Polyhydroxylated Phragmalins from the Fruit of a Chinese Mangrove, *Xylocarpus granatum*

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Three new polyhydroxylated phragmalins, named xyloccensins Y, Z_1, and Z_2 (**1**−**3**), were isolated from the fruit of a Chinese mangrove, *Xylocarpus granatum*, together with eight known compounds. The structures of these compounds were established on the basis of spectroscopic data and chemical methods.

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 are classified by the type of rings in the intact triterpene nucleus, and these are usually oxidized and designated as A, B, C, and D. Phragmalins, such as pseudrelones A_1 and A_2[1] isolated from *Pseudocedrela kotschyii* and khayanolides A−C[2] from *Khaya senegalensis*, 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.

The mangrove *Xylocarpus granatum* is distinguished for producing 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.[3−7] Recently, we have reported the isolation and identification of eight unique 8,9,30-phragmalin *ortho* esters and eight new mexicanolides from the bark and fruit of a Chinese mangrove, *X. granatum*.[8−14] Five new phragmalins,[15] among which four of the structures were the same as we reported,[11] were obtained from the bark of the same plant. In the current paper, we present the isolation and characterization of three new polyhydroxylated phragmalins, named xyloccensins Y, Z_1, and Z_2 (**1**−**3**), from the fruit of *X. granatum*. Additionally we isolated eight known compounds, spicatin,[16] aurantiamide,[17] daucosterol,[18] (+)-catechin,[19] α-tocopherol, abscisic acid, 4-hydroxybenzoic acid, and ethyl 3,4-dihydroxybenzoate.[20] The structures of these compounds were established on the basis of spectroscopic data and chemical methods.

Xyloccensin Y (**1**), a white amorphous powder, had a molecular formula of $C_{33}H_{42}O_{15}$, established by HRFABMS (*m*/*z* 701.2419, calcd for $[M + Na]^+$ 701.2416). Consequently, **1** had 13 degrees of unsaturation. From the ^1H and ^{13}C NMR data (Table 1), it was clear that seven of the 13 elements of unsaturation came from two carbon−carbon double bonds and five esters. Therefore, the molecule was hexacyclic. DEPT experiments revealed that **1** had seven tertiary methyls (a methoxy, three acetyls, and three methyls of the phragmalin nucleus), three methylenes, 11 methines (three olefinic), and 12 quaternary carbons (including five carbonyls). The presence of a methoxycarbonyl (δ_H 3.81 s, δ_C 53.5 CH_3, 172.6 qC), three acetyl groups (δ_H 2.12 s, δ_C 21.6 CH_3, 171.2 qC; 2.02 s, δ_C 21.7 CH_3, 172.2 qC; δ_H 2.19 s, δ_C 21.0 CH_3, 171.4 qC), and a *β*-furyl ring [δ_H 6.48 (br s), 7.59 (2H, br s); δ_C 110.8 CH, 123.1 qC, 142.0 CH, 145.0 CH] (Table 1) were recognized. All protons directly bonded to carbon atoms were assigned by analysis of HSQC data. A *δ*-lactone ring D, characterized by the following NMR data [δ_H 5.85 (s), 2.69 (d, 8.5 Hz), 2.78 (dd, 19.4, 8.5 Hz), 3.43 (d, 19.4 Hz), δ_C 79.3 CH, 42.0 qC, 43.7 CH, 28.8 CH_2, 173.1 qC], was confirmed by $^1H-^1H$ COSY correlations from H-14 to H_2-15 and HMBC correlations between H-14/C-13, H-14/C-15, H-14/C-16, H-14/C-17, H_2-15/C-16, H-17/C-13, H-17/C-14, and H-17/C-16 (Figure 1). The above NMR data and the 2D NMR studies including $^1H-^1H$ COSY, HSQC, and HMBC experiments strongly suggested that **1** was a phragmalin. Moreover, the chemical shifts of C-1 (δ_C 82.6 qC) and C-8, C-9, and C-12 (δ_C 78.2 qC, 80.0 qC, 71.3 CH) of ring C were almost the same as those (δ_C 84.7 qC, 78.4 qC, 77.6 qC, 71.3 CH) of tabulalide E[21] isolated from *Chukrasia tabularis*, and suggested that four hydroxyls were substituted at C-1, C-8, C-9, and C-12 like those in tabulalide E. An oxygenated methine (δ_H 5.22, d, 11.4 Hz; δ_C 78.3), showing HMBC correlations to C-1, C-2, C-4, and C-30 (Figure 1), was assigned to CH-3. Another oxygenated methine (δ_H 5.52, d, 2.5 Hz; δ_C 71.7), exhibiting HMBC correlations to C-1, C-2, C-3, and C-8 (Figure 1), was attributed to CH-30. An aliphatic methine (δ_H 2.20; δ_C 53.1), having $^1H-^1H$ COSY correlations to H-3 and H-30, respectively, was assigned to CH-2. The other oxygenated proton (δ_H 5.48 br s) coupled with H-5 (δ 3.32 br s) and showed an HMBC correlation to the carbon of the methoxycarbonyl group (Figure 1), and was thus unambiguously attributed to H-6. Furthermore, a pair of geminal protons (δ_H 1.45, d, 10.5 Hz; 2.26, d, 10.5 Hz), having HMBC correlations to C-1 and C-4 (Figure 1), was assigned as H_2-29. The presence of strong HMBC correlations between H-3 (δ 5.22, d, 11.4 Hz) and the acetyl carbon at δ 171.2, H-6 (5.48, br s), the acetyl carbon at δ 172.2, H-30 (5.52, d, 2.5 Hz), and the acetyl carbon at δ 171.4 (Figure 1) disclosed the location of the three acetyl groups at C-3, C-6, and C-30, respectively.

