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Xylogranatins A–D: Novel Tetranortriterpenoids with an Unusual 9,10-*seco* Scaffold from Marine Mangrove *Xylocarpus granatum*

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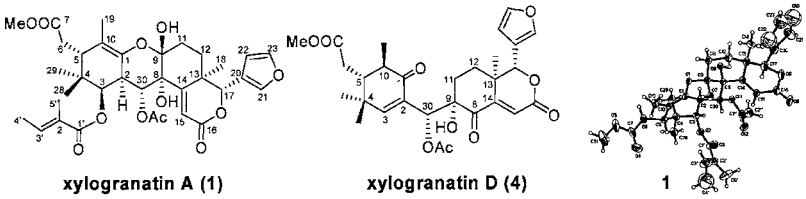
ABSTRACT



Four novel tetranortriterpenoids, xylogranatins A–D (1–4), with an unusual 9,10-*seco* skeleton were isolated from the seeds of a Chinese marine mangrove *Xylocarpus granatum*. Their structures were determined by spectroscopic and chemical means. Xylogranatin A (1) featured by a unique 1,9-oxygen bridge was confirmed by single-crystal X-ray diffraction, and xylogranatin D (4) with an unprecedented skeleton of C-30–C-9 linkage was postulated biogenetically from 3 via an α-hydroxyl ketone rearrangement and was chemically mimicked.

Diverse structures and significant biological activities of limonoids from plants of the Meliaceae family have been attracting considerable interest.[1] *Xylocarpus granatum* Koenig, a marine mangrove plant mainly distributed along the shore of the Indian Ocean and sea shores of Southeast Asia, is one of the three species in the genus *Xylocarpus* (Meliaceae). This plant has applications in the treatment of diseases, such as fever and malaria.[2i] Previous investigations on this plant have reported about 30 limonoids mainly belonging to the structural types of phragmalin and mexicanolide.[2] In a continuing search for structurally and biologically interesting metabolites from plant resources, we examined the seeds of *X. granatum* collected from Hainan island in the south of China, and four novel limonoid xylogranatins A−D (**1**−**4**), with an unusual 9,10-*seco* skeleton, were isolated. In addition, xylogranatin A (**1**) featured by a unique 1,9-oxygen bridge was confirmed by single-crystal X-ray diffraction, and xylogranatin D (**4**) with an unprecedented skeleton of C-30−

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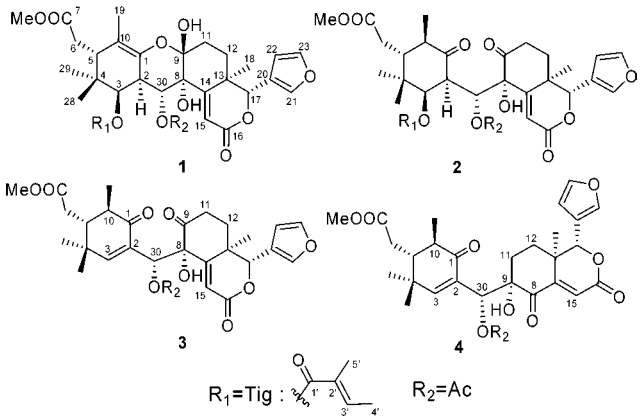
C-9 linkage was postulated biogenetically from **3** via an α-hydroxyl ketone rearrangement and was chemically mimicked. Compounds **1**−**4** showed moderate cytotoxicity against two tumor cell lines, P-388 and/or A-549.



