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Jatrocurcadiones A and B: two novel diterpenoids with an unusual 10,11-*seco*-premyrsinane skeleton from *Jatropha curcas*†

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Two novel diterpenoids, jatrocurcadiones A (1) and B (2), possessing an unusual 10,11-*seco*-premyrsinane skeleton were isolated from the twigs of *Jatropha curcas*. Their structures were determined by combined spectroscopic and chemical methods, and the absolute configurations were elucidated by quantum chemical calculations and $Rh_2(OCOCF_3)_4$-induced CD analysis. Jatrocurcadione A exhibited more potent inhibitory activity (IC_{50} = 10.0 μM) than the positive control (curcumin, IC_{50} = 25.0 μM) against thioredoxin reductase (TrxR), a potential target for cancer chemotherapy with redox balance and antioxidant functions.

Premyrsinanes are a group of highly oxygenated diterpenoids with a 5/7/6/3-tetracyclic carbon framework. So far about 30 premyrsinanes have been reported and all of them were isolated from the species of the family Euphorbiaceae.[1] Biosynthetically they are derived from the 6,12-cyclization of lathyrane diterpenes and serve as the precursors of myrsinane and cyclomyrsinane diterpenes.[2] The structural diversity of this compound class comes from the various oxidation patterns and substituents on the scaffold. In particular, the different oxidation patterns of Me-17 further divided them into three sub-classes.[2] Recently, their fascinating structures and important biological activities have attracted broad interests from both natural products and pharmaceutical chemists.[1,3]

Jatropha curcas LINN. (Euphorbiaceae) is a drought-resistant shrub widely distributed in many parts of Africa and Southeast Asia. It has become a famous natural resource plant for the production of biodiesel, animal feed, biopesticide, and

traditional medicine. Previous investigations have proved that it was a rich source of structurally diverse diterpenoids related to premyrsinanes, some of which featured unprecedented skeletons, such as jatrophalactam possessing a novel 5/13/3 tricyclic skeleton,[4] spirocurcasone with a rare "spirorahamnofolane" scaffold,[5] and jatrophadiketone possessing an unique 6/6/6 tricyclic framework.[6] These diterpenpoids showed broad range of bioactivities, such as anti-plasmodial,[7] anti-tuberculosis,[7] anti-inflammatory,[8] and especially cytotoxic activity.[6]

In our continuing efforts to discover the structurally unique and biologically significant metabolites from plant resources,[9–11] two novel diterpenoids, jatrocurcadiones A (**1**) and B (**2**), with an unusual 10,11-*seco*-premyrsinane skeleton were isolated from the twigs of *J. curcas* (Figure 1). Jatrocurcadione A exhibited significant inhibitory activity against thioredoxin reductase (TrxR) (IC_{50} = 10 μM), being more potent than the positive control (curcumin, IC_{50} = 25.0 μM). Herein, details of the isolation, structural elucidation, postulated biogenetic origin, and TrxR inhibitory activity of **1** and **2** are described.

The dried and powdered twigs of *J. curcas* were extracted with 95% EtOH at room temperature to give a crude extract, which was suspended in H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. Various column chromatographic separations of the EtOAc extract afforded compounds **1** (10.6 mg) and **2** (0.6 mg).

Compound **1**, a yellow oil, had a molecular formula $C_{20}H_{26}O_3$, as determined by HRESIMS ion at *m*/*z* 315.1964 $[M + H]^+$ (calcd 315.1955), corresponding to eight degrees of unsaturation. The IR absorption bands at 3441 and 1713 cm^{-1} indicated the presence of the hydroxyl and carbonyl groups, respectively. The ^1H NMR spectrum showed five methyl groups [δ_H 0.94 (3H, s), 1.02 (3H, d, J = 2.1 Hz), 1.04 (3H, d, J = 2.1 Hz), 1.35 (3H, d, J = 7.5 Hz), and 2.22 (3H, s)], an olefinic proton [δ_H 6.33 (1H, s)], an oxygenated methine [δ_H 5.31 (1H, dd, J = 2.3, 2.3 Hz)], and a series of aliphatic methylene or methine multiplets. The ^{13}C NMR spectrum, in combination with DEPT experiments, resolved 20 carbon resonances attributable to two carbonyl carbons, three double bonds, one sp^3 quaternary carbon, three sp^3 methines (one

bearing oxygen atom), three sp^3 methylenes, and five methyls. As the eight degrees of unsaturation were accounted for by three double bonds and two carbonyl groups, the remaining degrees of unsaturation required that **1** was tricyclic. Aforementioned information suggested that **1** possessed most of the structural features of premyrsinane diterpenoids, with the main difference being due to the absence of the cyclopropane ring.

