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Japodagricanones A and B, novel diterpenoids from *Jatropha podagrica*

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ABSTRACT

Two novel diterpenoids, japodagricanones A (**1**) and B (**2**), along with their biogenetically related diterpenoid 15-epi-*4E*-jatrogrossidentadion (**3**), were isolated from the leaves and twigs of *Jatropha podagrica*. Japodagricanones A (**1**) and B (**2**) are the first C-5-nor lathyrane-type diterpenoids. Their structures were established using spectroscopic data, including MS, NMR and ECD data. A plausible biosynthetic pathway for their generation was also proposed.

1. Introduction

The *Jatropha* genus (Euphorbiaceae) comprises approximately 175 species distributed mainly in the tropics and subtropics of Asia, Africa, and Latin America. Three species, *Jatropha curcas*, *Jatropha podagrica*, and *J. multifida* have been introduced to China [1]. In recent years, various structurally interesting natural products, especially the diterpenes including rhamnofolane, daphnane, tigliane, and lathyrane-type diterpenoids, have been isolated from this genus [2–5]. Some of these diterpenoids exhibit interesting bioactivities, including anti-tumor, anti-bacterial, and cytotoxic properties [3,6], which arose the chemists' interest for their total synthesis [7,8]. In the current study, the novel diterpenoids japodagricanones A (**1**) and B (**2**), along with the biogenetically related diterpenoid 15-epi-*4E*-jatrogrossidentadion (**3**) [9], were isolated from the leaves and twigs of *J. podagrica*. We report herein the isolation, structure elucidation, and bioactivities of the new compounds.

2. Experimental

2.1. General experiment procedure

Optical rotations were performed on a Jasco P-1020 polarimeter. UV spectra were measured with a Shimadzu UV 2401 PC spectrometer. IR spectra were recorded on a Bruker Tensor-27 infrared spectrophotometer with KBr disks. 1D and 2D NMR spectra were detected on a Bruker AV-600 MHz instrument with TMS as internal standard. EIMS and HREIMS were carried out on a Waters AutoSpec Premier P776 instrument. ECD spectra were obtained with an Applied Photophysics Chirascan spectrometer. Silica gel G (100–200 mesh, Qingdao Makall Group Co., Ltd.), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Corporation, Tokyo), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Silica gel H (10–40 m) were used for column chromatography. Semi-preparative HPLC was performed by using a CHIRALCEL OD-H 10 × 250 mm.

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2.2. Plant material

The leaves and twigs of *J. podagrica* were collected from Xishuangbanna of the Yunnan province, People's Republic of China, in August 2012. The plant was identified by Shuncheng Zhang (Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences). A voucher specimen (H20120808) was deposited at the Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powdered leaves and twigs (15 kg) of *J. podagrica* were extracted with MeOH (3 × 25 L) three times (4, 3, and 3 h, respectively) under reflux. The solvent were evaporated under reduced pressure to give the crude extracts, which were suspended in H_2O and then partitioned with petroleum ether and EtOAc successively to give two corresponding portions (236.3 and 60.8 g). The petroleum ether-soluble portion (236.3 g) was subjected to column chromatography over silica gel (80–100 mesh) using petroleum ether–EtOAc (20:1 → 0:1) to afford four fractions (A–D). Fraction B (24.6 g) was subjected to MCI silica gel, C18 silica gel, and Sephadex LH-20, and then further purified by semipreparative HPLC with a CHIRALCEL OD-H 10 × 250 mm (hexanes/2-propanol 96:4, flow 2.5 mL/min, UV detection at λ_{max} = 210 nm) to obtain japodagricanone A (**1**, 4.0 mg, t_R = 30.0 min), japodagricanone B (**2**, 2.8 mg, t_R = 34.0 min). Subfraction was partitioned by silica gel CC using chloroform–acetone (20:1) to afford compound **3** (30 mg).

Table 1
^{1}H and ^{13}C NMR data for 1 and 2 in $CDCl_3$.