Xylogranatin A (**1**), a colorless crystal, had a molecular formula of $C_{34}H_{42}O_{12}$ as established by ESI-MS at *m*/*z* 665.3 $[M + Na]^+$ and by HR-EIMS at *m*/*z* 642.2680 $[M]^+$ (calcd 642.2676). The IR spectrum displayed absorption bands at 3419 and 1716 cm^{-1}, indicating the presence of hydroxyl and ester functionalities. The UV absorption band at λ_{max} = 214 nm was suggestive of the presence of a furan ring.[2b] The ^{13}C NMR data and DEPT experiments showed that 9 of the 14 degrees of unsaturation came from five carbon−carbon double bonds and four carbonyls. The remaining five degrees of unsaturation were therefore indicative that compound **1** is pentacyclic. In addition, the NMR data (Table 1) and HSQC spectrum showed the presence of a methoxy (δ_H 3.69, s; δ_C 51.9), two hydroxyls (δ_H 3.29 and 2.76, each 1H, s), four methyls [δ_H 1.31 (s), 1.61 (d, J = 0.9 Hz), 0.91 (s), and 0.84 (s); δ_C 19.2, 12.8, 25.5, and 20.4], an acetyl (δ_H 2.16, s; δ_C 21.4 and 170.4), and a tigloyl [δ_H 6.83 (1H, qq, J = 6.9, 1.4 Hz), 1.79 (3H, d, J = 6.9 Hz), and 1.84 (3H, s); δ_C 137.4, 14.5, 12.2, 167.7, and 128.8], together with a β-furyl ring [δ_H 6.40 (d, J = 1.6 Hz), 7.40 (t, J = 1.6 Hz), and 7.44 (brs); δ_C 110.1, 142.9, 141.2, and 119.7]. The aforementioned data implied compound **1** possesses limonoid features.

Detailed 2D NMR studies (HSQC, $^1H-^1H$ COSY, and HMBC experiments) revealed that compound **1** was composed of two components, A and B (Figure 1a). The HMBC correlations suggested that component A (in blue) contained an intact C-ring and an α,β-unsaturated δ-lactone D-ring with a β-furyl at C-17, which was the same as that of utilins.[3] Furthermore, the two hydroxyl signals at δ_H 3.29 and 2.76 were observed in the HMBC to correlate with both C-8 and C-9, indicating that both carbons were hydroxylated. In component B (in red), two proton-bearing partial structures of C-5−C-6 and C-3−C-2−C-30 were readily recognized from the $^1H-^1H$ COSY spectrum; the two structural segments and the quaternary carbons (C-1, C-4, and C-10) were

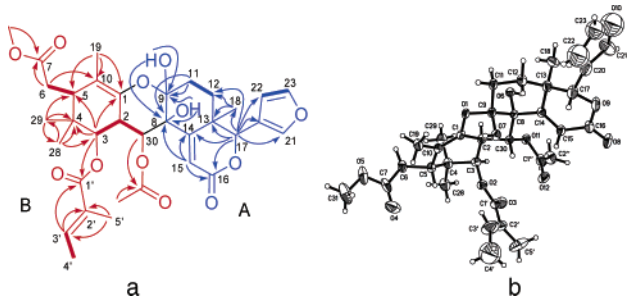


Figure 1. (a) Key $^1H-^1H$ COSY (−) and HMBC (→) correlations of **1**. (b) Single-crystal X-ray structure of **1**.

connected by the HMBC correlations of Me-28 (Me-29)/ C-3, C-4, and C-5; Me-19/C-1, C-5, and C-10; and H-3/C-1. The positions of the carbomethoxy group, the acetoxyl, and the tigloyloxyl groups were also located by HMBC correlations (Figure 1a).

Although there were no direct HMBC correlations available to link the two parts A and B, the presence of two oxygenated quaternary carbon signals at δ_C 137.6 (C-1, sp^2) and 74.6 (C-8, sp^3), a typical hemiketal at δ_C 99.2 (C-9), and the still "loose end" of a tertiary oxygenated carbon at δ_C 71.0 (C-30) only suggested that the two parts were connected via the C-8−C-30 bond and the C-1−O−C-9 ether bond to form a pyran ring. A planar structure as depicted in Figure 1 was thus proposed for **1**, which was fully consistent with its molecular composition. The single-crystal X-ray structure[4] (Figure 1b) of **1** confirmed its planar structure and allowed the determination of its relative configuration, which was also in good accordance with its relative configuration in solution as assigned by an NOESY spectrum.

