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Xylocarpanoids A and B, unique C_{28} skeleton limonoids from *Xylocarpus granatum*

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ABSTRACT

One novel tetranortriterpenoid derivative, xylocarponoid A, representing the first example of C28 skeleton limonoid, was isolated from the seeds of the Chinese mangrove, *Xylocarpus granatum*. Its C-1′-epimer, xylocarponoid B, was formed in $CDCl_3$. Their structures were elucidated by extensive spectroscopic analysis. A plausible biosynthetic pathway of xylocarponoid A was proposed.

1. Introduction

Limonoids, which have been found to date only in plants of the order Sapindales, are tetranortriterpenoids from a precursor with a 4,4,8-trimethyl-17-furylsteroid skeleton. They show a broad range of biological activities, such as antifeedant, antibacterial, antifungal, antiviral, antimalarial, and anticancer.[1] Previous studies on the genus of *Xylocarpus* have afforded a series of protolimonoids and limonoids.[2] In continuation of our efforts to identify biologically active components from medicinal plants, we made a thorough investigation on the constituents of seeds of the Chinese mangrove, *X. granatum*, resulting in the isolation of one novel limonoid with an unprecedented C_{28} skeleton, which afforded an epimeric mixture in $CDCl_3$. We describe here their isolation, structure elucidation, and plausible biosynthetic pathway.

Xylocarponoid A (**1**) was obtained as a colorless crystal. The molecular formula of **1**, $C_{29}H_{36}O_{10}$ (unsaturation index of 12), was inferred from its HR-FAB-MS analysis. The ^{13}C NMR spectrum revealed that **1** contains six olefinic carbons and three carbonyls. Therefore, the remaining six unsaturations required **1** to be hexacyclic. The ^{1}H NMR, ^{13}C NMR, and HSQC spectra (Table 1) showed the presence of six methyls, three methylenes, ten methines (two oxygenated and four olefinic ones), and 10 quaternary carbons (one ketone, two esters, and two olefinic carbons). In addition, two hydroxy groups [δ_H 4.40 (s), 2.65 (br s)], four tertiary methyls [δ_H 0.94 (s), 1.15 (s), 1.22 (s), and 1.80 (s); δ_C 20.7, 23.6, 28.4, and 25.4], one methoxy group (δ_H 3.69; δ_C 52.0), and a β-substituted furyl ring [δ_H 6.15 (br s), 7.26 (br s), and 7.36 (br s); δ_C 109.1, 122.5, 140.1, and 143.5] were distinguished by the ^{1}H and ^{13}C NMR data. The aforementioned spectroscopic data implied a limonoid feature of **1**.

Three structural fragments **1a**, **1b**, and **1c** (Fig. 2) were determined by analysis of the ^{1}H–^{1}H COSY, HSQC, and HMBC data of **1**. **1a** was elucidated by analysis of the spectroscopic data starting from a δ-lactone ring D, which was characterized by the following data [δ_H 2.02 (d, J = 10.4 Hz), 2.87 (d, J = 10.6 Hz), 4.84 (s); δ_C 34.2, 37.2, 46.7, 167.1, 82.5], and was further corroborated by the HMBCs between H-14/C-12, H-15/C-13, H-15/C-14, H-15/C-16, H-17/C-13, and H-17/C-16 (Fig. 2). The HMBC cross-peaks from H-17 to C-20, C-21, and C-22 indicated that the β-furan ring is connected to C-17. A methyl singlet at δ_H 1.15 ppm (Me-18) and the methylene proton of C-12 (H_{ax}-12), correlated in the HMBC spectrum to the C-13 of ring D, along with a ^{1}H–^{1}H COSY-related proton spin system, H_2-11–H_2-12, permitted us to establish this proton spin system and place Me-18 at C-13, thus establishing the substructure of **1a**. Likewise, fragment **1b** was also determined by analysis of the ^{1}H–^{1}H COSY and HMBC data. The proton spin system from H_2-6 to H_3-19 through H-5 and H-10 was established by interpretation of the ^{1}H–^{1}H COSY spectrum. The HMBC cross-peaks from both H_2-6 and an oxymethyl at δ_H 3.69 to the same ester carbon atom at δ_C 173.2 suggested that a methoxycarbonyl group [δ_H 3.69 (s); δ_C 52.0, 173.2] was connected to C-6. A characteristic α,β-unsaturated ketone moiety was also observed in its ^{1}H and ^{13}C NMR spectra [δ_H 6.74 (s); δ_C 203.6, 132.8, 157.8]. Furthermore, both the H-3 [δ_H 6.73 (s)] and two geminal methyl singlets resonated at δ_H 0.94 and 1.22 exhibited HMBCs to the same quaternary carbon atom (C-4) and a saturated methine carbon C-5, which implied that C-4 was located between C-3 and C-5, bearing two methyl groups. These features resemble those of a coexisting