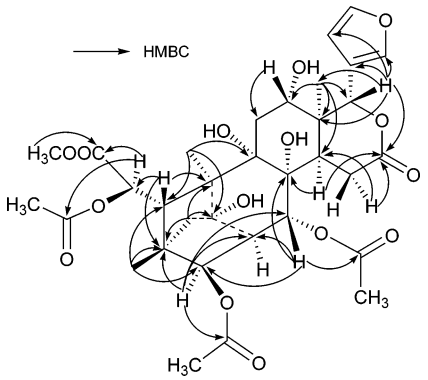

Figure 1. Selected HMBC correlations for compound **1**.

The relative stereochemistry of **1** was established on the basis of NOESY data. The configuration of H-14 was assigned to be α from its NOE correlation with 18-Me (Figure 2). The significant NOE interaction observed in **1** (Figure 2) from H-3 to H_{pro-R}-29, but not from H-3 to H-5, helped to establish this 3α-H and the corresponding 3*β*-OAc. Similarly, NOE interactions between H-30/

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

	1		**2**		**3**	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		82.6 qC		82.1 qC		82.4 qC
2	2.20[a]	53.1 CH	2.16[a]	53.7 CH	2.16 dd; 11.5; 2.0	53.9 CH
3	5.22 d; 11.4	78.3 CH	5.16 d; 11.5	78.7 CH	5.17 d; 11.5	78.9 CH
4		46.8 qC		47.0 qC		47.0 qC
5	3.32 br s	46.4 CH	2.97 br s	46.0 CH	3.11 br s	46.2 CH
6	5.48 br s	73.6 CH	4.34 br s	72.1 CH	4.42 br s	72.3 CH
7		172.6 qC		177.6 qC		177.8 qC
8		78.2 qC		75.5 qC		76.0 qC
9		80.0 qC	2.16[a]	50.6 CH	1.80 dd; 13.5; 4.0	58.0 CH
10		55.2 qC		47.1 qC		47.8 qC
11α	2.40 dd; 15.0; 3.0	36.3 CH_2	2.14[a]	30.0 CH_2	1.93 br d; 14.0	25.4 CH_2
11β	2.22[a]		1.91 br d; 13.5		1.71 dd; 13.5; 3.0	
12α	3.78 br s	71.3 CH	5.01 br s	72.9 CH	1.89 br d; 14.0	36.5 CH_2
12β					1.46 dd; 9.0, 3.0	
13		42.0 qC		37.1 qC		37.1 qC
14	2.69 d; 8.5	43.7 CH	2.39 d; 8.5	47.8 CH	2.04 d; 8.6	52.6 CH
15α	3.43 d; 19.4	28.8 CH_2	3.41 d; 19.0	29.3 CH_2	3.44 d; 19.0	29.3 CH_2
15β	2.78 dd; 19.4; 8.5		2.74 dd; 19.0; 8.5		2.81 dd; 19.0; 8.6	
16		173.1 qC		172.3 qC		173.3 qC
17	5.85 s	79.3 CH	5.82 s	78.6 CH	5.86 s	79.5 CH
18	1.09 s	20.1 CH_3	0.98 s	19.4 CH_3	0.98 s	24.3 CH_3
19	1.15 s	15.9 CH_3	1.37 s	24.2 CH_3	1.42 s	24.6 CH_3
20		123.1 qC		122.5 qC		123.2 qC
21	7.59 br s	142.0 CH	7.63 br s	142.0 CH	7.57 br s	141.7 CH
22	6.48 br s	110.8 CH	6.49 br s	110.5 CH	6.48 br s	110.7 CH
23	7.59 br s	145.0 CH	7.60 br s	145.2 CH	7.57 br s	144.7 CH
28	0.98 s	16.5 CH_3	0.94 s	16.5 CH_3	0.95 s	16.6 CH_3
29	2.26 d; 10.5	45.7 CH_2	2.41 d; 10.0	45.4 CH_2	2.44 d; 10.0	45.5 CH_2
	1.45 d, 10.5		1.34 d, 10.0		1.33 d, 10.0	
30	5.52 d; 2.5	71.7 CH	5.46 d; 2.0	71.7 CH	5.47 d; 2.0	72.3 CH
7-OMe	3.81 s	53.5 CH_3	3.77 s	53.0 CH_3	3.79 s	52.9 CH_3
3-Acetyl	2.12 s	21.6 CH_3	2.11 s	21.6 CH_3	2.13 s	21.5 CH_3
		171.2 qC		171.3 qC		171.5 qC
6-Acetyl	2.02 s	21.7 CH_3				
		172.2 qC				
12-Acetyl			2.09 s	20.9 CH_3		
				171.6 qC		
30-Acetyl	2.19 s	21.0 CH_3	2.01 s	21.7 CH3	2.02 s	21.6 CH_3
		171.4 qC		172.3 qC		172.4 qC