Xylogranatin B (**2**) was isolated as a colorless oil and was analyzed for the molecular formula of $C_{34}H_{42}O_{12}$ by an HR-EIMS spectrum ($[M]^+$, found 642.2664, calcd 642.2676). Thirty-four carbon signals resolved in the ^{13}C NMR spectrum (with DEPT experiments) were assigned as four ester carbonyls, two ketones, three sp^2 quaternary carbons, three sp^3 quaternary carbons, five sp^2 methines, six sp^3 methines, three sp^3 methylenes, and eight methyls. Ten out of the 14 degrees of unsaturation were consumed by the above unsaturated functionalities, suggesting that compound **2** possessed four rings, one less than **1**. Compared with compound **1**, the characteristic features of a β-furyl ring, an α,β-unsaturated δ-lactone, a tigloyl, an acetyl, and a methoxy were also recognized from compound **2** by analysis of its NMR spectra (Table 1). However, the quaternary carbon signals at δ_C 137.6 (C-1), 99.2 (C-9), and 117.3 (C-10) of **1** disappeared in compound **2**, and two ketone carbons and a tertiary sp^3 carbon signal were observed at δ_C 208.4, 209.4, and 47.0, respectively. This implied that **2** might be biosynthetically related with **1** and most likely via hydrolysis of

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(4) Crystallographic data for xylogranatin A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-615078). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

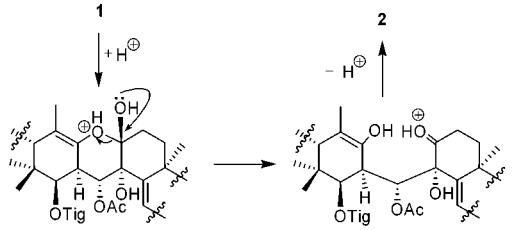
Table 1. ^{1}H and ^{13}C NMR Data of **1**−**4** (in $CDCl_3$)

	1		**2**		**3**		**4**	
no.	δ_H (mult., *J*, Hz)[a]	δ_C[b]	δ_H (mult., *J*, Hz)[a]	δ_C[b]	δ_H (mult., *J*, Hz)[a]	δ_C[b]	δ_H (mult., *J*, Hz)[a]	δ_C [b]
1		137.6		208.4		198.8		198.6
2	3.10 (m)	38.4	3.62 (dd, 8.8, 2.1)	48.2		128.4		130.1
3	4.80 (d, 3.7)	73.6	5.41 (d, 2.1)	80.6	7.00 (s)	162.0	7.00 (s)	161.5
4		37.8		39.5		36.8		36.7
5	2.88 (m)	40.2	2.21 (m)	46.5	2.28 (m)	45.2	2.28−2.34 (m)	45.2
6α	2.25 (dd, 16.7, 9.0)	32.7	2.25 (m)	34.2	2.27 (m)	34.6	2.30 (m)	34.7
6*β*	2.35 (dd, 16.7, 3.3)		2.45 (m)		2.46 (m)		2.49 (m)	
7		174.8		173.4		173.4		173.4
8		74.6		80.6		80.0		200.8
9		99.2		209.4		208.6		79.6
10		117.3	2.42 (m)	47.0	2.28 (m)	42.8	2.40 (m)	43.1
11α	2.54 (ddd,14.5, 14.5, 3.9)	28.1	2.61 (m)	33.5	2.50 (m)	33.0	1.78 (ddd, 14.6, 14.6, 3.4)	29.2
11*β*	1.71 (m)		3.35 (m)		3.03 (dd, 19.4, 5.6)		1.66 (ddd, 14.6, 3.4, 3.4)	
12α	1.42 (m)	30.6	1.57 (ddd, 6.5, 6.5, 6.5)	25.2	1.65 (m)	25.5	1.37 (ddd, 14.6, 3.4, 3.4)	28.3
12*β*	1.66 (m)		2.60 (m)		2.60 (m)		2.24 (ddd, 14.6, 14.6,3.4)	
13		38.8		38.4		38.4		41.4
14		163.0		165.3		164.1		156.2
15	5.98 (s)	116.5	6.13 (s)	119.0	6.17 (s)	118.1	6.35 (s)	121.6
16		165.1		163.2		163.3		163.3
17	5.03 (s)	81.9	5.24 (s)	79.9	5.32 (s)	80.1	5.51 (s)	81.3
18	1.31 (s)	19.2	0.95 (s)	18.7	0.97 (s)	18.4	1.07 (s)	18.5
19	1.61 (d, 0.9)	12.8	0.97 (d, 6.3)	11.7	1.03 (d, 6.2)	11.6	1.14 (d, 6.5)	12.2
20		119.7		119.6		119.5		119.2
21	7.44 (brs)	141.2	7.53 (s)	141.4	7.53 (s)	141.4	7.60 (s)	141.4
22	6.40 (d, 1.6)	110.1	6.44 (brs)	109.8	6.42 (d, 1.7)	109.8	6.49 (d, 1.8)	109.7
23	7.40 (t, 1.6)	142.9	7.43 (brs)	143.1	7.43 (d, 1.7)	143.2	7.44 (d, 1.8)	143.3
28	0.91 (s)	25.5	0.90 (s)	24.7	1.20 (s)	27.8	1.17 (s)	28.0
29	0.84 (s)	20.4	1.25 (s)	20.5	1.11 (s)	20.5	1.09 (s)	20.5
30	5.52 (d, 10.0)	71.0	5.87 (d, 8.8)	68.7	6.52 (s)	67.3	6.27 (s)	66.4
7-OMe	3.69 (s)	51.9	3.68 (s)	52.0	3.68 (s)	52.0	3.69 (s)	52.0
30-OAc	2.16 (s)	21.4	2.04 (s)	21.3	2.10 (s)	21.0	1.98 (s)	20.7
		170.4		171.0		169.9		168.9
1′		167.7		166.7				
2′		128.8		127.8				
3′	6.83 (qq, 6.9, 1.4)	137.4	6.70 (qq, 7.1, 1.4)	138.6				
4′	1.79 (d, 6.9)	14.5	1.80 (d, 7.1)	14.6				
5′	1.84 (s)	12.2	1.74 (s)	12.0				
8-OH	3.29 (s)		4.11 (s)		3.90 (s)			
9-OH	2.76 (s)						3.79 (s)	

