

Xylogranatin E, a New Phragmalin with a Rare Oxygen Bridge between C₁ and C₂₉, from the Fruit of a Chinese Mangrove *Xylocarpus granatum*

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Xylogranatin E, the second phragmalin with a rare oxygen bridge between C₁ and C₂₉ found in nature, was isolated from the fruit of a Chinese mangrove, *Xylocarpus granatum*. Its structure was determined by spectroscopic analysis. Complete assignment of ¹H and ¹³C NMR data of xylogranatin E was achieved by 2D NMR techniques, including ¹H–¹H COSY, HSQC, HMBC and NOESY spectra.

Key words: Phragmalin, *Xylocarpus granatum*, Complete Assignment of ¹H and ¹³C NMR Data

Introduction

Phragmalin with an oxygen bridge between C₁ and C₂₉ is a rare type of highly oxidized limonoids found in Meliaceae plants. To date, only one compound, named xylococcin L [1], has been isolated from the stem bark of *Xylocarpus granatum* by our group. Searching for potential drug leads from Chinese tropical mangrove plants, we have recently reported the isolation and identification of a mixture of butyrospermol fatty acid esters, 3 polyhydroxylated phragmalins, 8 unique 8,9,30-phragmalin *ortho* esters and 12 new mexicanolides from the stem bark and fruit of a Chinese mangrove *X. granatum* [2–10]. The structures of five phragmalins [11], among which four were the same as we reported [4], were obtained from the bark of the same plant. In this paper, we present the isolation and characterization of a new phragmalin with an oxygen bridge between C₁ and C₂₉. By the aid of 2D NMR techniques, exact and unambiguous ¹H and ¹³C NMR assignments were achieved.

Results and Discussion

The ethanolic extract of the fruit 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 and

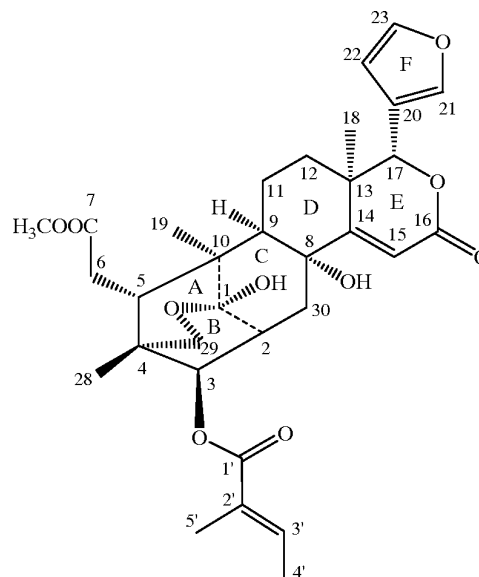


Fig. 1. Structure of xylogranatin E.

Sephadex LH-20 gel, followed by preparative reverse-phase C₁₈ HPLC to yield xylogranatin E (Fig. 1).

Xylogranatin E was isolated as an amorphous powder. Its molecular formula was determined as C₃₂H₄₀O₁₀ (unsaturation values of 13) from the HRESI-MS spectrum (*m/z* = 607.2520, calcd. for [M + Na]⁺ 607.2519). The UV maximum at 217 nm and the IR (KBr) absorption bands at 3600–3200,

