Structure, Absolute Configuration and Biological Evaluation of a New Labdane Diterpenoid from *Jatropha podagrica*

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Abstract: Jatrodagricaine A (**1**), a new labdane-type diterpenoid, together with three known derivatives (**2−4**) were isolated from the leaves of *Jatropha podagrica*. Their structures were elucidated by extensive spectroscopic methods. The absolute configuration of **1** was established by its biosynthetic pathway and comparison to known compound. All compounds were investigated for their anti-inflammatory and thioredoxin reductase (TrxR) inhibitory activities. Compound **1** showed potential inhibition in terms of nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW264.7 macrophages with IC_{50} value of $40.6 \pm 2.2 \mu$M.

1. Introduction

The genus *Jatropha* (Euphorbiaceae) consists of approximately 175 species extensively distributing in the tropic and subtropical areas of Africa, Asia, and Latin America. Some plants of this genus have been used for the treatment of various diseases such as skin infections, gonorrhea, jaundice and fever in folk medicine [1]. Previous studies showed that this genus is rich in structurally intriguing diterpenes with remarkable bioactivities, such as cytotoxicity, antibacterial, and anti-inflammatory effects [2].

Jatropha podagrica Hook. (Euphorbiaceae), a multipurpose shrub, is extensively planted as an ornamental plant in tropical countries. This plant is used as a folk medicine to treat the rheumatic condition, fever, constipation, hepatitis, itch, and parasitic skin diseases in African [3]. Previous investigation of *J. podagrica* resulted in the separation of some diterpenes and alkaloids, several of which showed potential biological activities including hypotensive, antibacterial, and neuromuscular-blocking effects [3]. During the course of our search for structurally unique and biologically interesting components of natural medicines [4-6], jatrodagricaine A (**1**) (Figure 1), a new labdane-type diterpenoid, along with three known derivatives (**2−4**) were obtained from the dried leaves of *J.*

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podagrica. Herein, the isolation, structural elucidation, and biological investigation of these isolates are described.
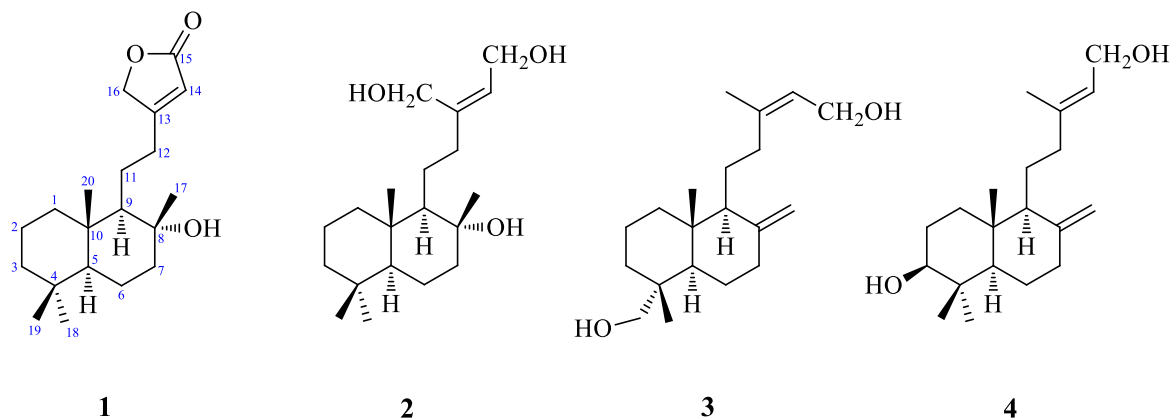

Figure 1. Structures of compounds **1−4**

2. Materials and Methods

2.1. Plant Material

The dried leaves of *J. podagrica* were collected in June 2018 from South China Botanical Garden, Chinese Academy of Sciences, Xingke Road, Tianhe District, Guangzhou city, Guangdong Province, China, and identified by Associate Professor Daogeng Yu, Chinese Academy of Tropical Agricultural Science, Danzhou, P. R. China. A voucher specimen (No. 20180610) was deposited at the Natural Product Research Centre of Shaanxi University of Chinese Medicine.

