Supporting Information

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

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S1. Experimental Section

For compound 1:

S2. ^1H NMR spectrum (400 MHz) of xylogranatin A (1) in CDCl_3 .

S3. ^{13}C NMR spectrum (100 MHz) of xylogranatin A (1) in CDCl_3 .

S4. HSQC spectrum (400 MHz) of xylogranatin A (1) in CDCl_3 .

S5. ^1H - ^1H COSY spectrum (400 MHz) of xylogranatin A (1) in CDCl_3 .

- S6. HMBC spectrum (400 MHz) of xylogranatin A (1) in CDCl₃.**
- S7. NOESY spectrum (400 MHz) of xylogranatin A (1) in CDCl₃.**
- S8. EIMS spectrum of xylogranatin A (1).**
- S9. IR (KBr disc) spectrum of xylogranatin A (1).**
- S10. Key NOESY correlations and X-ray structure of xylogranatin A (1).**

For compound 2:

- S11. ¹H NMR spectrum (400 MHz) of xylogranatin B (2) in CDCl₃.**
- S12. ¹³C NMR spectrum (100 MHz) of xylogranatin B (2) in CDCl₃.**
- S13. HMQC spectrum (400 MHz) of xylogranatin B (2) in CDCl₃.**
- S14. HMBC spectrum (400 MHz) of xylogranatin B (2) in CDCl₃.**
- S15. NOESY spectrum (400 MHz) of xylogranatin B (2) in CDCl₃.**
- S16. EIMS spectrum of xylogranatin B (2)**
- S17. IR (KBr disc) spectrum of xylogranatin B (2).**
- S18. Selected HMBC and key NOESY correlations of xylogranatin B (2).**

For compound 3:

- S19. ¹H NMR spectrum (400 MHz) of xylogranatin C (3) in CDCl₃.**
- S20. ¹³C NMR spectrum (100 MHz) of xylogranatin C (3) in CDCl₃.**
- S21. HSQC spectrum (400 MHz) of xylogranatin C (3) in CDCl₃.**
- S22. HMBC spectrum (400 MHz) of xylogranatin C (3) in CDCl₃.**
- S23. NOESY spectrum (400 MHz) of xylogranatin C (3) in CDCl₃.**
- S24. EIMS spectrum of xylogranatin C (3).**

S25. IR (KBr disc) spectrum of xylogranatin C (3).

S26. Selected HMBC and key NOESY correlations of xylogranatin C (3).

For compound 4:

S27. ^1H NMR spectrum (400 MHz) of xylogranatin D (4) in CDCl_3 .

S28. ^{13}C NMR spectrum (100 MHz) of xylogranatin D (4) in CDCl_3 .

S29. HSQC spectrum (400 MHz) of xylogranatin D (4) in CDCl_3 .

S30. HMBC spectrum (400 MHz) of xylogranatin D (4) in CDCl_3 .

S31. NOESY spectrum (400 MHz) of xylogranatin D (4) in CDCl_3 .

S32. NOESY spectrum (Enlargement) of xylogranatin D (4) in CDCl_3 .

S33. ESIMS spectrum of xylogranatin D (4).

S34. HR-ESIMS spectrum of xylogranatin D (4).

S35. IR (KBr disc) spectrum of xylogranatin D (4).

S36. Selected HMBC correlations of 4.

S1. Experimental Section

General Experimental Procedures: Melting points were measured on an SGW X-4 melting instrument and uncorrected. Optical rotations were determined on a Perkin-Elmer 341 polarimeter, and CD was obtained on a Jasco 810 spectrometer. UV was recorded on a Shimadzu UV-2550 Spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were measured on a Bruker AM-400 spectrometer. EIMS and HR-EIMS (70 eV) were made on a Finnigan MAT 95 mass spectrometer and ESI-MS on a Finnigan LC Q^{DECA} instrument. Semi-preparative HPLC was performed on a Waters 515 pump with a Waters 2487 detector (254 nm), and a YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm). All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.). Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co. Ltd.), C₁₈ reversed-phase silica gel (150-200 mesh, Merck), MCI gel (CHP20P, 75-150 μm, Mitsubishi Chemical Industries Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography (CC).