In the ^1H–^1H COSY spectrum three structural fragments **a** (C-1–C-2–C-16), **b** (C-7–C-8), and **c** (C-18–C-10–C-19) were first established by the observed correlations (Figure 2). The connectivities of the three fragments, quaternary carbons, and other functional groups were mainly achieved by analysis of the HMBC spectrum (Figure 2). The HMBC correlations from a tertiary methyl (CH_3-17) to C-5, C-6, C-7, and C-12 allowed the connections of C-5, C-7, C-12, and C-17 to the quaternary carbon C-6. The C-8 and C-11 were linked via C-9 by the correlations from H-10 to C-8, C-9, and C-11, and from H_2-8 and H-11 to C-9. The HMBC correlations from H-11 to C-6 and C-12 further linked C-11 to C-12. Thus, the six-membered C-ring with an isopropyl group at C-9 was constructed. The HMBC correlations from the olefinic methyl (CH_3-20) to C-12, C-13, and C-14, together with the correlations from H_2-5 to C-4 and C-15 constructed the seven-membered ring B with a dienone system. This was further supported by the chemical shifts of C-4 (δ_C 142.3), C-15 (δ_C 163.4), C-12 (δ_C 155.8), C-13 (δ_C 132.1), and C-14 (δ_C 191.3) as compared with the known analogues sharing the same dienone system, such as 1,5-dioxo-2,3-dihydroxyrhamnofola-4(10),6,11(18),15-tetraene,[7] and jatrogrossidione.[12] The C-1 in the fragment **a** was linked to C-15 by HMBC correlations of H-1/C-15 and C-4, while C-2 was linked to C-4 via C-3 by HMBC correlations from H-2 and H_2-5 to C-3, which finally constructed ring A. Thus, the planar structure of **1** was determined as depicted. It was worth noting that the homoallylic coupling (^5J coupling) between H-1 and H-5b was observed in **1**, which led to the doublet splits of these signals in ^1H NMR spectrum (H-1, dd, J = 2.3, 2.3 Hz and H-5b, dd, J = 15.4, 2.3 Hz). This phenomenon was extensively observed in the compounds containing this fragment.[12,13]

The geometry of $\Delta^{9,11}$ and Δ^{12} double bonds were assigned as *E* and *Z*, respectively, by NOESY correlations of H-11/Me-18 and Me-20. The relative configuration of ring A was assigned by analysis of NOE experiment and ^{1}H NMR analysis. The strong NOE correlation between CH_3-16 and H-1 indicated the *trans*-relationship of H-1 and H-2, further supported by the small coupling constant between H-1 and H-2 (J = 2.3 Hz). *Cis*-oriented protons would give a coupling constant around 7 Hz.[12] The relative configuration of CH_3-17 at C-6 was left unassigned, since no NOE effect was observed with either H-1 or Me-16. The determination of the absolute configuration of C-1 by Mosher's method was hampered due to the occupation of the quaternary carbons in the right side of OH-1 (C-15, C-14, C-13, and C-12). Thus, the absolute configuration of **1** was determined by quantum chemical calculations combined with $Rh_2(OCOCF_3)_4$-induced CD analysis. The theoretical OR and ECD data of four possible isomers of **1** (**1a**–**1d**, figure 3) was calculated. Firstly, conformational analysis were carried out via Monte Carlo searching using molecular mechanism with MMFF94 force field in the Spartan 08 program.[14] The results showed two lowest energy conformers for each of **1a** and **1b**, whose relative energy within 2.0 Kcal/mol (Fig. S2†). Subsequently, the conformers were reoptimized using DFT at theB3LYP/6-311++G(2d,2p) level in vacuum with the Gaussian 09 program.[15] The B3LYP/6-311++G(2d,2p) harmonic vibrational frequencies were further calculated to confirm their stability. Based on the above optimized geometries, the specific optical rotations were calculated at the B3LYP-SCRF(PCM, dichloromethane) /6-311++G(2d,2p) level. For ECD calculation, the energies, oscillator strengths, and rotational strengths of the first 60 electronic excitations were calculated using the TDDFT methodology at the B3LYP-SCRF(PCM, acetonitrile)/aug-cc-pVDZ level. The ECD spectra were simulated by the overlapping Gaussian function[16] in which velocity rotatory strengths of the first 5 exited states for conformers of **1a** and first 4 ones for conformers of **1b** were adopted. To get the final spectrum for a compound, the calculated data for the lowest energy conformers of a compound were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). The OR and ECD data of **1c** and **1d** were obtained by directly inverse of those

of **1b** and **1a**, respectively.