2.2. General Experimental Procedures

Optical rotation was recorded on a SGM-533 polarimeter. Bruker Avance III-600 or -500 spectrometer was used to perform NMR experiments with TMS as an internal standard. Bruker TENSOR-II ATR instrument was used to record infrared spectrum. Bruker APEX-II mass spectrometer was used to obtain HR-ESI-MS spectrum. Silica gel 60 (300−400 mesh, Qingdao Marine, China), MCI gel (75−150 μm, GE Healthcare, Japan), ODS (20−45 μm, Fuji Silysia, Japan), and Sephadex LH-20 (18−111 μm, GE Healthcar, Japan) was used for Column chromatographies (CC). Waters 1525 binary pump system equipped with a Waters 2489 detector and a Waters-Xbrige C18 (250 × 10 mm, i.d. 5 μm, Waters Co. Ltd., Ireland) column was used for semipreparative HPLC separations.

2.3. Extraction and Isolation

The dried-leaves of *J. podagrica* (0.9 kg) were powdered and extracted with 80% MeOH (5 L × 10 days × 4 times) under room temperature. Under reduced pressure, the MeOH extract give a concentrated residue (150.9 g) which was dispersed in water (1.0 L) and further extracted with EtOAc (1.0 L x 4 times). The EtOAc fraction (10.7 g) was separated by a column with MCI gel as the stationary phase and eluted with aqueous MeOH (50, 60, 70, 80, 90, and 100%, v/v) to afford six crude fractions (Fr. 1–6). Fr. 5 (0.5 g) was transferred to a Sephadex LH-20 column (CHCl$_3$/MeOH as the eluent, v/v = 1:1) to yield subfractions 5.1 and 5.2, and the latter (0.2 g) was further purified by semipreparative HPLC to yield **1** (3.6 mg; MeCN/H$_2$O, v/v = 48:52, t_R = 38.9 min, flow rate = 3.0 mL/min). Fr. 3 (1.2 g) was chromatographed on a column with silica gel as the stationary phase (petroleum ether/acetone as the eluent, 20:0–0:1) to yield **2** (20.3 mg). Fr. 4 (1.5 g) was separated by an ODS column (CH$_3$OH/H$_2$O as the eluent, 2:8–1:0) to obtain nine subfractions (Fr. 4.1–Fr. 4.9). Fr. 4.7 (0.4) was subjected to a silica gel column (petroleum ether/EtOAc as the eluent, 20:0–0:1) to yield **3** (2.6 mg) and **4** (1.2 mg).

2.4. Spectroscopic Data

Colorless amorphous solid; $[\alpha]26$ D -6.2 (*c* 2.25, MeOH); IR (KBr) v_{max}: 3507, 2922, 2854, 1730, 1626, 1443, 1389, 1339, 1301, 1259, 1233, 1171, 1125, 1076, 1045, 1011 cm^{-1}; HRESIMS *m/z* 343.2246 [M + Na]$^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2249). ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1.

2.5. TrxR Inhibitory Activity Assay

All isolates were assessed for their TrxR inhibition using a microplate reader under room temperature [7]. Briefly, the mixture (50 μL) of 170 nM NADPH-reduced TrxR and various concentrations of test samples was pre-incubated for the indicated times under room temperature in a 96-well plate. Then, 50 μL of a master mixture in TE buffer containing DTNB (final concentration, 2 mM) and NADPH (final concentration, 0.2 mM) was added, and the optical density was recorded at 412 nm during the initial 3 min. The control experiments were added with the same volume of DMSO and the percentage of the control was used to express the activity.

