

Jatroidaine A, A New Tetranortirucallane Type Triterpene from *Jatropha multifida*

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Abstract: Jatroidaine A (**1**), a new tetranortirucallane-type triterpene, and two known analogues (**2–3**) were isolated from the leaves and branches of *Jatropha multifida*. Their structures were fully elucidated by extensive spectroscopic methods and comparison to known compounds. The absolute configuration of **1** was assigned by single-crystal X-ray diffraction analysis. All compounds were evaluated for their anti-inflammatory and thioredoxin reductase (TrxR) inhibitory activities. Unfortunately, no significant activity was observed.

Keywords: *Jatropha multifida*; tirucallane triterpene; anti-inflammatory activity; thioredoxin reductase. © 2021ACG Publications. All rights reserved.

1. Introduction

Jatropha multifida (Euphorbiaceae), commonly called “Shan-Hu-Hua” in China, is a multipurpose shrub widely cultivated as an ornamental plant in South America and South China [1], and its roots, stems, leaves, and seeds have been traditionally used to treat oral candidiasis, gonorrhoea, fever, astriction, wounds and skin infections in African folk medicine [2-4]. Investigation on *J. multifida* showed that this plant was a rich source of structurally attractive diterpenoids with diverse biological activities including cytotoxic, antibacterial, antileishmanial, antimalarial, antiviral, larvicidal, and thioredoxin reductase inhibitory (TrxR) effects [1, 5-8]. However, no previous studies on triterpenoids have been reported from the plant. In continuation of our work on the *Jatropha* species [9-10], a new tetranortirucallane-type triterpene, jatroidaine A (**1**), and two known analogues (**2–3**) (Figure 1) were isolated from *J. multifida*. Herein, the isolation, structural elucidation, and the anti-inflammatory and TrxR inhibitory effects of these isolates are described.

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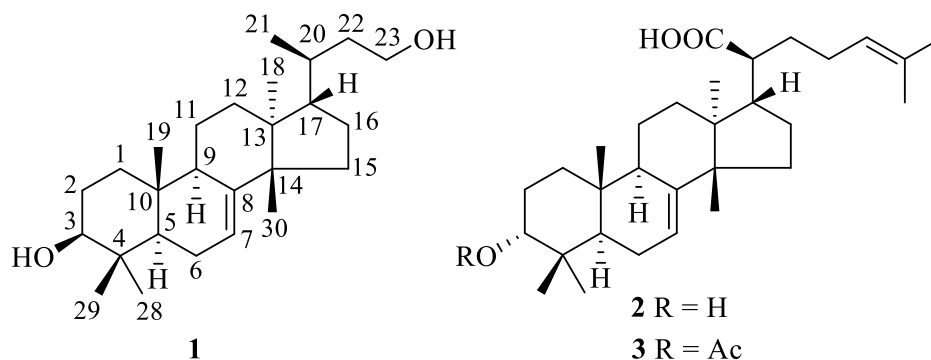


Figure 1. Structures of compounds **1–3**

2. Materials and Methods

2.1. Plant Material

The leaves and branches of *J. multifida* were gathered from Mengla County, Yunnan Province, P. R. China, in July 2019 and identified by Associate Professor Daogeng Yu, Chinese Academy of Tropical Agricultural Science, Danzhou, P. R. China. A voucher specimen (No. 20190711) was deposited at the Natural Product Laboratory of Shaanxi Collaborative Innovation Center of Chinese Medicinal Resource Industrialization, Shaanxi University of Chinese Medicine.

2.2. General Experimental Procedures

NMR spectra were recorded on a Bruker Avance III-600 spectrometer. X-ray diffraction experiment was collected on a Bruker APEX-II CCD diffractometer with Cu K α radiation. Melting point was measured on a X-4 microscopic melting point meter. Infrared spectrum was recorded on a Bruker Tensor II spectrometer with an ATR sensor. HRESIMS data was measured on a Bruker APEX II mass spectrometer. Column chromatography (CC) was performed on silica gel (Shanghai Titan Scientific Co., Ltd, Shanghai, China), Sephadex LH-20 (St. Louis, MO, USA), and ODS (Mitsubishi Chemical Industries, Tokyo, Japan). Fractions were monitored by TLC and spots were visualized by sprayed with 5% H₂SO₄ in EtOH followed by heating.