[a] Overlapped signals assigned by ^{1}H−^{1}H COSY, HSQC, and HMBC spectra without designating multiplicity.

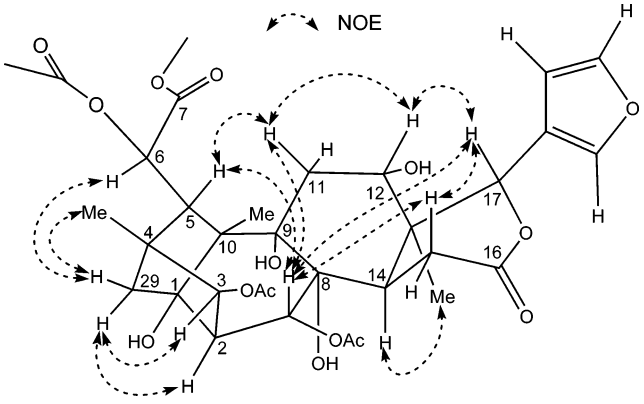
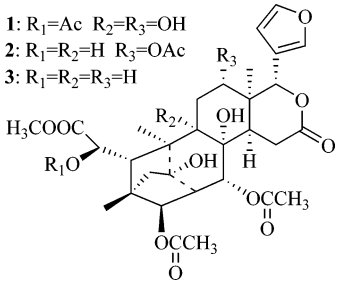


Figure 2. Diagnostic NOE correlations for compound **1**.

H-5, H-30/H-17, H-30/H-11β, H-30/H-15β, H-12/H-11β, H-12/H-17, H-5/H-11β, and H-17/H-15β (Figure 2) indicated the *cis* junctures between rings B/C and C/D and the α-configuration of the 12-hydroxyl as in tabulalide E.[21] The configuration of H-6 was also determined to be α from its NOE correlation with 29-H$_{pro-s}$ (Figure 2). This result was in agreement with two broad singlet peaks for H-5 and H-6 in the ^{1}H NMR spectrum of **1**, and these were the same as those in phragmalin xyloccensin O.[10] On the basis of the above results, the structure of **1**, named xyloccensin Y, was elucidated as shown in Chart 1.

Xyloccensin Z_1 (**2**) was isolated as a white amorphous powder. Its molecular formula was established as $C_{33}H_{42}O_{14}$ by HRFABMS

Chart 1. Structures of Compounds **1**−**3**



(*m*/*z* 685.2462, calcd for [M + Na]$^+$ 685.2466). The NMR data of **2** were almost the same as those of **1**, except for the absence of a hydroxyl group. A methine (δ_H 2.16; δ_C 50.6), having ^{1}H−^{1}H COSY correlations to H_2-11 and HMBC correlations to C-10, C-8, and C-11, was assigned to CH-9. Its configuration was determined as α from an NOE correlation with H-14 (SI). Moreover, the location of the acetyl group (δ_H 2.09 s, δ_C 20.9 CH_3, 171.6 qC) was assigned to be not at C-6 but at C-12, by the upfield shift of H-6 (δ 4.34 in **2** and 5.48 in **1**) and HMBC correlations from H-12 (δ 5.01 br s) to the carbonyl carbon of this acetyl group. Its α-configuration was established by an NOE correlation from H-12 to H-17 and H-11β (SI). Therefore, compound **2** was identified as 6-deacetyl-9-deoxy-12-acetylxyloccensin Y and given the trivial name xyloccensin Z_1.