2.6. Anti-inflammatory Assay

The anti-inflammatory effects of compounds (**1**−**4**) were also assessed in terms of nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW264.7 mouse macrophage cells (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China). Cell culture, Griess and MTT procedures, and data analysis for the inhibition of NO production were the same as in the previous protocol [8]. Briefly, RAW 264.7 macrophages cells (8×10^4 cells/well) were suspended in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal bovine serum. The cells were pre-incubated with or without various concentrations of test samples for 1 h and then stimulated by 1.0 μg/mL LPS for 24 h. The equivalent supernatant (100 μL) and Griess reagent (100 μL, 0.1% *N*-[1-naphthyl] ethylene-diamine and 1% sulfanilamide in 5% H_3PO_4) were mixed. RAW 264.7 macrophages cell viability was conducted by MTT assay and minocycline was used as a positive control.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1**, colorless amorphous solid, had a molecular formula of $C_{20}H_{32}O_3$ as determined by the positive HR-TOF-MS (*m/z* 343.2246 [M + Na]$^+$, calcd. 343.2249), accounting for five indices of hydrogen deficiency. The ^{1}H NMR spectrum (Table 1) of **1** showed one olefinic proton at δ_H 5.85 (1H, br s, H-14), one oxygenated methylene at δ_H 4.78 (1H, dd, *J* = 17.3, 1.6 Hz, H-16a) and 4.73 (1H, dd, *J* = 17.3, 1.6 Hz, H-16b), and four methyl singlets at δ_H 1.17 (3H, s, Me-17), 0.87 (3H, s, Me-18), 0.81 (3H, s, Me-20), and 0.79 (3H, s, Me-19). The ^{13}C NMR spectrum (Table 1) showed a total of 20 carbon signals, including one ester carbonyl at δ_C 174.4 (s, C-15), one trisubstituted double bond at δ_C 171.3 (s, C-13) and 115.0 (d, C-14), one oxygenated quaternary carbon at δ_C 74.1 (s, C-8), one oxygenated methylene at δ_C 73.2 (t, C-16), as well as four methyl carbon signals in the upfield region. The ^{1}H,^{1}H-COSY correlations (Figure 2) of **1** revealed three isolated spin coupling systems as follows: H$_2$-1/H$_2$-2/H$_2$-3, H-5/H$_2$-6/H$_2$-7, and H-9/H$_2$-11/H$_2$-12. The HMBC correlations (Figure 2) from H$_2$-16 to C-13 (δ_C 171.3), C-14 (δ_C 115.0), and C-15 (δ_C 174.4) and from H-14 to C-12 (δ_C 31.6), C-13, C-15 and C-16 (δ_C 73.2) indicated the presence of an α,β-unsaturated γ-lactone moiety. The above NMR features showed high similarities to those of 9-hydroxylabd-13-en-15,16-olide [9], a recently reported labdane diterpene lactone. Careful comparison of the NMR data of **1** with those of the diterpene lactone suggested the different hydroxylated position in their structures. The HMBC correlations from Me-17 (δ_H 1.17, s) to C-7 (δ_C 44.9, t), C-8 (δ_C 74.1, s) and C-9 (δ_C 60.8, d) confirmed the linkage of the hydroxy group at C-8. The relative configuration of **1** was assigned on the basis of the following NOESY correlations (Figure 3): Me-17↔Me-20/H-7β, H-5↔H-7α/H-9, H-6β↔Me-19/Me-20, and H-6α↔Me-

18, which was in consistent with that of 9-hydroxylabd-13-en-15,16-olide. The absolute configuration of **1** was deduced by its plausible biogenetic pathway (Scheme 1). As shown, compound **2** underwent an repeated oxidation and subsequent esterification reaction to produce **1**. Furthermore, the specific rotation of **1** was determined to be $[\alpha]_D^{20} -6.2$ (*c* 2.25, MeOH), which was similar to the value of $[\alpha]_D^{20} -5.8$ (*c* 2.5, MeOH) observed for 8α,15,16-trihydroxy-labd-13E-ene (**2**) [10]. Taken together, the absolute configuration of **1** was defined as shown in Figure 1, and named as jatrodagricaine A.

Known compounds 8α,15,16-trihydroxy-labd-13E-ene (**2**) [10], kayadiol (**3**) [11], and labda-8(17),13E-diene-3,15-diol (**4**) [12] were authenticated by matching their 1D NMR spectra with those reported in the literatures. ^{13}C NMR data of known compounds **2**−**4** were summarized in Table S1. Compounds (**2**−**4**) were firstly isolated from the genus of *Jatropha*.