The above calculation showed positive specific optical rotations for **1a** (+197.6) and **1b** (+412.6). Comparing to the experimental OR data (+268.8), **1c** and **1d** were excluded for their negative OR values. Structures of **1a** and **1b** both showed a *S* configuration at C-6, which was consistent with those of premyrsinanes and myrsinanes from the biosynthetic point of view. However, the ECD calculations of **1a** and **1b** generated similar results (Fig. S3†), which could not be used to ambiguously select one of the isomers. Thus, the absolute configuration of C-1 was further determined by $Rh_2(OCOCF_3)_4$-induced CD analysis.

On the basis of the bulkiness rule for secondary alcohols, a positive Cotton effect around 350 nm (the band E) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum indicated a *S*-configuration, while negative Cotton effect implied a *R*-configuration.[17] Thus, a positive Cotton effect at around 350 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum of **1** assigned the 1*S* configuration (Figure 4). Consequently, the 2*S* configuration was defined by the *trans*-relationship of CH_3-16 and OH-1. Thus, the absolute configuration of **1** was assigned as 1*S*, 2*S*, 6*S*, and compound **1** was named jatrocurcadione A.

Compound **2**, a light yellow oil, had the molecular formula $C_{22}H_{28}O_4$, as determined by HRESIMS. The NMR spectra of **2** showed high similarity to those of **1** except for the presence of an additional acetyl group [δ_H 2.04 (3H, s); δ_C 20.8, 170.8], implying that **2** was an acetylated derivative of **1**. The additional acetyl group was located at 1-OH by the downfield-shifted H-1 signal in **2** with respect to that in **1** (δ_H 6.26 in **2**; δ_H 5.31 in **1**), although the HMBC correlation from H-1 to the acetyl carbonyl was not observed. The structure of **2** was further confirmed by chemical correlation of **1** to **2** via acetylation. Compound **2** was given the trivial name jatrocurcadione B. The presence of both **1** and its acetylated derivative raises the possibility that **2** could be artifacts formed by acetylation of **1** with traces of AcOH present in EtOAc during the extraction process. This possibility was not investigated further.

Compound **1** and **2** represented the first example of the cleavage of C_{10}-C_{11} bond of

the cyclopropane ring in the premyrsinanes. The previously reported 9,10-*seco*-premyrsinanes were known as myrsinanes. So far more than ten skeletons related to premyrsinane have been isolated from the species *J. curcas*, and their biogenetic pathways were summarized in scheme 1. From the scheme it is not difficult to reason the existence of 10,11-*seco*-premyrsinanes in the plant, as rhamnofolanes, derived from tigliane skeleton by cleavage the C_{10}-C_{11} bond of the cyclopropane ring, already set the precedents.

Thioredoxin reductase (TrxR) is the major regulator of the intracellular redox homeostasis, which plays important roles in diverse biological processes.[18–20] Overexpressed TrxR in cancer cells is potentially related to the imbalanced deoxynucleotide pools and may accelerate the development of the malignant phenotype by gene amplification, genetic rearrangements, and even therapy resistance.[21,22] On the contrary, deactivation of TrxR does not only change the redox state and activity of Trx, but may also convert TrxR into a reactive oxygen species generator,[23] and further lead to the inhibition of proliferation, and even induction of necrosis or apoptosis of cells.[24] Thus, TrxR was a potential target for cancer chemotherapy. Compounds with α,β-unsaturated ketone motif were believed to be the potential TrxR inhibitors,[10] as the functional group may serve as an alkylator reacting with the Sec residue of TrxR. Jatrocurcadione A was tested for inhibitory activity against TrxR, and curcumin, a well-known natural TrxR inhibitor,[25] was used as positive control. Compound **2** obtained only as trace amount (0.6 mg) was not subjected to this assay. As a result (Table S1†), jatrocurcadione A showed potent inhibitory activities with IC_{50} value at 10.0 μM, being more active than the positive control (curcumin, IC_{50} = 25.0 μM).