No.	1		2	
	δ_C[a]	δ_H (mult; *J*, Hz)[a]	δ_C[a]	δ_H (mult; *J*, Hz)[a]
1	154.5 (d)	6.84 (s)	154.7 (d)	6.81 (s)
2	146.3 (s)		146.2 (s)	
3	205.5 (s)		205.7 (s)	
4a	45.8 (d)	2.82 (d, 18.6)	46.3 (t)	2.71 (d, 18.6)
4b		2.48 (d, 18.6)		2.53 (d, 18.6)
6	209.2 (s)		209.0 (s)	
7	44.2 (t)	2.39 (m, 2H, overlapped)	44.1 (t)	2.37 (t, 2H, 7.2)
8a	18.8 (t)	1.48–1.42 (m)	18.9 (t)	1.30 (m)
8b		1.35–1.27 (m)		1.40 (m)
9	26.0 (d)	0.36 (ddd, 9.4, 9.0, 6.0)	26.1 (d)	0.36 (ddd, 9.4, 9.0, 6.6)
10	17.8 (s)		17.7 (s)	
11	24.8 (d)	0.20 (dd, 15.0, 9.0)	24.1 (d)	0.27 (ddd, 9.4, 8.4, 6.6)
12a	29.1 (t)	1.66 (m)	29.5 (t)	1.34 (m)
12b		1.25 (m)		1.28 (m)
13	39.8 (d)	2.42 (m)	39.2 (d)	2.49 (m)
14	213.3 (s)		213.6 (s)	
15	82.6 (s)		82.7 (s)	
16	10.5 (q)	1.85 (s)	10.5 (s)	1.83 (s)
17	30.3 (q)	2.09 (s)	30.3 (q)	2.08 (s)
18	29.3 (q)	0.92 (s)	29.4 (q)	0.93 (s)
19	14.8 (q)	0.80 (s)	14.8 (q)	0.75 (s)
20	19.0 (q)	0.96 (d, 6.6)	18.4 (q)	1.10 (d, 6.6)

[a] ^{1}H NMR spectra were recorded at 600 MHz, and ^{13}C NMR spectrum were recorded at 150 MHz.

2.4. Japodagricanone A (**1**)

Colorless oil, [α]24 D −150.5 (*c* 1.2, MeOH); UV (MeOH) λ_{max} (log ε) 210 (0.40) nm; IR ν_{max} (KBr) 3428, 2926, 1713, 1633, and 1384 cm^{-1}; ECD (2.06 × 10^3 μM, MeOH), λ_{max} (Δε) 300 (−4.8), 234 (+12.3), 205 nm (−12.6); ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 343 $[M + Na]^+$, 663 $[2\,M + Na]^+$; HREIMS *m/z* 320.1988 $[M]^+$ (calcd for 320.1988).

2.5. Japodagricanone B (**2**)

Colorless oil, [α]24 D +76.1 (*c* 1.2, MeOH); UV (MeOH) λ_{max} (log ε) 211 (0.38) nm; IR ν_{max} (KBr) 3422, 2926, 2857, 1712, 1635, and 1384 cm^{-1}; ECD (2.06 × 10^3 μM, MeOH), λ_{max} (Δε) 302 (+4.0), 233 (−12.0), 204 nm (+11.2); ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 343 $[M + Na]^+$, 663 $[2\,M + Na]^+$; HREIMS *m/z* 320.1993 $[M]^+$ (calcd for 320.1988).

2.6. Bioassay

Compounds **1** and **2** were screened for cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human cancer cell lines using the MTT method [10]. Moreover, the two compounds were tested for their antimicrobial activity against three microorganisms, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and MRSA 98#, using the agar plate punch assay. The minimum inhibitory concentrations (MICs) of compounds **1** and **2** against MRSA 98# were determined by the two-fold dilution method [11].