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Table 1
The ^{1}H, ^{13}C NMR, HMBC, and NOESY data for 1 and 2 in $CDCl_3$ (500 MHz for ^{1}H, 125 MHz for ^{13}C)

Position	1				2		
	δ_H (mult, *J* Hz)[a]	δ_C[b]	HMBC	NOESY	δ_H (mult, *J* Hz)	δ_C	HMBC
1	—	203.6			—	199.8	
2	—	132.8			—	130.0	
3	6.74 (s)	157.8	2, 4, 5, 6, 28, 29, 30	14^{w}, 15^{s}, 28^{s}, 29^{s}, 30^{w}, 2^{w}	6.86 (s)	160.3	2, 4, 5, 28, 30
4	—	36.5			—	36.3	
5	2.35 (o)	46.0	4, 7, 29		2.35 (o)	45.3	
6a	2.48 (dd, 15.9, 2.3)	34.7	4, 5, 7, 10	$6b^{s}$, 19^{s}, 28^{s}, 29^{s}		34.2	
6b	2.27 (dd, 16.4, 7.6)		4, 5, 7, 10	$6a^{s}$			
7	—	173.2			—	173.5	
8	—	68.9			—	74.1	
9	—	96.9			—	212.5	
10	2.39 (quint, 6.3)	44.0	1, 5, 6, 19	19^{s}, 28^{s}	2.30 (o)	42.8	
11a	2.33 (o m)	29.1	9, 12	$11e^{s}$, $12e^{m}$, 18^{s},	2.72	32.5	12, 13
11e	1.68 (br dd, 13.4, 3.6)		8, 9, 13	8-OH^{w}, $11a^{s}$, $12a^{s}$, $12e^{w}$	2.37		
12a	2.14 (td, 14.4, 4.7)	28.4	11, 13, 17	$11e^{s}$, $12e^{s}$, 17^{s}	2.70 (m)	27.5	11, 13, 17
12e	1.41 (br d, ~14.5)			$11a^{m}$, $11e^{s}$, $12a^{s}$, 17^{s}, 18^{s}	1.63 (m)		
13	—	34.2			—	35.2	
14	2.02 (d, 10.4)	37.2	8, 9, 12, 13, 15, 18, 30, 1′	3^{w}, 15^{s}, 17^{w}, 18^{s}, 21^{w}, 22^{s}, 28^{w}, 30^{w}, 8-OH^{s}	2.17 (d, 8.7)	46.7	8, 15, 30, 1′
15	2.87 (d, 10.6)	46.7	1′, 13, 14, 16	3^{s}, 14^{s}, 21^{m}, 22^{m}, 2^{s}	3.23 (d, 8.4)	44.1	1′
16	—	167.1			—	170.1	
17	4.84 (s)	82.5	8, 12, 13, 14, 16, 18, 20, 21, 22	$12a^{s}$, $12e^{s}$, 14^{w}, 18^{s}, 21^{s}, 22^{m}	6.32 (s)	78.8	12, 13, 14, 16, 18, 20, 21, 22
18	1.15 (s)	23.6	12, 13, 14, 17	$11a^{s}$, $12e^{s}$, 14^{s}, 17^{s}, 21^{s}, 22^{s}, 8-OH^{m}	0.95 (s)	24.1	17
19	1.08 (o d, 6.3)	12.0	1, 5, 10	$5/6b^{s}$, 10^{s}, OMe^{w}	1.02 (d, 6.3)	20.0	1, 5, 10
20	—	122.5			—	120.4	
21	7.26 (br s)	140.1	20, 22, 23	14^{w}, 15^{m}, 17^{s}, 18^{s}, 28^{w}	7.45 (s)	140.5	20, 22, 23
22	6.15 (br s)	109.1	20, 21	14^{s}, 15^{m}, 17^{m}, 18^{s}, 23^{s}, 28^{s}	6.44 (s)	109.8	20, 21
23	7.36 (br s)	143.5	20, 21, 22	22^{s}, 28^{s}	7.41 (s)	142.7	20, 21
28	0.94 (s)	20.7	3, 4, 5, 29	3^{s}, $6a^{s}$, 10^{s}, 14^{w}, 21^{w}, 22^{s}, 23^{w}	1.09 (s)	20.3	3, 4, 5, 29
29	1.22 (s)	28.4	3, 4, 5, 28	3^{s}, $6a^{s}$	1.19 (s)	27.7	3, 4, 5, 28
30	5.26 (s)	70.2	2, 3, 4, 8, 9, 14, 1′	3^{w}, 8-OH^{s}, 9-OH^{w}, 14^{w}	5.60 (s)	64.0	1, 2, 3, 8, 14, 1′
1′	—	98.3			—	95.7	
2′	1.80 (s)	25.4	1′, 15, 30	3^{w}, 15^{s}	1.76 (s)	29.5	15, 30, 1′
8-OH	4.40 (s)	—	8, 9, 14, 30	$11a^{w}$, 14^{s}, 18^{m}, 30^{s}	3.89 (s)	—	8, 9, 14
9-OH	2.65 (br s)	—	8, 9	30^{w}			
1′-OH					2.62 (s)	—	15, 1′, 2′
OMe	3.69 (s)	52.0	7	19^{w}	3.68 (s)	52.0	7