In summary, two novel diterpenoids, jatrocurcadione A (**1**) and B (**2**), with an unusual 10,11-*seco*-premyrsinane skeleton were isolated from the twigs of *J. curcas*. Their structures including the absolute configurations were determined by comprehensive spectroscopic analysis, chemical methods, and ECD calculations. The significant inhibitory activity against TrxR of jatrocurcadione A was observed. The isolation of **1** and **2** in the current research not only expand the structural diversity of

the premyrsinane categories, but also provide a novel structural motif for further developing TrxR inhibitors.

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† Electronic supplementary information (ESI) available: General experimental procedures, plant material, extraction and isolation, chemical correlation of **1** to **2**, evaluation of the TrxR inhibitory activities, IR, HRESIMS, CD, 1D and 2D NMR spectra of **1** and **2** as well as the quantum chemical calculations.

Acknowledgments

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Table1. ^{1}H and ^{13}C NMR data of compounds **1** and **2** (in Pyridine-d_5, δ in ppm, J in Hz)

No.	**1**[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
1	5.31, dd (2.3, 2.3)	75.7, CH	6.26, dd (2.0, 2.0)	77.0, CH
2	2.84, qd (7.5, 2.3)	51.0, CH	2.66, qd (7.6, 2.0)	48.3, CH
3		209.2, C		207.9, C
4		142.3, C		145.3, C
5a	2.61, d (15.4)	35.5, CH_2	2.63, d (15.7)	35.3, CH_2
5b	2.48, dd (15.4, 2.3)		2.50, dd (15.7, 2.0)	
6		39.8, C		39.3, C
7a	1.64, m	39.3, CH_2	1.65, m	39.0, CH_2
7b	1.52, m		1.52, ddd (7.1, 5.1, 1.5)	
8a	2.11, m	24.3, CH_2	2.13, m	24.0, CH_2
8b	1.99, m		2.02, m	
9		157.7, C		158.0, C
10	2.34, m	36.7, CH	2.35, m	36.5, CH
11	6.33, s	121.8, CH	6.33, s	121.3, CH
12		155.8, C		155.7, C
13		132.1, C		131.6, C
14		191.3, C		188.6, C
15		163.4, C		158.4, C
16	1.35, d (7.5)	14.3, CH_3	1.43, d (7.6)	14.7, CH_3
17	0.94, s	20.7, CH_3	0.95, s	20.7, CH_3
18	1.02, d (2.1)[c]	21.9[c], CH_3	1.02, d (2.3)[c]	21.2[c], CH_3
19	1.04, d (2.1)[c]	21.6[c], CH_3	1.03, d (2.3)[c]	21.4[c], CH_3
20	2.22, s	16.2, CH_3	2.21, s	16.0, CH_3
1-OAc			2.04, s	170.8, C
				20.8, CH_3

[a]400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. [b]600 MHz for ^{1}H NMR and ^{13}C chemical shifts obtained from 2D NMR experiments. [c]May be interchanged in each column.

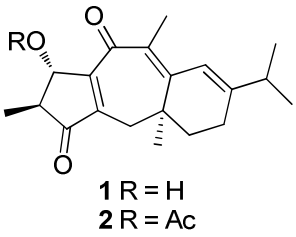


Figure 1. Structures of **1** and **2**.

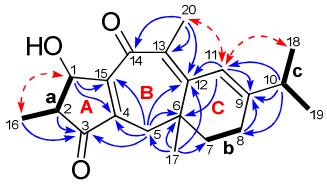
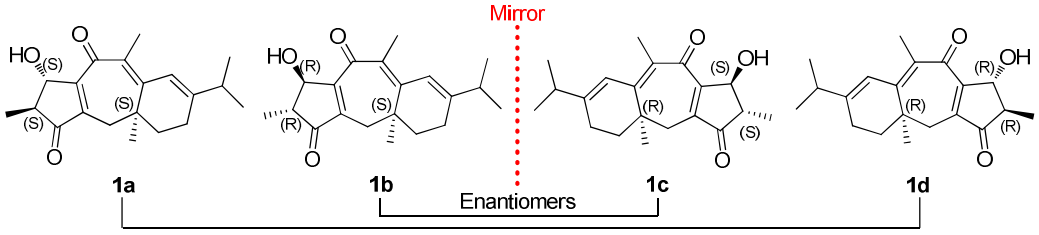


Figure 2. Selected ^{1}H–^{1}H COSY (▬), HMBC (→) and Key NOE correlations (⇢) correlations of **1**.