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

the C-1/C-9 hemiketal of **1**, followed by an isomerization of the enol at C-1 to form **2** (Scheme 1). Further evidence

Scheme 1. Hypothetical Biogenetic Route from **1** to **2**



was obtained from the HMBC spectrum of **2**, in which C-1 (δ_C 208.4) correlated with H-2, H-3, H-30 and Me-19, and C-9 (δ_C 209.4) correlated with H-11, H-12, and OH-8. The stereochemistry at C-10 of **2** was resolved by an NOESY experiment, where the correlations of H-10/H-2 and Me-29 established the α-configuration of H-10. The biogenetic transformation of compound **1** to **2** was finally mimicked chemically (Scheme 3) to confirm the structure of **2**.

Xylogranatin C (**3**) was isolated as a colorless oil, and the molecular formula of $C_{29}H_{34}O_{10}$ was established by HR-EIMS at *m*/*z* 542.2149 $[M]^+$ (calcd 542.2152) and ^{13}C NMR spectroscopy. The NMR data of **3** were very similar to those of **2**, with differences only attributable to a *β*-elimination of the tigloyloxyl moiety to form a Δ^2 double bond. This was revealed by the absence of the tigloyl signals and the appearance of two additional sp^2 carbon signals at δ_C 128.4 (C-2) and 162.0 (C-3), together with the upfield shifted C-1 carbon signal at δ_C 198.8 (δ_C 208.4 in **2**), and was supported by the HMBC correlations of H-3/C-1, C-2, C-4, C-28, C-29, and C-30 and of H-30/C-1, C-2, and C-3. The relative

stereochemistry of **3** was established by an NOESY spectrum. The chemical transformation of **2** to **3** (Scheme 3) further confirmed the structure.

