ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 14:5 (2020) 355-360

records of natural products

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

Dongbo Zhang[†], Jingao Yu[†], Zhen Zhang, Yanni Liang, Zhishu Tang[*] and Zheng Wang[*]

Shaanxi Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Shaanxi Province Key Laboratory of New Drugs and Chinese Medicine Foundation Research, State Key Laboratory of Research & Development of Characteristic Qin Medicine Resources (Cultivation), Shaanxi University of Chinese Medicine, Xianyang 712046, P. R. China

(Received December 10, 2019; Revised January 28, 2020; Accepted February 17, 2020)

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 ± 2.2 μM.

Keywords: *Jatropha podagrica*; labdane diterpenoid; anti-inflammatory activity; thioredoxin reductase. © 2020 ACG Publications. All rights reserved.

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.*

[†] These authors contributed to this work equally.

[*] Corresponding author: E-Mail: tzs6565@163.com (Zhishu Tang) and wazh0405@126.com (Zheng Wang).

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products September-October 2020 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.172.1912.1495

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

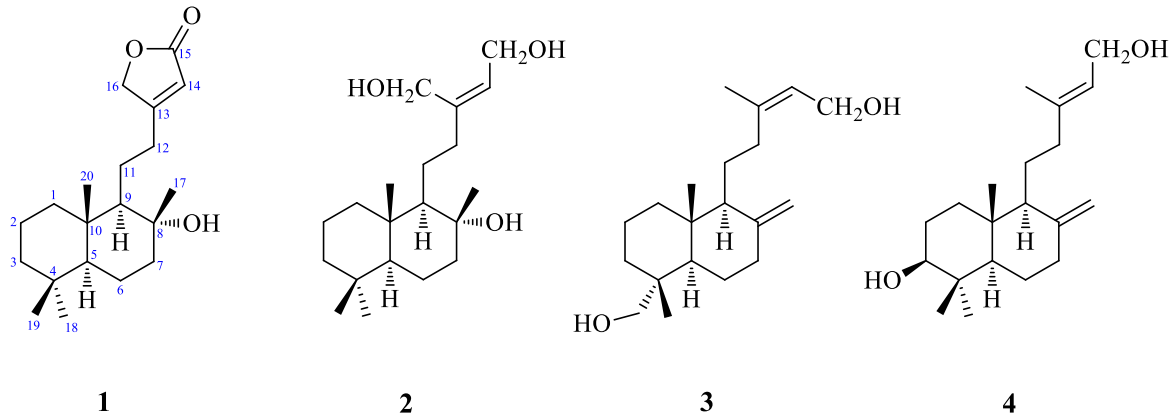


Figure 1. Structures of compounds **1**−**4**

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_2O, 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_3OH/H_2O 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; [α]26 D –6.2 (*c* 2.25, MeOH); IR (KBr) ν_{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). ^1H (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 ^1H 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 ^1H,^1H-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-

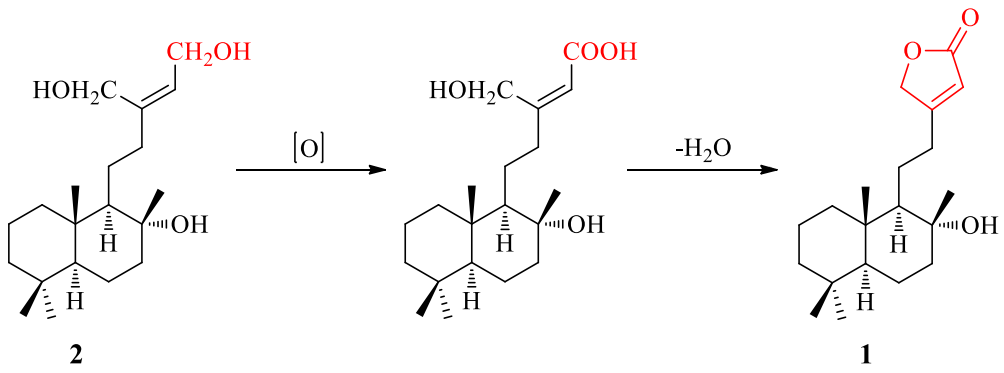
18, which was in consistent with that of 9-hydroxylabd-13-en-15,16-olide. The absolute configuration of **1** was deduced by its plausible biogenetic pathway (Scheme 1). As shown, compound **2** underwent an repeated oxidation and subsequent esterification reaction to produce **1**. Furthermore, the 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	δc (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)
6*β*	1.27 (1H, qd-*like*, 13.1, 3.1)	20.5 (t)
6*α*	1.66 (1H, m)	—
7*α*	1.41 (1H, m)	44.9 (t)
7*β*	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.



2 **1**

Scheme 1. Proposed biosynthetic pathway of compound **1**

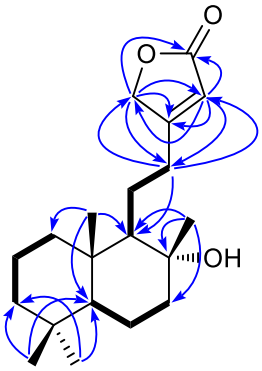


Figure 2. Key HMBC (arrows) and $^{1}H-^{1}H$ COSY (bold) correlations of compound **1**

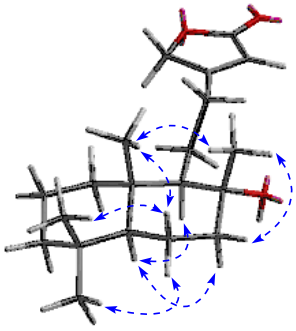


Figure 3. Key NOESY correlations of compound **1**

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 ± 1.7 μM). MTT assay implied that **1** showed no cytotoxicity at high concentrations (150 μM).