[a] Multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet; o, overlapped; and br, broad.
[b] The numbers with underline represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm).

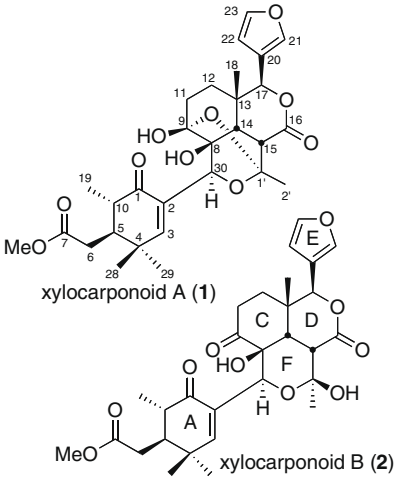


Figure 1. Structures of xylocarponoids A and B.

known limonoid xylogranatin C (**3**).[3] The remaining fragment **1c** was assembled as a tetrasubstituted 1,3-dioxane [δ_H 5.26 (s); δ_C 68.9, 96.9, 70.2, 98.3] as suggested by HMBC cross-peaks (H-30/C-8, H-30/C-9, and H-30/C-1′). Moreover, the HMBCs from two hydroxy protons to both C-8 and C-9 indicated that two hydroxy groups were located at C-8 and C-9. The HMBCs from one methyl at δ_H 1.80 (s) to C-1′ allowed a reasonable connection of Me-2′ to C-1′. The long-range HMBCs observed from H-30 to C-14, H-2′ to C-15, H-14 to C-8, H-14 to C-9, H-14 to C-30, H-14 to C-1′, 8-OH to C-14, 8-OH to C-30, H-15 to C-1′, H_2-11 to C-9, and H_{eq}-11 to C-8 not only further supported substructure **1c**, but also connected substructures **1a** and **1c** through C-9–C-11, C-8–C-14, and C-1′–C-15. The connections between **1b** and **1c** could also be elucidated by

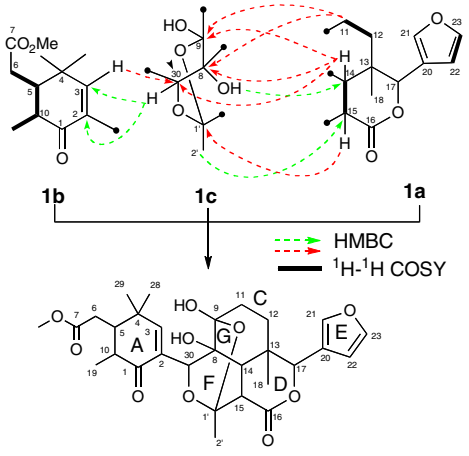


Figure 2. Partial structures, the key ^{1}H-^{1}H COSY (solid line) and HMBCs of xylocarponoid A.