No.	δH ; mult.; $J(\text{Hz})$	δC ; mult.	HMBC	$^1\text{H}-^1\text{H}$ COSY
1		98.0; s		
2	2.86, dt, 10.0, 4.0	41.8; d	1, 3, 4, 8, 30	3, 30 α , 30 β
3	4.90, d, 10.0	77.2; d	1', 2, 4, 5, 29, 30	2
4		37.4; s		
5	2.73, d, 10.0	34.5; d	1, 3, 4, 6, 7, 10, 19	6a, 6b
6a	2.39, br s		4, 5, 7, 10	5
6b	2.44, d, 10.0	32.2; t	4, 5, 7, 10	5
7		173.8; s		
8		72.2; s		
9	2.09, dd, 9.0, 3.5	53.0; d	5, 8, 10, 11	11 α , 11 β
10		42.8; s		
11 α	1.43, dd, 13.5, 3.0		9, 12, 13	9, 11 β , 12 α , 12 β
11 β	1.83, br d, 14.0	18.7; t	8, 9, 13	9, 12 α , 11 α , 12 β
12 α	1.26, dd, 9.0, 3.0		9, 11, 17, 18	11 α , 11 β , 12 β
12 β	1.83, brd, 14.0	31.7; t	9, 11, 13, 17	11 α , 11 β , 12 α
13		38.6; s		
14		167.0; s		
15	6.07, s	116.3; d	8, 13, 14, 16	
16		165.4; s		
17	5.42, s	79.8; d	12, 13, 14, 16, 18, 20, 21, 22	
18	1.33, s	22.1; q	12, 13, 14, 17	
19	1.12, s	17.1; q	1, 5, 9, 10	
20		120.1; s		
21	7.43, br s	141.8; d	20, 22, 23	22, 23
22	6.49, br s	110.6; d	20, 21, 23	21, 23
23	7.49, br s	142.9; d	20, 21, 22	21, 22
28	0.62, s	15.3; q	3, 4, 5, 29	
29 _{pro-R}	3.45, d, 9.5		1, 3, 4, 5, 28	29 β
29 _{pro-S}	3.95, d, 9.5	68.3; t	3, 4, 5	29 α
30 α	2.23, dd, 15.0, 9.0		1, 2, 3, 8	2, 30 β
30 β	2.48, dd, 15.0, 4.0	31.6; t	1, 2, 8, 14	2, 30 α
7-OMe	3.71, s	52.1; q	7	
tigloyl				
1'		167.7; s		
2'		128.5; s		
3'	6.93, q, 7.0	139.5; d	1', 2', 4', 5'	4'-Me
4'	1.85, d, 7.0	14.7; q	2', 3'	3'
5'	1.89, s	12.3; q	1', 2', 3'	

Table 1. ^1H and ^{13}C NMR data, HMBC and $^1\text{H}-^1\text{H}$ COSY correlations for xylogranatin E (500 MHz for ^1H and 125 MHz for ^{13}C , CDCl_3).

2985 and 1740–1710 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 seven of the thirteen units of unsaturation refer to four carbon-carbon double bonds and three carbonyl groups. Therefore, the other six units of unsaturation result from the presence of six rings.

DEPT experiments revealed that xylogranatin E possesses six methyls (including a methoxy, a doublet and four tertiary methyls), five methylenes, ten methines (five olefinic) and eleven quaternary carbons (including three carbonyls). The presence of a methoxy-carbonyl ($\delta_{\text{H}} = 3.71$ s, $\delta_{\text{C}} = 52.1$ q, 173.8 s), a tiglate moiety [$\delta_{\text{H}} = 6.93$ (q, 7.0 Hz), 1.85 (d, 7.0 Hz) and 1.89 s; $\delta_{\text{C}} = 167.7$ s, 128.5 s, 139.5 d, 14.7 q and 12.3 q], and a β -furyl ring [$\delta_{\text{H}} = 6.49$ br s, 7.43 br s, 7.49 br s; $\delta_{\text{C}} = 110.6$ d, 120.1 s, 141.8 d, 142.9 d] (Ta-

ble 1) were recognized. All protons directly bonded to carbon atoms were assigned by analysis of HSQC data. An α,β -unsaturated δ -lactone ring E, characterized by the following NMR data [$\delta_{\text{H}} = 5.42$ s, 6.07 s; $\delta_{\text{C}} = 79.8$ d, 38.6 s, 167.0 s, 116.3 d, 165.4 s], was confirmed by HMBC correlations between H-15 / C-13, H-15 / C-14, H-15 / C-16, H-17 / C-13, H-17 / C-14, H-17 / C-16 (Table 1). The above NMR data and the 2D NMR studies including $^1\text{H}-^1\text{H}$ COSY, HSQC and HMBC experiments strongly suggested that xylogranatin E was a phragmalin. Moreover, the chemical shifts of C-1, C-2, C-3, C-4 and C-29 ($\delta_{\text{C}} = 98.0$ s, 41.8 d, 77.2 d, 37.4 s, 68.3 t) of ring B were almost the same as those ($\delta_{\text{C}} = 97.8$ s, 43.1 d, 77.1 d, 37.8 s, 67.8 t) of xyloccensin L [1] isolated from the stem bark of the same plant. It suggested that xylogranatin E has a rare oxygen bridge between C₁ and

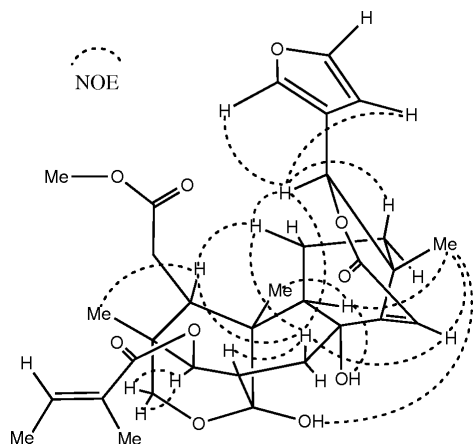


Fig. 2. Significant NOE correlations in xylogranatin E.

