21 s
4	46.7 s		45.4 s	
5	46.3 d	2.61 br s	45.5 d	2.48 br s
6	72.1 d	6.24 br s	71.8 d	6.22 br s
7	172.8 s		172.7 s	
8	82.9 s		84.9 s	
9	87.6 s		87.8 s	
10	49.6 s		49.7 s	
11	33.0 t	2.34 dd; 14.0; 4.0 2.04[a]	33.2 t	2.35 dd; 14.0; 4.0 2.01[a]
12	69.1 d	4.99 dd; 13.5; 4.5	69.0 d	5.02 dd; 13.5; 4.5
13	43.6 s		43.7 s	
14	154.4 s		153.8 s	
15	124.5 d	6.67 s	124.8 d	6.54 s
16	163.8 s		163.6 s	
17	79.4 d	5.92 s	79.6 d	5.89 s
18	15.0 q	1.60 s	15.0 q	1.59 s
19	16.5 q	1.23 s	16.4 q	1.31 s
20	122.5 s		122.3 s	
21	142.8 d	7.54 br s	142.8 d	7.54 br s
22	111.1 d	6.64 dd; 2.0; 1.0	111.1 d	6.65 dd; 2.0; 1.0
23	144.3 d	7.62 br s	144.4 d	7.62 br s
28	16.0 q	0.93 s	15.9 q	0.91 s
29	44.5 t	1.49 dd; 10.5; 1.5 2.25 d; 10.5	41.5 t	1.81 dd; 11.0; 1.0 2.26 d; 11.0
30	75.2 d	4.97 d; 4.0	74.8 d	5.38 s
1-OH		3.46 s		3.40 s
2-OAc			21.6 q	2.09 s
			168.8 s	
3-OAc	21.8 q	1.95 s	21.8 q	1.99 s
	170.5 s		170.9 s	
6-OAc	21.0 q	2.21 s	21.0 q	2.22 s
	169.3 s		169.3 s	
12-OAc	19.8 q	1.52 s	19.8 q	1.52 s
	170.2 s		170.2 s	
7-OMe	53.6 q	3.80 s	53.7 q	3.80 s
ortho esters				
31	120.4 s		120.5 s	
32	16.6; q	1.68 s	16.6 q	1.71 s

[a] Overlapped by the solvent of acetone-d_6 without designating multiplicity.

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(8) *X. granatum* was collected from the sea coast of Sanya of Hainan Province in southern China in July 2001.

(9) **Xyloccensins O (1):** colorless needles; $[\alpha]^{25}_D$ −22 (*c* 0.8, acetone); UV (acetonitrile) λ_{max} nm 214; IR (KBr) 3600−3200, 2985, 1740−1715 cm^{-1}; ESI-MS *m*/*z* 701.4 $[M + H]^+$, 723.3 $[M + Na]^+$; HRESI-MS *m*/*z* 701.2447, (calcd for $[M + H]^+$ 701.2445).

suggested that compound **1** was a limonoid of *seco*-B,D rings. There was no signal observed in the ^{1}H NMR spectrum for the two tertiary methyls at positions 4β (C-29) and 8β (C-30) in the basic limonoid skeleton, whereas two non-equivalent 29-methylene proton signals at δ 1.49 (dd, J = 10.5, 1.5 Hz), 2.25 (d, J = 10.5 Hz) and a methine proton at δ 4.97 (d, J = 4.0 Hz, H-30) were presented instead. These data showed that compound **1** was a phragmalin.

The H-5 signal at δ 2.61 showed the long-range ^{1}H−^{13}C correlations with C-3, C-4, C-7, C-9, C-10, C-28, and C-29. A methine proton at δ 4.97 (d, J = 4.0 Hz, H-30) displayed HMBC correlations with the C-1, C-8, and C-9. The 29-meththylene protons exhibited HMBC correlations with C-1, C-3, C-4, C-10, and C-28 (Figure 2). Further, H-2 at δ 3.09

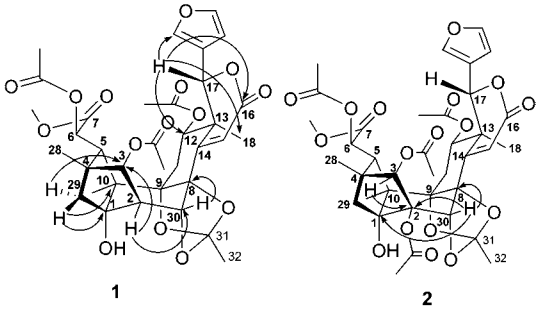


Figure 2. Selected HMBC correlations for compounds **1** and **2**.

(dd, J = 12.0, 4.0 Hz) showed strong ^{1}H−^{1}H COSY correlations with H-3 at δ 5.33 (d, J = 12.0 Hz) and H-30 at δ 4.97 (d, J = 4.0 Hz). One proton of the 29-methylene at δ 1.49 exhibited *W*-type long-range coupling (J = 1.5 Hz) with H-5 at δ 2.61. These evidences clearly characterized the left-hand tricyclo[3.3.1^{2,10}.1^{1,4}]decane ring system in compound **1**.