Plant Material: The plant material of *Xylocarpus granatum* was collected from Hainan island, People's Republic of China, in August of 2004 and was identified by Prof. S.-M. Huang, Department of Biology, Hainan University, P. R. China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Accession number Xg-2004-1Y).

Extraction and Isolation: The air-dried powder of the seeds of *X. granatum* (15.0 kg) was extracted with 95% EtOH for 3 times to give 412 g of crude extract, which was suspended in water (1.5 L) and then partitioned with ethyl acetate to give the ethyl acetate soluble fraction (172 g). The ethyl acetate soluble fraction was subjected to silica gel CC eluted successively with a petroleum ether/acetone gradient system (100:0→0:100) to obtain 15 fractions (Fr 1–15) according to TLC monitoring. Fr 15 (16 g) was further purified by a silica gel CC eluted with CHCl₃/MeOH 40:1 to give **1** (23 mg). Frs 13 and 14 were combined and extensively subjected to CC over silica gel, MCI gel, and Sephadex LH-20 to obtain five major components, each of which was purified by Semi-preparative HPLC using CH₃CN/H₂O 30:70 (3 mLmin⁻¹) to give compounds **2** (18 mg), **3** (150 mg) and **4** (35 mg) in turn.

Xylogranatin A (1): Colorless rhomboid crystals (ethyl acetate), mp 177-178 °C; $[\alpha]_D^{20} +62.2$ (c 0.325,

CH₃CN); UV (CH₃CN) $\lambda_{\max}$ (log ϵ) 213.7 (4.43) nm; CD (CH₃CN) 225 ($\Delta\epsilon$ +23.66), 269 ($\Delta\epsilon$ +1.57) nm; IR (KBr disc) $\nu_{\max}$ 3419, 2948, 1716, 1653, 1437, 1371, 1267, 1164, 1036 cm⁻¹; EIMS 70 eV m/z (relative intensity): 642 [M]⁺ (3), 482 (33), 381 (26), 262 (100), 239 (62), 166 (4); HREIMS m/z 642.2680 [M]⁺ (calcd for C₃₄H₄₂O₁₂, 642.26766).

Xylogranatin B (2): Colorless gum; $[\alpha]_D^{20}$ +51.6 (c 0.215, CH₃CN) UV (CH₃CN) $\lambda_{\max}$ (log ϵ) 214.7 (4.27) nm; CD (CH₃CN) 198 ($\Delta\epsilon$ -11.41), 223 ($\Delta\epsilon$ +10.66), 243 (-4.91), 278 (+6.17) nm; IR (KBr disc) $\nu_{\max}$ 3438, 2974, 1738, 1722, 1466, 1373, 1263, 1226, 1051 cm⁻¹; EIMS 70 eV m/z (relative intensity) 642 [M]⁺ (18), 482 (16), 368 (25), 262 (94), 239 (61), 166 (100), 138 (39); HREIMS m/z 642.2664 [M]⁺ (calcd for C₃₄H₄₂O₁₂, 642.2676).

Xylogranatin C (3): Colorless gum; $[\alpha]_D^{20}$ -20.0 (c 0.095, CH₃CN); UV (CH₃CN): $\lambda_{\max}$ (log ϵ) 225.9 (4.14) nm; CD (CH₃CN) 199 ($\Delta\epsilon$ +9.0), 240 ($\Delta\epsilon$ -14.8), 275 ($\Delta\epsilon$ +3.61) nm; IR (KBr disc) $\nu_{\max}$ 3446, 2970, 1732, 1670, 1371, 1229, 1024, 875 cm⁻¹; EIMS 70 eV m/z (relative intensity) 542 [M]⁺ (3), 368 (23), 308 (17), 262 (100), 239 (24), 165 (36); HREIMS m/z 542.2149 [M]⁺ (calcd for C₂₉H₃₄O₁₀, 542.2152).