3. Results and discussion

3.1. Structure elucidation

Compound **1** was obtained as a colorless oil. The HREIMS (*m/z* 320.1988 $[M]^+$, calcd 320.1988) indicated a molecular formula of $C_{19}H_{28}O_4$ (six unsaturations). The IR absorptions at 3428 and 1713 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. The ^{1}H NMR data (Table 1) indicated the presence of four methyl singlets at δ_H 1.85 (s), 2.09 (s), 0.92 (s), and 0.80 (s); one methyl doublet at δ_H 0.96 (d 6.6); and one olefinic proton signal at δ_H 6.84 (s). The ^{13}C and DEPT NMR spectra (Table 1) and HSQC data revealed 19 carbon signals, including five methyl groups, four methylenes, four methines, and six quaternary carbons (three ketone carbonyls, one olefinic, and one oxygenated). The three carbonyl groups and one double bond occupied four of the six degrees of unsaturation, and the remaining two degrees of unsaturation were due to the two rings in the molecule.

Extensive comparison of the ^{1}H and ^{13}C NMR data with those of a known lathyrane-type diterpene, 15-epi-4*E*-jatrogrossidentadion (**3**) [9], suggested that both compounds possess the same gem-dimethyl cyclopropane ring and cyclopentenone ring. The striking differences between the compounds were the absence of the $\Delta^{4(5)}$ double bond and the presence of an additional ketone carbonyl group (δ_C 209.2) in **1**. Considering the existence of two rings in **1**, the structure of japodagricanone A (**1**) was tentatively considered to contain an open ring system due to the cleavage of the C-4/C-5 double bond, as confirmed by 2D NMR studies. The ^{1}H–^{1}H COSY correlations of H-13/H_2-12,

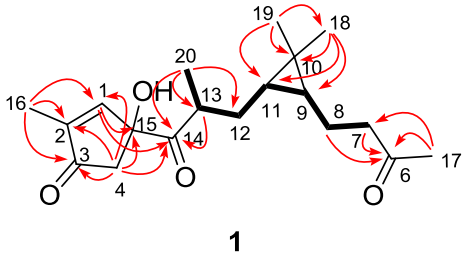
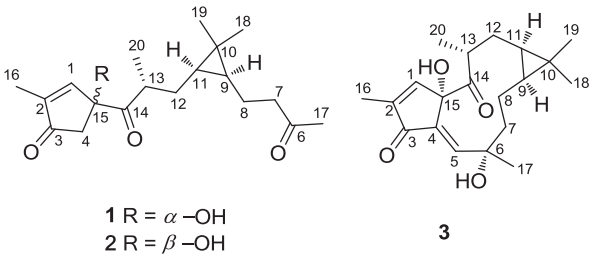


Fig. 1. Structures of compounds **1**–**3**.



Scheme 1. Plausible biosynthetic pathway for **1** and **2**.

H_2-12/H-11, H-11/H-9, H-9/H_2-8, H-8a/H-7, and H-13/H_3-20 established the fragment of one side chain, as drawn in bold in Fig. 2. The presence of a carbonyl group at C-14 (δ_C 213.3) was evident from the HMBC correlation of H_3-20 and H-13 to C-14. The HMBC correlation of H_3-17 to C-6 (δ_C 209.2) and C-7 and that of H-7 and H-8 to C-6 indicated that the Me-17 was linked to C-7 via a carbonyl group of C-6. The location of the gem-dimethyl cyclopropane ring system was confirmed by HMBC correlation from H_3-18 to C-9, C-10, and C-11 and from H_3-19 to C-9, C-10, C-11, and C-18. The HMBC correlations from H_3-16 to C-1, C-2, and C-3; H_2-4 to C-1, C-2, C-3, C-14, and C-15; H-1 to C-14 and C-15 established the cyclopentenone ring, which also indicated that C-15 was the core linkage of the long side chain and cyclopentenone ring. Thus, the planar structure of **1** was assigned as shown in Fig. 2.