A singlet methyl signal at δ 1.60 (Me-18) showed HMBC correlations with C-12, C-13, C-14, and C-17, and a singlet olefinic proton at δ 6.67 (H-15) was correlated to C-8, C-13, C-14, and C-16. The H-17 singlet at δ 5.92 was coupled to C-12, C-14, C-16, and C-18 and furan carbons C-21 and C-22 (Figure 2). These correlations fully illustrated the structure of the C and D rings in compound **1**. In addition, the presence of 1-hydroxyl and 3,6,12-triacetyl groups was also confirmed by HMBC experiment.

The location of the orthoacetate group and the stereochemistry of compound **1** remained to be determined. All the phragmalin ortho esters have been reported as 1,8,9- or 8,9,14-orthoacetates. However, in compound **1**, there were a free 1-hydroxyl group and a quaternary nonoxygenated carbon C-14. The phragmalin ortho ester might be a completely new type of orthoacetate, whereas the position of orthoacetate was difficult to be determined from the NMR experiments.

After considerable effort, suitable crystals were obtained. Thus, X-ray crystallographic analysis of **1**[10] confirmed our proposed structure and the position of orthoacetate. A computer-generated perspective drawing of the final X-ray model of compound **1** was given in Figure 3. The result

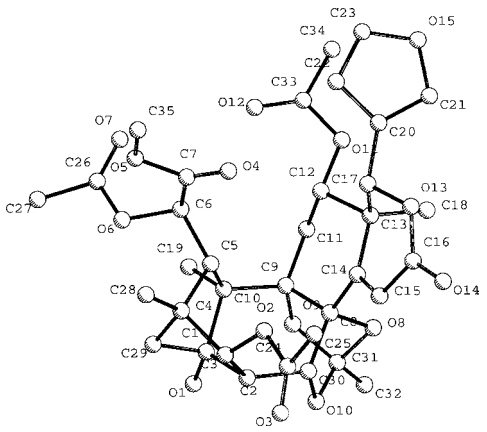


Figure 3. X-ray structure of xyloccensin O (**1**).

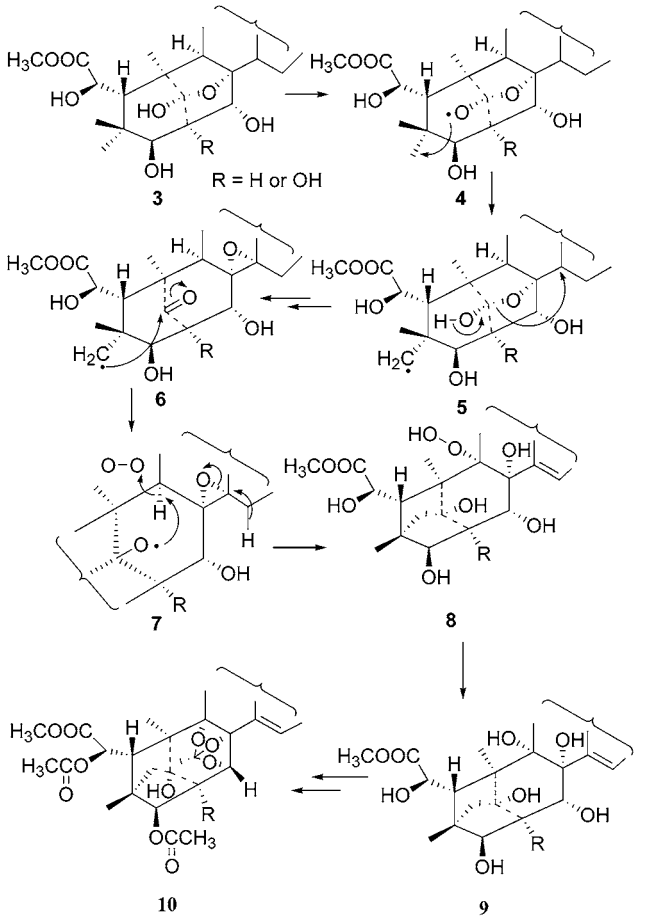
showed that the orthoacetate group was bridged at positions of 8, 9 and 30. So far, compound **1** turned out to be a new type of phragmalin, consisting of eight rings, designated as A$_1$, A$_2$, B, C, D, E, F, and G (Figure 1). The two five-carbocyclic rings (A$_1$ and A$_2$) and the two dioxolane rings (F and G) adopt envelope conformations. The two six carbocyclic rings, B and C, appeared as chairs. The unsaturated δ-lactone ring D exhibited a half-chair conformation and the furan ring E was planar. Rings A$_1$/B, A$_2$/B, B/C, B/F, B/G and C/F were all cis-fused. The whole shape of the molecule was like a basket. Because the configuration of H-17 exhibited exclusively β-orientation in all known phragmalins, the absolute stereochemistry of **1** can be derived as shown in Figure 1. In this study, the configurations of the chiral centers C-6 and C-12 have been determined to be *R* and *S*, respectively.