Xylogranatin D (4): Colorless oil; $[\alpha]_D^{20}$ +102.0 (c 0.125, CH₃CN); UV (CH₃CN) $\lambda_{\max}$ (log ϵ) 234.3 (4.23) nm; CD (CH₃CN) 230 ($\Delta\epsilon$ +15.48), 346 ($\Delta\epsilon$ +2.4) nm; IR (KBr disc) $\nu_{\max}$ 3439, 2966, 1736, 1670, 1371, 1232, 1167, 1038; positive ESIMS m/z (relative intensity) 565.5 [M + Na]⁺ (100); HRESIMS m/z 565.2025 [M + Na]⁺ (calcd for C₂₉H₃₄O₁₀Na, 565.2050).

Transformation of 1 to 2: 5% aq. HCl (0.6 mL) was added dropwise to a solution of compound **1** (10 mg, 0.0156 mmol) in acetone (5 mL), then refluxed for 30 min. After removal of the solvent under reduced pressure, the residue was purified by silica gel column (eluted by CHCl₃) to afford the desired product (8 mg), which was identified to be **2** on the basis of ¹H, ¹³C NMR and MS spectra, and optical rotation measurements.

Transformation of 2 to 3: A solution of **2** (10 mg, 0.0156 mmol) in acetonitrile (3 mL) containing Et₃N (0.1 mL) was stirred at rt for 2 h. After work-up, the crude product was purified by TLC to give **3** (6 mg), which was identified by NMR data, MS and optical rotation measurements.

Transformation of 3 to 4: Compound **3** (20 mg) was heated neat at about 140 °C under an atmosphere of N₂ until the starting material was completely exhausted (ca. 40 min) as monitored by using TLC. The crude product was purified by preparative HPLC (CH₃CN/H₂O 80:20) to give **4** (15 mg) as identified by ¹H, ¹³C NMR, MS, and optical rotation measurements.

X-ray Crystal data and structure refinement for xylogranatin A (1):

Empirical formula	C ₃₈ H ₅₀ O ₁₄
Formula weight	730.78
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Hexagonal
Space group	P6(5)
Unit cell dimensions	a=23.446 (16) Å, α= 90 ° b=23.446 (16) Å, β= 90 ° c=14.036 (14) Å, γ= 120 °
Volume	6682 (9) Å ³
Z	6
Calculated density	1.090 Mg/m ³
Absorption coefficient	0.083 mm ⁻¹
F(000)	2340
Crystal size	0.429 × 0.347 × 0.289 mm ³
Theta range for data collection	1.76 to 26.00 °
Limiting indices	-28<=h<=20, -24<=k<=28, -17<=l<=16
Reflections collected / unique	36628/ 8324 [R(int) = 0.0768]
Completeness to theta = 26.00	99.9 %
Absorption correction	Empirical
Max. and min. transmission	1.00000 and 0.79609
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	8324 / 13 / 442
Goodness-of-fit on F ²	0.958
Final R indices [I>2σ(I)]	R1 = 0.0806, wR2 = 0.2035
R indices (all data)	R1 = 0.1190, wR2 = 0.2233
Absolute structure parameter	0.4 (15)
Extinction coefficient	0.0105 (11)
Largest diff. peak and hole	0.550 and -0.336 e. Å ⁻³

Colorless crystals of **1** were obtained by recrystallization in EtOAc. Crystal data were obtained on a Bruker SMART CCD detector employing graphite monochromated Mo-K α radiation ($\lambda=0.71073$ Å) at 293(2) K and operating in the ϕ - ω scan mode. The structure was solved by direct methods and refined with full-matrix least-squares calculations on F^2 using SHELX-97.¹ All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. 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 or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK. [fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk].