The relative stereochemistry of **1** was assigned by ROESY data and comparison of the NMR spectroscopic data with that of compound **3**. The ROESY correlations of H-9/CH_3-18 and CH_3-18/H-11 indicated that H-9 and H-11 were cofacial and were arbitrarily assigned as α-oriented. However, the observation of the correlations of CH_3-20/H-11 and H-11/H-13 indicated that the linear chain (C-6–C-14) could rotate freely due to its flexibility. Meanwhile, the proton signal of OH-15 was unable to be observed in NMR experiments with Pyridine-d_5 and DMSO-d_6 as solvents. Thus, the relative configurations of H-13 and OH-15 could not be solved from the available NMR data. The ECD spectrum of **1** revealed a negative Cotton effect at 300 nm ($\Delta\varepsilon$ −4.8), 234 nm ($\Delta\varepsilon$ +12.3), and 205 nm ($\Delta\varepsilon$ −12.6), due to the n → π* transition of the α,β-unsaturated carbonyl moiety. Therefore, the absolute configuration of C-15 in **1** was determined to be 15*R* according to Snatzke's rule [12,13]. The other three chiral centers of C-9, C-11, and C-13 might have the same absolute configurations as those of 15-epi-*4E*-jatrogrossidentadion (**3**) from a biogenetic perspective [4,9].

Compound **2**, obtained as a colorless oil, had the same molecular formula as **1**. The IR, UV, and ^{1}H and ^{13}C NMR spectra of 2 were very similar to those of **1**, indicating that both compounds had the same planar structure, which was further confirmed by a detailed analysis of its 2D NMR (HSQC, ^{1}H–^{1}H COSY, and HSQC) spectra. The reversed ECD data (300 nm, $\Delta\varepsilon$ (+4.0); 233 nm, (−12.0); 205 nm, (+11.2)) compared with those of **1** (302 nm, $\Delta\varepsilon$ (−4.8); 234 nm, (+12.3); 205 nm, (−12.6)) indicated that **2** was a C-15 epimer of **1** since the ECD curves of **1** and **2** were contributed mainly by the chromophore of cyclopentenone [14]. Thus, the absolute configuration of **2** was unambiguously established as shown in Fig. 1.

The biogenetic origin of japodagricanone A (**1**) might be derived from isojatrogrossidion, a lathyrane-type diterpenoid from the title genus [15] (Scheme 1). The key biochemical reactions were deduced to be the oxidation of the double bond (C-5/C-6) to form intermediate (ii) with a β-keto aldehyde, which was subsequently oxidated and decarboxylated to yield intermediate (iii). Further oxidation would finally generate **1** and **2**.

3.2. Bioassay results

Compounds **1** and **2** showed no inhibitory activity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human cancer cell lines with $IC_{50} > 40$ μM. Only compound **2** exhibited weak activity against MRSA 98# with an MIC value of 25.0 μg/mL.

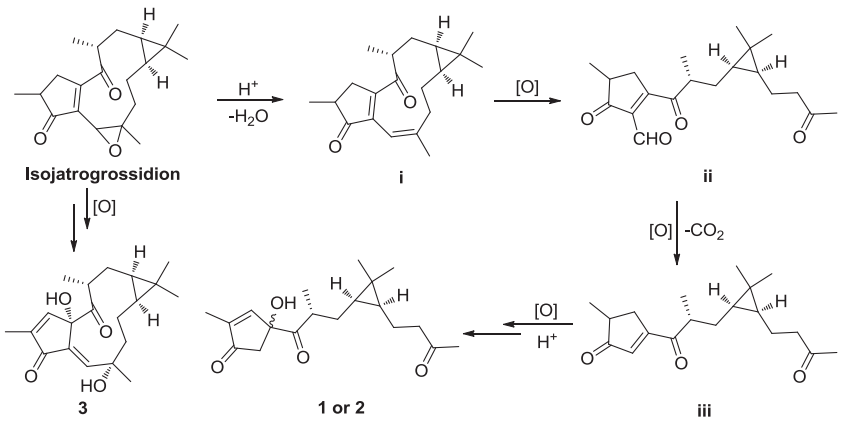


Fig. 2. ^{1}H–^{1}H COSY (bold) and key HMBC (arrow) correlations for compound **1**.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.07.021.

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