Firure 3. Four possible isomers of **1** (**1a**–**1d**) for quantum chemical calculations.

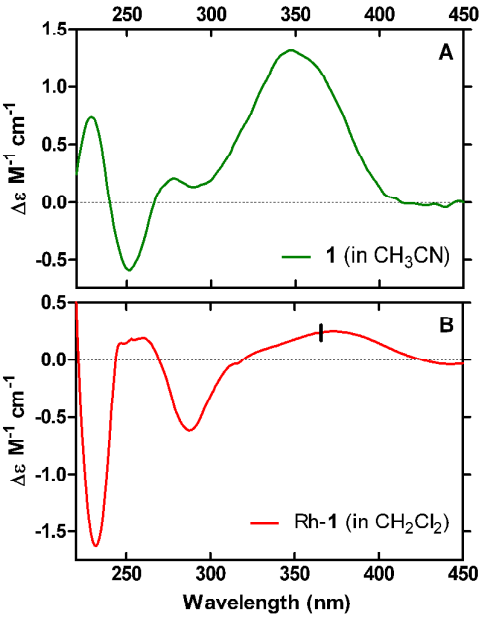
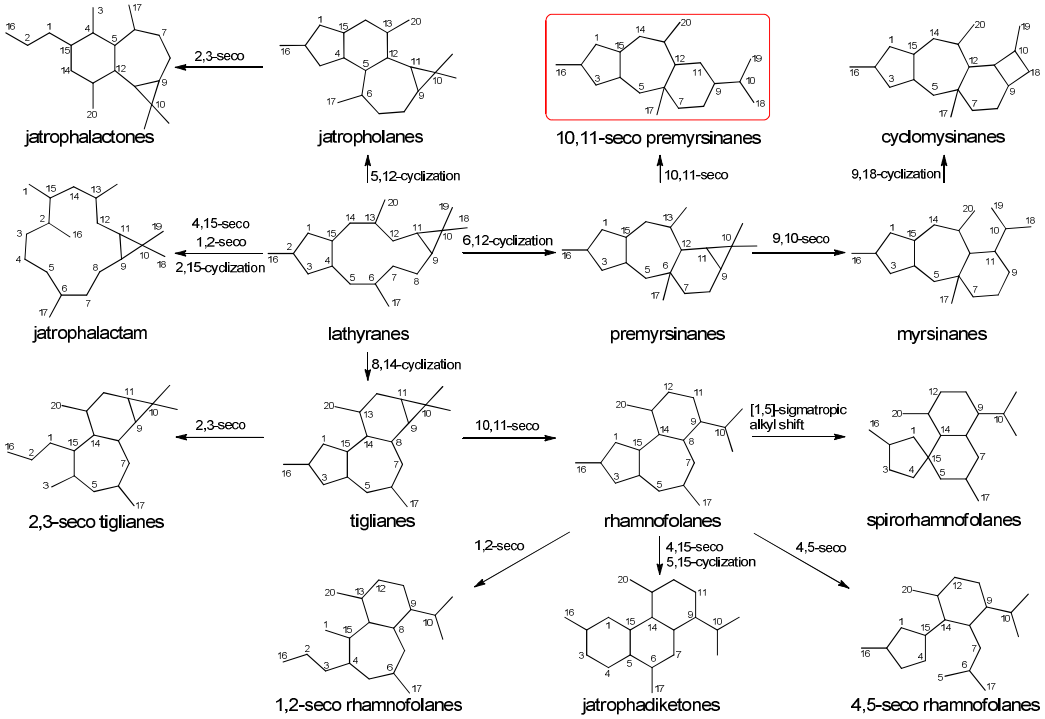


Figure 4. (**A**) The CD spectrum of **1** in CH_3CN; (**B**) The $Rh_2(OCOCF_3)_4$ induced CD spectrum of **1** in CH_2Cl_2.

Scheme 1. Proposed Biogenesis of skeletons related to premyrsinanes in the plant of *Jatropha curcas*.



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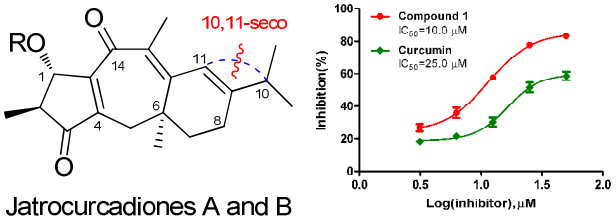

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