Cytotoxicity assay: The in vitro cytotoxicity against the P-388 and A-549 cell lines was evaluated by using the MTT and SRB methods, respectively, and with pseudolaric acid B as a positive control ($IC_{50} = 0.74$ μ M against P-388). The manipulation followed those described in previous literatures.²

References:

- (1) Sheldrick, G. M. *SHELX-97: Program for X-ray Crystal Structure Solution and Refinement*; University of Göttingen: Göttingen, Germany, 1997.
- (2) (a) Wang, X. N.; Yin, S.; Fan, C. Q.; Wang, F. D.; Lin, L. P.; Ding, J.; Yue, J. M. *Org. Lett.* **2006**, 8, 3845–3848. (b) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, 48, 589–601. (c) Skehan, P. A.; Storeng, R.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Can. Inst.* **1990**, 82, 1107–1112. (d) Pan, D. J.; Li, Z. L.; Hu, C. Q.; Chen, K.; Chang, J. J.; Lee, K. H. *Planta Med.* **1990**, 56, 383–385.

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S6. HMBC spectrum (400 MHz) of Xylogranatin A (**1**) in CDCl₃.

S7. NOESY spectrum (400 MHz) of Xylogranatin A (**1**) in CDCl₃.

YXK-9
NOESY

S8. EIMS spectrum of xylogranatin A (1).

S9. IR (KBr disc) spectrum of xylogranatin A (1).

S10. Key NOESY correlations and X-ray structure of xylogranatin A (**1**).

Key NOESY correlations of xylogranatin A (**1**)

X-ray structure of xylogranatin A (**1**)

S11. ^1H NMR spectrum (400 MHz) of xylogranatin B (**2**) in CDCl_3 .

S12. ^{13}C NMR spectrum (100 MHz) of xylogranatin B (**2**) in CDCl_3 .

S13. HMQC spectrum (400 MHz) of xylogranatin B (**2**) in CDCl₃

S14. HMBC spectrum (400 MHz) of xylogranatin B (**2**) in CDCl₃

Xylogranatin B (**2**)

yxk-24
GC

S15. NOESY spectrum (400 MHz) of xylogranatin B (**2**) in CDCl₃

YAG-29
NOESY

S16. EIMS spectrum of xylogranatin B (2).

S17. IR (KBr disc) spectrum of xylogranatin B (2).

S18. Selected HMBC and key NOESY correlations of xylogranatin B (**2**).

a. Selected HMBC ($\rightarrow$) correlations of **2**

b. Key NOE ($\leftrightarrow$) correlations of **2**

S19. ^1H NMR spectrum (400 MHz) of xylogranatin C (**3**) in CDCl_3 .

yx6-18

S20. ^{13}C NMR spectrum (100 MHz) of xylogranatin C (**3**) in CDCl_3 .

YXG-18 CDCl₃ BB-DEPT-135 Nov 16 2005

S21. HSQC spectrum (400 MHz) of xylogranatin C (**3**) in CDCl₃.

S22. HMBC spectrum (400 MHz) of xylogranatin C (**3**) in CDCl₃.

S23. NOESY spectrum (400 MHz) of xylogranatin C (**3**) in CDCl₃

YXK-18
NOESY

SPEC:	161122		Rapise:	03:15.6	31
Scmp:	YXC-18		Start :	15:47:05	39
Comm:	Finnigan/MAT95//70ev/Tsou:200C/R:1000				
Mode:	EI +VE +LMR BSCAN (EXP) UP LTR NRM				
Oper:	WANG_JesimM.CAS Client : S/N:SM01-0001	Inlet :			
Base:	262.1 Inten : 5685606	Masses:			
Norm:	262.1 RIC : 44706329	#peaks:			
Peak:	1000.00 mmu

S25. IR (KBr disc) spectrum of xylogranatin C (3).

S26. Selected HMBC and key NOESY correlations of xylogranatin C (**3**).

a. Selected HMBC ($\rightarrow$) correlations of **3**

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S27. ^1H NMR spectrum (400 MHz) of xylogranatin D (**4**) in CDCl_3 .

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S30. HMBC spectrum (400 MHz) of Xylogranatin D (4) in CDCl₃.

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S33. ESIMS spectrum of xylogranatin D (4).

S34. HR-ESIMS spectrum of xylogranatin D (4).

S35. IR (KBr disc) spectrum of xylogranatin D (4).

S36. Selected HMBC ($\rightarrow$) correlations of **4**

Selected HMBC correlations of **4**.