2.3. Extraction and Isolation

The fresh leaves and branches of *J. multifida* (1.0 kg) were cut and extracted with 70% MeOH (5 \times 3 L, 2 h each) under reflux to give 38.0 g of crude extract. The extract was suspended in H₂O and partitioned with EtOAc (1.0 L \times 3). The EtOAc extract (10.2 g) was subjected to ODS gel medium pressure liquid chromatography with H₂O–MeOH (1:0–0:1) to afford fractions 1–10. Fraction 8 (0.5 g) was chromatographed on a silica gel CC eluted with petroleum ether/EtOAc (10:1) to obtain compound **1** (1.7 mg). Fraction 6 (1.3 g) was separated by performing Sephadex LH-20 CC eluted with MeOH to afford subfractions 6.1–6.8. Subfraction 6.3 (0.19 g) was purified by a silica gel CC eluted with petroleum ether/acetone (2:1) to yield compounds **2** (3.1 mg) and **3** (1.9 mg).

2.4. Crystal Data of **1**

Crystallographic Data for **1** was collected on a Bruker APEX-II CCD diffractometer with Cu K α radiation ($\lambda = 1.54178 \text{ \AA}$) and refined with the SHELXL refinement package using Least Squares minimisation. The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre as CCDC 2042110 for **1**. Crystal Data: C₂₆H₄₄O₂ ($M = 388.61 \text{ g/mol}$): triclinic, space group *P1*(no.1), $a = 10.9288(3) \text{ \AA}$, $b = 11.7408(4) \text{ \AA}$, $c = 15.7438(5) \text{ \AA}$, $\alpha = 84.345(2)^\circ$, $\beta = 69.749(2)^\circ$, $\gamma = 66.639(2)^\circ$, $V = 1738.14(10) \text{ \AA}^3$, $Z = 3$, $T = 162.0 \text{ K}$, $\mu(\text{CuK}\alpha) = 0.513 \text{ mm}^{-1}$, $D_{\text{calc}} = 1.114 \text{ g/cm}^3$, 46387 reflections measured ($5.99^\circ \leq 2\theta \leq 136.916^\circ$), 12355 unique ($R_{\text{int}} = 0.0777$, $R_{\text{sigma}} = 0.0634$)

which were used in all calculations. The final R_1 was 0.0562 ($I > 2\sigma(I)$) and wR_2 was 0.1658 (all data), Flack parameter = 0.03(14).

2.5. Spectroscopic Data

Jatroidaine A (1): Colorless crystal (MeOH); mp 186-188 °C; IR (KBr) ν_{\max} : 3430, 2957, 2928, 2859, 1631, 1460, 1383, 1261, 1057, 804 cm^{-1} ; UV (MeOH) λ_{\max} : 203 nm; HRESIMS m/z 411.3226 [$M + Na$]⁺ (calcd for $C_{26}H_{44}O_2Na$, 411.3239). ¹H-NMR (600 MHz, CD₃OD) and ¹³C-NMR (150 MHz, CD₃OD) data, see Table 1.

2.6. TrxR Inhibitory Activity Assay

All compounds were determined for their TrxR inhibitory capacities by the previous report [11]. Briefly, the TrxR and various concentrations of test samples were mixed and incubated at room temperature in a 96-well plate. A master mixture in TE buffer containing DTNB and NADPH was added, and the absorbance at 412 nm was recorded during the initial 3 min. The equivalent DMSO was used to the control experiments.

2.7. Anti-inflammatory Assay

The anti-inflammatory properties of compounds **1–3** were evaluated by inhibition of lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 macrophages according to the previous literature [12]. All experiments were conducted in triplicates.