Acknowledgments

This work was supported by the Program for the National Natural Science Foundation of China (81703392), the Key Research and Development Program of Shaanxi Province (2017SF-365), the Natural Science Foundation of Shaanxi Provincial Department of Education (16JK1196), and the Youth Innovation Team of Shaanxi Universities.

Supporting Information

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

ORCID
Dongbo Zhang: 0000-0002-5108-9514
Jingao Yu: 0000-0003-0260-3334
Zhen Zhang: 0000-0001-5246-2686
Yanni Liang: 0000-0002-8002-6555
Zhishu Tang: 0000-0001-7795-9322
Zheng Wang: 0000-0002-1655-0889

References

[1] W. Ghali, D. Vaudry, T. Jouenne and M.N. Marzouki (2013). Assessment of cyto-protective, antiproliferative and antioxidant potential of a medicinal plant *Jatropha podagrica*, *Ind. Crop. Prod.* **44**, 111-118.

[2] L. Chen, J. L. Huang, L. Zhang, H.Y. Tian and S. Yin (2019). Jatrogricaine A: a new diterpenoid with a 5/6/6/4 carbon ring system from the stems of *Jatropha podagrica*, *Chin. J. Nat. Med*. **17**, 298-302.

[3] T. N. Minh, T. D. Xuan, H. D. Tran, T. M. Van, Y. Andriana, T. D. Khanh, N. V. Quan and A. Ahmad (2019). Isolation and purification of bioactive compounds from the stem bark of *Jatropha podagrica*, *Molecules* **24**, 889 (15 pages).

[4] D.B. Zhang, D.G. Yu, M. Sun, X. X. Zhu, X.J. Yao, S.Y. Zhou and K. Gao (2015). Ervatamines A–I, anti-inflammatory monoterpenoid indole alkaloids with diverse skeletons from *Ervatamia hainanensis*, *J. Nat. Prod*. **78**, 1253-1261.

[5] J. Zeng, D. B. Zhang, P. P. Zhou, Q. L. Zhang, L. Zhao, J. J. Chen and K. Gao (2017). Rauvomines A and B, two monoterpenoid indole alkaloids from *Rauvolfia vomitoria*, *Org. Lett*. **19**, 3998-4001.

[6] D. B. Zhang, Z. S. Tang, P. Xie, Y. N. Liang, J. G. Yu, Z. Zhang, D. Z. Duan, C. L. Cui, Z. X. Song, L. Ren, Z. Wang and D. G. Yu (2019). A pair of new *neo*-clerodane diterpenoid epimers from the roots of *Croton crassifolius* and their anti-inflammatory, *Nat. Prod. Res*.1-7.doi:10.1080/14786419.2019.1601193

[7] D. Z. Duan, B. X. Zhang, J. Yao, Y. P. Liu, J. Y. Sun, C. P. Ge, S. J. Peng and J. G. Fang (2014). Gambogic acid induces apoptosis in hepatocellular carcinoma SMMC-7721 cells by targeting cytosolic thioredoxin reductase, *Free Radic. Biol. Med*. **69**, 15-25.

[8] Z. H. Wang, H. J. Xu, Y. Y. Zhai, L. P. Yang, Q. Li, M. Huang, X. Y. Chen, W. K. Zhang, Y. L. Meng, Q. L. Zhang, C. Niu and Y. R. Zhang (2019). Three new labdane-type diterpenoids from *Callicarpa macrophylla* Vahl, *Nat. Prod. Res*. **33**, 2904-2910.

[9] K. Du, M. De Mieri, M. Neuburger, P. C. Zietsman, A. Marston, S. F. van Vuuren, D. Ferreira, M. Hamburger and J. H. van der Westhuizen (2015). Labdane and clerodane diterpenoids from *Colophospermum mopane*, *J. Nat. Prod*. **78**, 2494-2504.

[10] J. Jakupovic, A. Schuster, U. Ganzer, F. Bohlmann and P. E. Boldt (1990). Sesqui-and diterpenes from *Baccharis species*, *Phytochemistry* **29**, 2217-2222.

[11] Y. B. Ryu, H. J. Jeong, J. H. Kim, Y. M. Kim, J. Y. Park, D. Kim, T. T. H. Naguyen, S. J. Park, J. S. Chang, K. H. Park, M. C. Rho and W. S. Lee (2010). Biflavonoids from *Torreya nucifera* displaying SARS-CoV 3CLpro inhibition, *Bioorg. Med. Chem*. **18**, 7940-7947.

[12] T. Konishi, M. Azuma, R. Itoga, S. Kiyosawa, Y. Fujiwara and Y. Shimada (1996). Three new labdane-type diterpenes from wood, *Excoecaria agallocha*, *Chem. Pharm. Bull*. **44**, 229-231.

A C G
publications
© 2020 ACG Publications