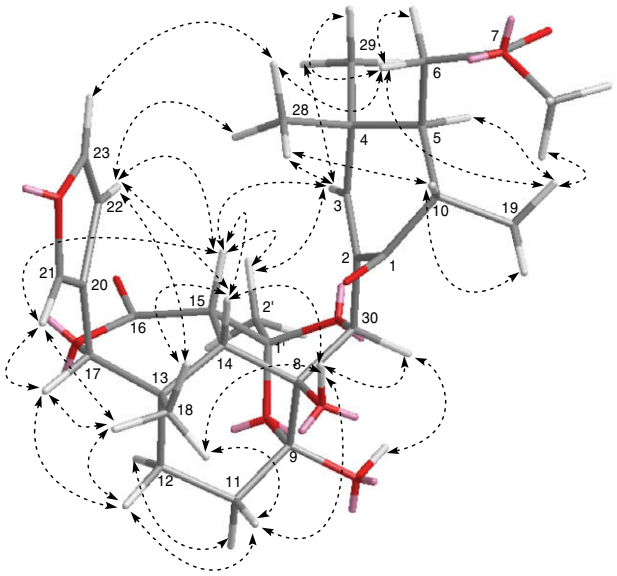


Figure 3. Calculated conformation by MM2 and significant NOESY correlations of xylocarponoid A (**1**).

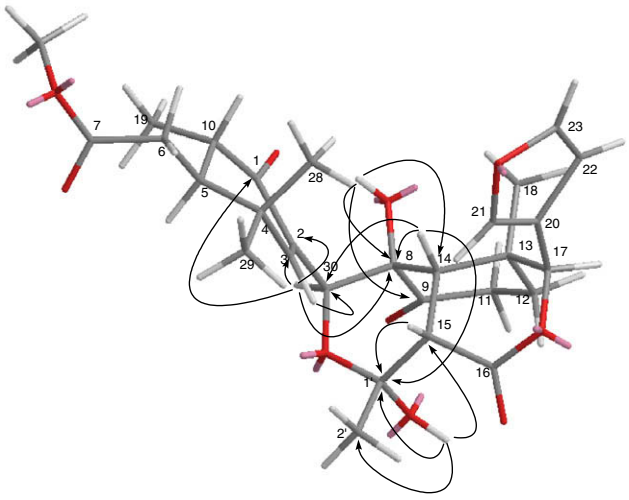
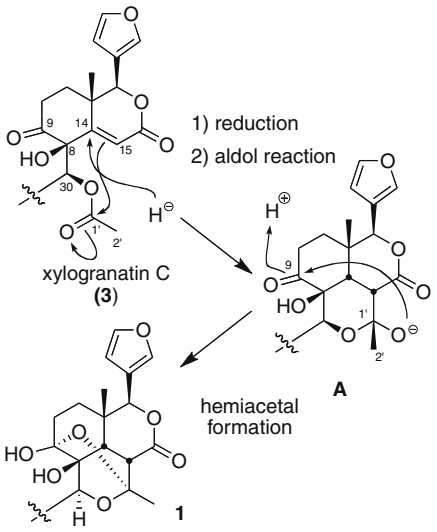


Figure 4. Key NOESY correlations of xylocarponoid B (**2**).



Scheme 1. Hypothetical biogenetic route from **3** to **1**.

HMBCs. The observed HMBC cross-peaks H-30/C-2, H-30/C-3, and H-3/C-30 connected the substructure moieties **1b** and **1c** through C-2 and C-30.

The relative configuration of **1** was defined on the basis of the NOESY spectrum as shown in a three-dimensional drawing (Fig. 3) generated by MM2 calculation.[4] The proton H-3 (ring A) had strong NOE correlation with both H-14 and H-15. This suggested that ring F adopted a boat form with H-14 and H-15 cis to ring A. These protons exhibited strong NOE with the protons of furan ring E (H-21 and H-22) indicating that ring D is also a boat form. The H-17 had NOE with both H-12$_{ax}$ and H-12$_{eq}$, but only showed stronger NOE with the equatorial one. The Me-18 also had strong NOE with H-12$_{eq}$ as well as H-14. A strong NOE between H-11$_{ax}$ and Me-18 indicates that Me-18 is axial in chair ring C and equatorial in boat ring D. Regarding ring A, Me-28 had NOE with the furan protons and with H-10, which means that Me-28 occupies a pseudo-axial position facing furan and cis to axial H-10. Therefore, Me-19 is pseudo equatorial. The fact that Me-28 had NOE with H_2-6 means that the CH_2-6 groups must be equatorial. Based on the above results, the relative stereochemistry of **1** was elucidated as shown in Figure 2, which includes a rigid cage-like structure.