Xylogranatin D (**4**), a colorless oil, was assigned the molecular formula of $C_{29}H_{34}O_{10}$ by HR-ESIMS at *m*/*z* 565.2025 $[M+Na]^+$ (calcd 565.2050), suggesting that **4** was an isomer of **3**. Comparison of its ^1H and ^{13}C NMR spectra with those of **3** indicated that the structures of both compounds were closely related, and the main differences occurred at C-8 and C-9. The crucial HMBC correlations of H-15/C-8 (δ_C 200.8) and H-11/C-30 showed that the C-30 methine migrated to C-9 in **4**, which resulted in the formation of the hydroxylated quaternary carbon at C-9 (δ_C 79.6) and the conjugated ketone group at C-8. Consequently, the chemical shift of H_2-11 at δ_H 1.78 (ddd, J = 14.6, 14.6, 3.4 Hz) and 1.66 (ddd, J = 14.6, 3.4, 3.4 Hz) in **4** was obviously upfield shifted by comparison with those of compound **3**.

The relative stereochemistry of **4** was determined by an NOESY experiment and chemical correlation with **3**. The NOESY correlations of H-30/H-11β and H-12β in **4** indicated that the C-9−C-30 bond was β-oriented (Figure 2). The biogenetic synthesis of compound **4** was

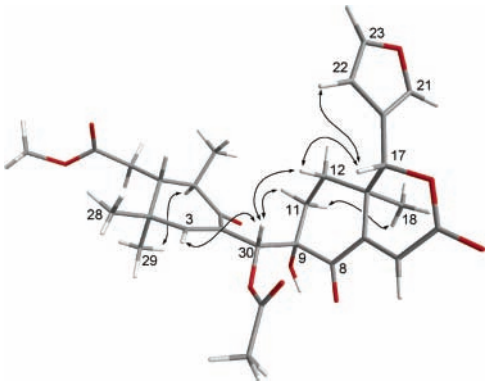
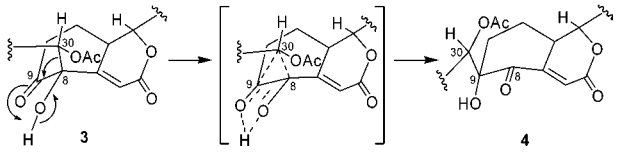


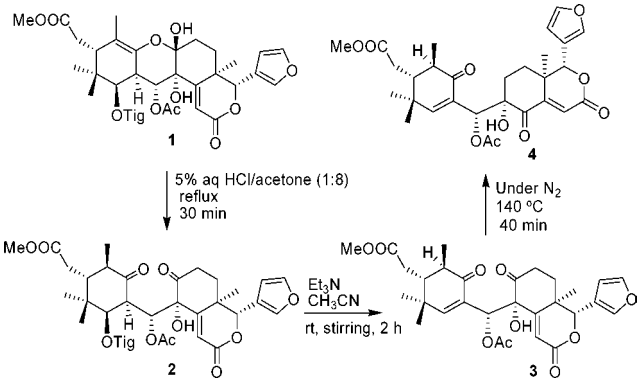
Figure 2. Key ROESY correlations of **4**.

hypothetically postulated from **3** via an α-hydroxyl ketone rearrangement (Scheme 2) and was chemically mimicked to

Scheme 2. Hypothetical Biogenetic Route from **3** to **4**



Scheme 3. Chemical Correlations of Compounds **1**−**4**



confirm the structure of **4** (Scheme 3),[5] in which the stereochemistry of the migratory group at C-30 was theoretically maintained. The rearrangement from **3** to **4** was also simulated in other conditions, e.g., stirring (even refluxing) **3** in different solvents in the presence of silica gel (mimicking the column chromatography conditions) for several days, and **4** was not produced as monitored by HPTLC, indicating that compound **4** is a genuine natural product. To the best of our knowledge, this α-hydroxyl ketone rearrangement with such a large migratory group is rare in both natural and synthetic aspects.[6]

The in vitro cytotoxic activities of the xylogranatins **1**−**4** against two tumor cell lines, P-388 murine leukemia and A-549 human lung carcinoma, were evaluated. Compounds **2**−**4** showed moderate cytotoxicity against the P-388 cell line with the corresponding IC_{50} values of 8.9, 6.3, and 14.6 μM, respectively, whereas compounds **1** and **2** exhibited cytotoxicity against the A-549 cell line with IC_{50} values of 15.7 and 11.3 μM, respectively.

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Supporting Information Available: Experimental procedures; physical and spectral data of **1**−**4**; and CIF data for the crystal structure of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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