C₂₉ just like xylococcin L. The strong HMBC correlation from 29-H_{pro-R} (3.45, d, 9.5 Hz) to C-1 (98.0 s) (Table 1) confirmed the connection by this oxygen bridge. Furthermore, the chemical shifts of C-8 and C-9 (δ_C = 72.2 s, 53.0 d) of ring D were quite similar to those (δ_C = 75.5 s, 50.6 d) of xylococcin Z₁ [8] isolated from the fruit of the same plant, disclosing that a hydroxyl group was substituted at C-8. The strong HMBC correlation from H-3 (δ_H = 4.90, d, 10.0 Hz) to the carbonyl carbon atom (δ_C = 167.7 s) (Table 1) of the tigloyl moiety revealed that it was attached to C-3.

The relative stereochemistry of xylogranatin E was established on the basis of NOESY data. The significant NOE interaction (Fig. 2) from H-3 to H₂-29, but not from H-3 to H-5, helped to establish 3 α -H and the corresponding 3 β -tiglate. Similarly, NOE interactions between H-30 β / H-5, H-30 β / H-17, H-30 β / H-11 β , H-30 β / H-15, H-5 / H-11 β , H-9 / H₃-18, H-9 / H₃-19 (Fig. 2), indicated a *cis* orientation between these respective pairs of protons. The orientation of 8-OH remained to be determined. Fortunately, its active proton appeared as a high and narrow peak at δ = 5.76 in the ¹H NMR spectrum detected in acetone-d₆. The strong NOE correlations between 8-OH / H₃-18 and 8-OH / H-9 disclosed its α position (Fig. 2). Based on the above results, the structure of xylogranatin E, was elucidated as shown in Fig. 1. To our knowledge, it is the second phragmalin with an oxygen bridge between C₁ and C₂₉ found in nature.

Experimental Section

General experimental procedures

UV spectra were obtained on a Beckman DU-640 UV spectrophotometer and IR spectra recorded on a Perkin-

Elmer FT-IR 1760X spectrophotometer. Electrospray ionization (ESI)-mass 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 ¹³C, respectively equipped with an inverse-detection 5 mm probe (TBI probe, ¹H 90° pulse width = 6.1 μ s, ¹³C 90° pulse width = 12.3 μ s) and operating at r. t. with tetramethylsilane as internal standard. About 10 mg samples were dissolved in CDCl₃ (0.5 mL) to record the NMR spectra.

Plant material

The fresh fruit of the mangrove *Xylocarpus granatum* was 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. GKLM-002-3) is maintained in the Herbarium of the South China Sea Institute of Oceanology.

Extraction and isolation

The dried fruit (5 kg) of *X. granatum* was extracted with hot 95% ethanol three times. 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 ethyl acetate extract (120 g) was chromatographed on S gel CC and eluted using a chloroform-methanol system (100:0 ~ 2:1) to yield 50 fractions. Fractions 13 to 16 (2.8 g) were combined and subjected to CC on silica gel, octadecylsilyl silica gel and Sephadex LH-20 gel, followed by preparative reverse-phase C₁₈ HPLC using acetonitrile-water system (35:65) to yield xylogranatin E (12 mg).

Xylogranatin E: An amorphous powder, $[\alpha]_D^{25}$ = -42° (*c* = 0.6, acetonitrile). – UV (MeCN): λ_{max} = 217 nm. – IR (KBr): ν = 3600–3200, 2980 and 1740–1710, 1635, 875 cm⁻¹. – ¹H NMR and ¹³C NMR (CDCl₃): see Table 1. – HR-ESI-MS: *m/z* = 607.2520 [M + Na]⁺ (C₃₂H₄₀O₁₀Na requires 607.2519).

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