Xyloccensin P (**2**)[11] was obtained as white powder. HRESIMS (*m*/*z* 759.2481, calcd for [M + H]$^+$ 759.2500) showed the molecular formula of C$_{37}$H$_{42}$O$_{17}$ (unsaturation value of 17). The UV, IR and NMR spectra of compound **2** were similar to that of compound **1**. This revealed that compound **2** might have the same phragmalin nucleus as compound **1**. However, an aliphatic proton at δ 3.09 (dd, J

(10) **X-ray Crystallographic Analysis of 1.** All measurements were made on a DIP-2030K diffractometer with graphite-monochromated Mo Kα radiation λ = 0.710 69 Å at 293 K. Crystal data: monoclinic, $(C_{35}H_{40}O_{15})_2 \cdot [(CH_3OH)_{0.5}]_2 \cdot (H_2O)_{0.5}$ (M = 700.69, solvent molecules not included), space group $P2_1$ with a = 10.692(1) Å, b = 11.727(1) Å, c = 31.535(3) Å, V = 3903.0(5) Å^3, Z = 2, and D_{calcd} = 1.232 g/cm^3. The structure was solved by direct methods (SHELXS-86) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. A total of 7680 unique reflections (2θ < 50°) were collected, of which 5379 observed reflections ($|F|^2 \geq 8\sigma|F|^2$) were used. The final agreement factors of R = 0.064 and R_w = 0.064 (w = $1/\sigma|F|^2$).

(11) **Xyloccensins P (2):** an amorphous powder; [α]$^{25}_D$ −36 (*c* 0.6, acetone) UV (acetonitrile) λ_{max} nm 214; IR (KBr) 3600−3210, 2980, 1745−1710 cm^{-1}; ESI-MS *m*/*z* 781.3 [M + Na]$^+$; HRESI-MS *m*/*z* 759.2481 (calcd for [M + H]$^+$, 759.2500).

Scheme 1. Proposed Biosynthetic Pathway to 8,9,30-Phragmalin Orthoesters (**10**) from a Mexicanolide (**3**) (Double Arrows Indicate Multisteps in the Conversion)



= 12.0, 4.0 Hz, H-2 in 1) disappeared. Instead, one more acetyl signal at δ_H 2.09 (s) and δ_C 168.8 s, 21.6 q (Table 1) appeared. It indicated that an acetoxy group might replace this aliphatic proton. Confirming evidence was obtained from the $^1H-^1H$ COSY and HMBC studies. There were no $^1H-^1H$ COSY correlations observed in compound **2** between H-2/H-3, and H-2/H-30. However, a quaternary oxygenated carbon at δ 84.3 (C-2) exhibited strong HMBC correlations to H-3 at δ 5.21(s) and H-30 at δ 5.38 (s) (Figure 2). Therefore, the structure of compound **2** was identified as 2-acetoxy xyloccensin O, named xyloccensin P.

On the basis of the previous work of Taylor,[12] a possible biosynthetic pathway for 8,9,30-phragmalin ortho esters is proposed, as shown in Scheme 1. Compound **3**, a ketal derivative of the xyloccensin M,[7a] may be the biosynthetic precursor of 8,9,30-phragmalin ortho esters. It could yield oxygen radical **4**, which may oxidize the C-29 methyl leading to the formation of radical **5**. Radical methylene in **6**, transformed from radical **5** in multisteps,[12] may attack the C-1 ketone to yield oxygen radical **7**. It may oxidize C-9 methine and form a carbon radical. The resultant C-9 carbon radical can trap molecular oxygen to generate the hydroperoxide **8**, which can be reduced to C-9 alcohol **9**. The opening of 8,14-epoxy ring from **7** to **8** may be initiated by proton abstraction at C-15. The resultant polyhydroxy compound **9** could involve in the formation of 8,9,30-phragmalin ortho esters as **10**.

In summary, xyloccensins O and P, from the stem bark of the mangrove plant *X. granatum* in southern China, were isolated and identified as 8,9,30-phragmalin ortho esters. These two compounds are a unique class of highly oxidized phragmalins. This study also demonstrated that *X. granatum* is a new source for the production of limonoids with a novel carbon framework. The preliminary test showed these two compounds had the antifeedant activities.[13] Further studies are in progress.

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Supporting Information Available: One- and two-dimensional NMR spectra of **1** and **2** and X-ray data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Antifeedant activity was tested by a conventional leaf disk method against the third larvae of *Pieris brassicae*. Preliminary tests showed that xyloccensins O and P were active at a concentration of 1000 ppm.