It is worth noting that a ring cleavage isomer of **1**, compound **2**, formed gradually during the NMR experiments of **1** in $CDCl_3$, and they finally reached an equilibrium in a ratio of 4:1 (**1**:**2**). The ^{13}C NMR data of **2** resemble those of **1** with the exception of the signals around fragment **1c**. Especially, a carbonyl carbon (δ_c 212.5) emerged instead of C-9 hemiacetal carbon (δ_c 96.9) in **1**. In addition, a hydroxy proton (δ_H 2.62) emerged, which exhibited strong HMBCs to C-15, C-1′, and C-2′. The above data suggested that the cyclic di-hemiacetal moiety constituted by C-30–O–C-1′–O–C-9–C-8 in **1** opened to 1′-hydroxy-9-keto moiety in **2**, and C-1′ was epimerized (Fig. 1). On the basis of weak signals reasonable in COSY, HMBC, HSQC, and NOESY (Fig. 4), we can elucidate a structure for **2** as shown in Figure 1, and named it as xylocarponoid B.

To the best of our knowledge, most limonoids found in nature possess a C_{26} skeleton except for those degraded. Xylocarponoid A (**1**) is the only C_{28} limonoid found as yet. Moreover, naturally 9,10-*seco*-mexicanolide-type limonoids are very rare.[2,3,5] The biogenetic precursor of **1** might be xylogranatin C (**3**), found also in this plant. Aldol condensation between the α,β-unsaturated ketone and C-30 acetoxy group gives pentacyclic intermediate **A**. Successive intramolecular hemiacetal formation constructs the hexacyclic skeleton **1** (Scheme 1).

Compound **1** showed no in vitro cytotoxicity against the MDA-MB-231 (human breast adenocarcinoma) and SW-620 (human colon carcinoma) cell lines by MTT[6] methods.

2. Experimental section

2.1. General

MS: Thermo Finnigan MAT95XP. Optical rotations: Perkin–Elmer 243B. NMR: Bruker Avance DRX-500. Chromatography: Silica gel 200–300 mesh (Qingdao Marine Chemical Factory, China). Preparative HPLC: Waters Delta Prep 3000 pump, 2996 PDA detector.

2.2. Plant material

Seeds of *X. granatum* were collected in March 2006 from Hainan Island, Southern China, dried at ambient temperature, and identified by Dr. Wen-Qing Wang, School of Life Sciences, Xiamen University in China. A voucher specimen (No. HEBNMC-2006-1)

has been deposited in the herbarium of School of Pharmaceutical Sciences, Hebei Medical University, PR China.

2.3. Extraction and isolation

Dried seeds (3.0 kg) of *X. granatum* were extracted with 95% ethanol at room temperature. After evaporation of the solvent, the residue was suspended in water and extracted with petroleum ether and dichloromethane, successively. The dichloromethane extract (65.8 g) was chromatographed on silica gel column and eluted using petroleum ether–acetone system (95:5–2:3) to yield 10 fractions. Fraction 8 (6.3 g) was further purified by repeated silica gel column chromatography to yield **1** (2.5 mg) as a colorless crystal, mp 160–162 °C, $[\alpha]_D^{25}$ −37 (*c* 0.090, CH_3COCH_3). HR-EI-MS: *m*/*z* ($M^{+\cdot}$) 544.2304 (calcd 544.2308). HPLC-PDA analysis detected **1** eluting at 16.05 min, and the maximum wavelength of peak was at 238.5 nm [Diamonsil C18 ODS-2, 4.6 × 250 mm; MeOH–H_2O (55:45→100:0), 40 min]. **2** was formed during the NMR detection of **1** in $CDCl_3$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.11.123.

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