3. Results and Discussion

3.1. Structure Elucidation

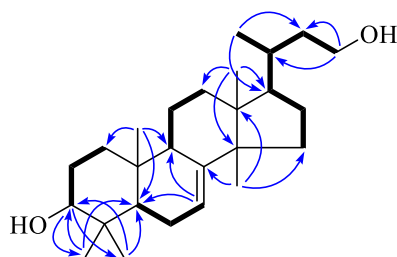
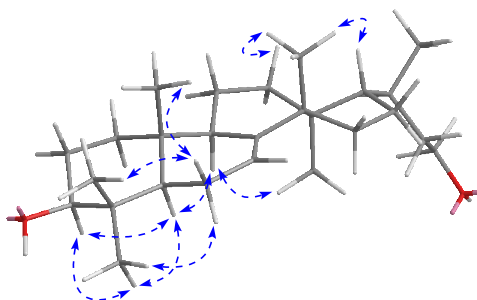
Compound **1**, obtained as colorless crystal in MeOH, possessed a molecular formula of $C_{26}H_{44}O_2$ by the (+)-HR-ESI-MS (m/z 411.3226 [$M + Na$]⁺, calcd. 411.3239), requiring five indices of hydrogen deficiency. The ¹H NMR spectrum (Table 1) in methanol-*d*₄ of **1** showed one olefinic proton at δ_H 5.27 (1H, dd, $J = 6.5, 2.9$ Hz, H-7), one oxygenated methylene at δ_H 3.54 (1H, dt, $J = 10.6, 7.6$ Hz, H-23a) and 3.62 (1H, ddd, $J = 10.6, 8.2, 4.6$ Hz, H-23b), one oxygenated methine proton at δ_H 3.17 (1H, dd, $J = 11.0, 4.8$ Hz, H-3), five methyl singlets at δ_H 0.77, 0.85, 0.85, 0.94, 1.00, as well as one methyl doublet at δ_H 0.91 (3H, d, $J = 6.0$ Hz, Me-21). The ¹³C NMR spectrum (Table 1) showed a total of 26 carbon signals, including one trisubstituted double bond at δ_C 119.3 (d, C-7) and 147.0 (s, C-8), one oxygenated methine carbon at δ_C 79.9 (d, C-3), one oxygenated methylene at δ_C 60.9 (t, C-23), and six methyl carbon signals in the upfield region. The above NMR features showed high similarities to those of 24,25-epoxy-3 β ,23-dihydroxy-7-tirucallene [13], a tetracyclic triterpene. Careful comparison of the NMR data of **1** with those of the tirucallane-type triterpene suggested the difference of the side-chain at C-17 in their structures. Considering its molecular formula, this compound could be inferred to be a tetranortirucallane-type triterpenoid. This deduction was well supported by the following 2D NMR analysis: (a) the ¹H,¹H-COSY correlations (Figure 2) of Me-21/H-20/H₂-22/H₂-23; (b) the HMBC correlations (Figure 2) from Me-21 to C-17 (δ_C 54.7) and C-22 (δ_C 39.9), and from H₂-23 to C-20 (δ_C 34.4) and C-22. The relative configuration of **1** was further verified by the following NOESY correlations (Figure 3): H-3 \leftrightarrow H-5/Me-28, H-9 \leftrightarrow H-5/Me-18, H-6 β \leftrightarrow Me-19/Me-29, Me-28 \leftrightarrow H-5/H-6 α , and Me-30 \leftrightarrow H-12 β /H-17, which was in consistent with that of 24,25-epoxy-3 β ,23-dihydroxy-7-tirucallene. Therefore, the structure of **1** was established as 24,25,26,27-tetranortirucall-7-ene-3 β ,23-diol. To our delight, the high-quality crystals were obtained in MeOH, which allowed a successful performance of X-ray crystallography study using Cu $K\alpha$ radiation (Figure 4). Finally, the absolute configuration of **1** was assigned as 3*S*, 5*R*, 9*R*, 10*R*, 13*S*, 14*S*, 17*S*, 20*S*, and named as jatroidaine A (Figure 1).

The two known compounds were identified as 3 α -hydroxy-7,24-dienetirucallic acid (**2**) [14] and 3 α -acetoxy-7,24-dienetirucallic acid (**3**) [15] by comparing their spectroscopic data with those reported in the literatures.

Table 1. The NMR data for **1** in CD₃OD (δ in ppm, J in Hz).

No.	¹ H NMR	¹³ C NMR
1a	1.14 (1H, td, 12.6, 4.6)	38.6 (t)
1b	1.69 (1H, m)	—
2	1.57–1.68 (2H, m)	28.4 (t)
3	3.17 (1H, dd, 11.0, 4.8)	79.9 (d)
4	—	40.0 (s)
5	1.30 (1H, dd, 12.0, 5.7)	52.2 (d)
6 β	1.98 (1H, m)	25.1 (t)
6 α	2.14 (1H, br d, 17.3)	—
7	5.27 (1H, dd, 6.5, 2.9)	119.3 (d)
8	—	147.0 (s)
9	2.23 (1H, m)	50.5 (d)
10	—	36.0 (s)
11	1.49–1.61 (2H, m)	19.3 (t)
12 β	1.83 (1H, br dd, 13.6, 9.6)	35.2 (t)
12 α	1.66 (1H, m)	—
13	—	44.8 (s)
14	—	52.4 (s)
15a	1.46 (1H, ddd, 12.1, 9.5, 2.2)	35.1 (t)
15b	1.54 (1H, m)	—
16a	1.33 (1H, m)	29.3 (t)
16b	1.99 (1H, m)	—
17	1.52 (1H, m)	54.7 (d)
18	0.85 (3H, s)	22.4 (q)
19	0.77 (3H, s)	13.6 (q)
20	1.53 (1H, m)	34.4 (d)
21	0.91 (3H, d, 6.0)	19.0 (q)
22a	1.19 (1H, m)	39.9 (t)
22b	1.74 (1H, m)	—
23a	3.54 (1H, dt, 10.6, 7.6)	60.9 (t)
23b	3.62 (1H, ddd, 10.6, 8.2, 4.6)	—
28	0.94 (3H, s)	28.3 (q)
29	0.85 (3H, s)	15.4 (q)
30	1.00 (3H, s)	27.8 (q)

Data were measured at 600 MHz. Assignments were based on DEPT, HSQC, ¹H-¹H COSY, and HMBC experiments.