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of compound **1** (δ in ppm, *J* in Hz) in CDCl$_3$*

Position	δ_H	δ_C (type)
1	0.93, 1.63 (each 1H, m)	39.9 (t)
2	1.44, 1.60 (each 1H, m)	18.4 (t)
3	1.14, 1.39 (each 1H, m)	41.8 (t)
4	—	33.2 (s)
5	0.92 (1H, dd, 12.1, 2.0)	56.0 (d)
6β	1.27 (1H, qd-*like*, 13.1, 3.1)	20.5 (t)
6α	1.66 (1H, m)	—
7α	1.41 (1H, m)	44.9 (t)
7β	1.87 (1H, dt, 12.3, 3.2)	—
8	—	74.1 (s)
9	1.11 (1H, t-*like*, 4.2)	60.8 (d)
10	—	39.0 (s)
11	1.54, 1.71 (each 1H, m)	23.1 (t)
12a	2.46 (1H, ddd, 16.1, 11.0, 5.2)	31.6 (t)
12b	2.61 (1H, ddd, 16.1, 10.9, 5.9)	—
13	—	171.3 (s)
14	5.85 (1H, br s)	115.0 (d)
15	—	174.4 (s)
16a	4.78 (1H, dd, 17.3, 1.6)	73.2 (t)
16b	4.73 (1H, dd, 17.3, 1.6)	—
17	1.17 (3H, s)	24.2 (q)
18	0.87 (3H, s)	33.4 (q)
19	0.79 (3H, s)	21.5 (q)
20	0.81 (3H, s)	15.4 (q)

*Assignments were based on 1D and 2D NMR experiments.
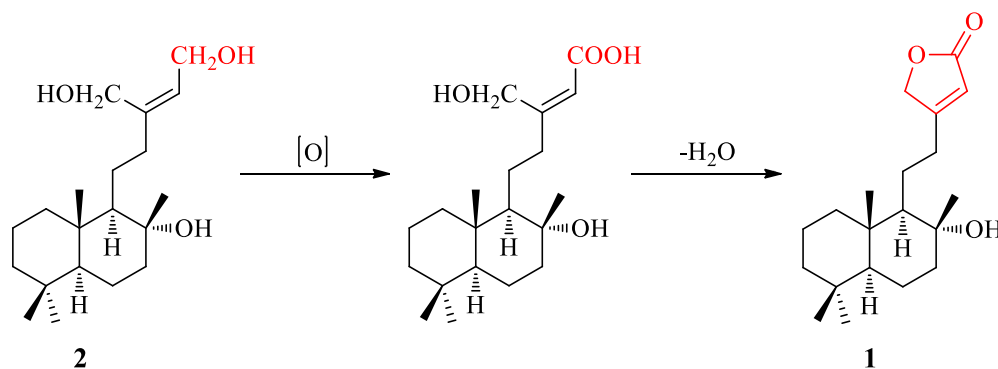
Scheme 1. Proposed biosynthetic pathway of compound **1**

Figure 2. Key HMBC (arrows) and ^{1}H−^{1}H COSY   **Figure 3.** Key NOESY correlations of compound **1**
(bold) correlations of compound **1**

3.2. TrxR Inhibitory and Anti-inflammatory Activity Assays

All compounds (**1−4**) were assessed for their TrxR inhibition, and no significant activity was observed. Additionally, these isolates, except **3** and **4** which were not available in sufficient quantities, were also tested for their anti-inflammatory effects. As a result, compound **1** showed potential inhibition of NO production in LPS-induced RAW264.7 mouse macrophage cells with IC_{50} value of 40.6 ± 2.2 μM, being comparable to the positive control (Minocycline, $IC_{50} = 31.9 \pm 1.7$ μM). MTT assay implied that **1** showed no cytotoxicity at high concentrations (150 μM).

Acknowledgments

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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