Xyloccensin Z_2 (**3**), a white amorphous powder, had the molecular formula $C_{31}H_{40}O_{12}$, established by HRFABMS (*m*/*z*

627.2410, calcd for $[M + Na]^+$ 627.2412). The NMR data of **3** were highly similar to those of **2**, except for the absence of the 12-acetoxy group. $^1H-^1H$ COSY correlations from H_2-11 to H_2-12 confirmed this result. The relative configuration of **3** was established to be the same as that of **2** on the basis of the NOE correlations (SI). Consequently, the structure of **3** was identified as 12-deacetoxyxyloccensin Z_1 and given the name xyloccensin Z_2.

Phragmalins with an 8,9-dihydroxy group are scarce in the meliaceae plants. From a biosynthetic perspective, it is the precursor of the phragmalin *ortho* ester[10] (including 1,8,9-, 8,9,14-, and 8,9,30-subtypes) that is the most complicated type of limonoid found in nature. To date, only four such compounds, named tabulalin and tabulalides A, B, and E,[21] have been reported from the root bark of *Chukrasia tabularis*, and the configuration of 8-OH and 9-OH in these compounds was always found to be α. To our knowledge, xyloccensin Y, the first 8,9-dihydroxyphragmalin discovered from the plant genus *Xylocarpus*, is the fifth one to be found in nature.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Polaptronic HNQW5 automatic high-resolution polarimeter (Schmidt & Haensch Co. Ltd.). UV spectra were obtained on a Beckman DU-640 UV spectrophotometer, and IR spectra were recorded on a Perkin-Elmer FT-IR 1760X spectrophotometer. Fast atom bombardment (FAB)-MS spectra were measured on a Bruker APEX II spectrometer in positive or negative ion mode. NMR spectra were recorded in methanol-d_4 using a Bruker AV-500 spectrometer (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) with tetramethylsilane as the internal standard. 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.) was used.

Plant Material. The fresh fruit of the mangrove *Xylocarpus granatum* was collected in June 2005 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 maintained in the Herbarium of the South China Sea Institute of Oceanology.

Extraction and Isolation. The dried fruits (8 kg) of *X. granatum* were extracted with hot 95% ethanol three times. The extract was concentrated under reduced pressure, followed by suspension in water. After defatting with *n*-hexane, the aqueous layer was further extracted with ethyl acetate. The ethyl acetate extract (220 g) was chromatographed on Si gel CC and eluted using a chloroform−methanol system (100:0−2:1) to yield 120 fractions. Fractions 4−10 were combined and further purified with Si gel CC (petroleum ether−ethyl acetate, 30:1) to afford a mixture of butyrospermol and β-sitosterol fatty acid esters (1.5 g). Fractions 26−38 (5.2 g) were combined and further purified with preparative HPLC (YMC-Pack ODS-5-A, 250 × 20 mm i.d., methanol−water, 20:80 to 50:50) to yield xyloccensins Y (**1**, 10 mg), Z_1 (**2**, 12 mg), and Z_2 (**3**, 8 mg), spicatin (20 mg), daucosterol (50 mg), aurantiamide (6 mg), (+)-catechin (100 mg), α-tocopherol (5 mg), abscisic acid (7 mg), 4-hydroxybenzoic acid (5 mg), and ethyl 3,4-dihydroxybenzoate (6 mg).

Xyloccensin Y (1): white, amorphous powder; $[\alpha]^{25}_D$ +65 (*c* 1.6, methanol); UV (MeCN) λ_{max} 212 nm; IR (KBr) ν_{max} 3410, 2985, 1730, 1710, 1620, 1430, 1384, and 1145 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRFABMS *m*/*z* 701.2419 [calcd for $C_{33}H_{42}O_{15}Na$ $[M + Na]^+$, 701.2416].

Xyloccensin Z_1 (2): white, amorphous powder; $[\alpha]^{25}_D$ +55 (*c* 1.2, methanol); UV (MeCN) λ_{max} 212 nm; IR (KBr) ν_{max} 3408, 2985, 1730, 1710, 1620, 1432, 1386, and 1140 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRFABMS *m*/*z* 685.2462 [calcd for $C_{33}H_{42}O_{14}Na$ $[M + Na]^+$, 685.2466].

Xyloccensin Z_2 (3): white, amorphous powder; $[\alpha]^{25}_D$ +48 (*c* 1.8, methanol); UV (MeCN) λ_{max} 212 nm; IR (KBr) ν_{max} 3406, 2985, 1730, 1710, 1624, 1433, 1388, and 1138 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); HRFABMS *m*/*z* 627.2410 [calcd for $C_{31}H_{40}O_{12}Na$ $[M + Na]^+$, 627.2412].

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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