**Figure 2.** Key HMBC (arrows) and ¹H-¹H COSY (bold) correlations of compound **1****Figure 3.** Key NOESY correlations of compound **1**

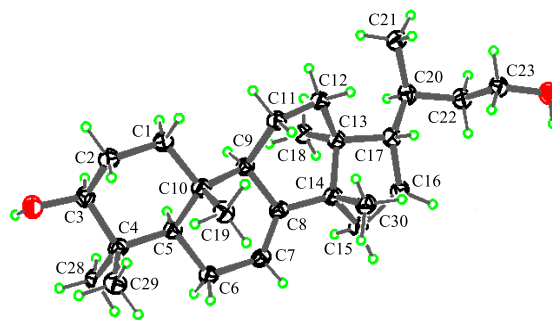


Figure 4. ORTEP drawings of compound **1**

3.2. Activity Assays

The anti-inflammatory and TrxR inhibitory effects of isolates **1–3** were evaluated. Nevertheless, no significant activity was observed.

Acknowledgments

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Supporting Information

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

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References

- [1] J. S. Zhang, Y. Zhang, S. Li, A. Ahmed, G. H. Tang and S. Yin (2018). Cytotoxic macrocyclic diterpenoids from *Jatropha multifida*, *Bioorg. Chem.* **80**, 511-518.
- [2] B. Dehgan (1982). Novel *Jatrophas* for Florida landscapes, *Proc. Fla. State Hort. Soc.* **95**, 277-280.
- [3] O. O. Aiyelaagbe (2001). Antibacterial activity of *Jatropha multifida* roots, *Fitoterapia* **72**, 544-546.
- [4] J. Kayode and M. A. Omotoyinbo (2009). Ethnobotanical utilization and conservation of chewing sticks plants species in Ekiti state, Nigeria, *Res. J. Bot.* **3**, 107-115.
- [5] S. Rampadarath, D. Puchooa and V. M. Ranghoo-Sanmukhiya (2014). Antimicrobial, phytochemical and larvicidal properties of *Jatropha multifida* Linn, *Asian. Pac. J. Trop. Med.* **7**, S380-S383.
- [6] A. Falodun, V. Imieje, O. Erharuyi, A. Joy, P. Langer, M. Jacob, S. Khan, M. Abaldry and M. Hamann (2014). Isolation of antileishmanial, antimalarial and antimicrobial metabolites from *Jatropha multifida*, *Asian. Pac. J. Trop. Biomed.* **4**, 374-378.
- [7] R. Chokchaisiri, J. Srijun, W. Chaichompoo, S. Cheenpracha, L. Ganranoo and A. Suksamrarn (2020). Anti-herpes simplex type-1 (HSV-1) activity from the roots of *Jatropha multifida* L, *Med. Chem. Res.* **29**, 328-333.
- [8] J. Y. Zhu, C. Y. Zhang, J. J. Dai, K. Rahman and H. Zhang (2017). Diterpenoids with thioredoxin reductase inhibitory activities from *Jatropha multifida*, *Nat. Prod. Res.* **31**, 2753-2758.

- [9] D. B. Zhang, Z. Wang, Y. N. Liang, J. G. Yu, Z. Zhang, S. J. Liu, Z. Zhang, Z. X. Song, Z. S. Tang and D. Z. Duan (2020). Jatrophainolides A-C, new cembrane-type diterpenoids with PTP1B inhibitory activity from the root bark of *Jatropha integerrima*, *Phytochem. Lett.* **36**, 166-170.
- [10] D. B. Zhang, J. G. Yu, Z. Zhang, Y. N. Liang, Z. S. Tang and Z. Wang (2020). Structure, absolute configuration and biological evaluation of a new labdane diterpenoid from *Jatropha podagrica*, *Rec. Nat. Prod.* **14**, 355-360.
- [11] 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.
- [12] 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.
- [13] X. D. Luo, S. H. Wu, Y. B. Ma and D. G. Wu (2000). Tirucallane triterpenoids from *Dysoxylum hainanense*, *Phytochemistry* **54**, 801-805.
- [14] A. J. Mora, G. Delgado, G. D. de Delgado, A. Usubillaga, N. Khouri and A. Bahsas (2001). 3-Hydroxytirucalla-7,24-dien-21-oic acid: a triterpene from *protium crenatum* sandwith, *Acta Cryst.* **C57**, 638-640.
- [15] M. Sawadogo, A. M. Vidal-Tessier and P. Delaveau (1985). Oleoresin of *Canarium schweinfurthii* Engl, *Ann. Pharm. Fr.* **43**, 89-96.

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