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An Undescribed Ingenane Glucoside Isolated from the Roots of *Euphorbia fischeriana* Steud. and Its Anti-inflammatory Activity

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Abstract: A new compound, ingenol 3-O-β-D-glucopyranoside (**1**) was isolated from the roots of *Euphorbia fischeriana* Steud. (Euphorbiaceae), together with four known compounds (**2**–**5**). The structure of compound **1** was characterized by a combination of spectroscopic techniques and X-ray crystallography. Moreover, biological evaluation revealed that compound **1** exhibited potent inhibitory effect on NO production in lipopolysaccharide-stimulated RAW 264.7 macrophages, with IC_{50} value of 25.24 ± 3.48 μM.

Keywords: *Euphorbia fischeriana* Steud.; Ingenol 3-O-β-D-glucopyranoside; Anti-inflammatory activity. © 2025 ACG Publications. All rights reserved.

1. Plant Source

The roots of *Euphorbia fischeriana* Steud. (Euphorbiaceae) were collected in August 2021 from Qinling Mountains, Shanxi Province, China and were identified by Ling-Ling Cai of Beijing University of Chinese Medicine. A voucher specimen (No. 202101) was deposited in Beijing University of Chinese Medicine, Peking, China.

2. Previous Studies

The roots of *E. fischeriana* Steud. are named 'Lang Du' and used as a traditional Chinese medicine for the treatment of tuberculosis, dermatitis, and tinea [1]. Previously chemical investigation led to over a hundred of diterpenoids being isolated from *E. fischeriana*, including ingenane [2], tigliane [2], *ent*-rosane [2], jatrophane [3], lathyrane [3], *ent*-atisane [4], *ent*-abietane [5] diterpenoids and so on. Ingenane-type diterpenoids commonly occur in nature in esterified form and show potent biological activities [6–8].

3. Present Study

In this study, a new ingenane glucoside **1** was isolated from the roots of *E. fischeriana*, together with its aglycone and three phenolic compounds (Figure 1). The structure of compound **1** was elucidated by extensive spectroscopic and X-ray crystallographic analysis. The known compounds were identified as ingenol (**2**) [9], 2,4-dihydroxy-6-methoxy-3-methylacetophenone 4-O-β-D-glucopyranoside (**3**) [10], 2,4-dihydroxy-6-methoxy-3-methylacetophenone (**4**) [10], and 2,4-dihydroxy-6-methoxy-3-aldehydoacetophenone (**5**) [11], based on the comparison of their spectroscopic data with those reported in the literatures. Herein, the isolation, structural elucidation, and biological activity of the isolates were reported.

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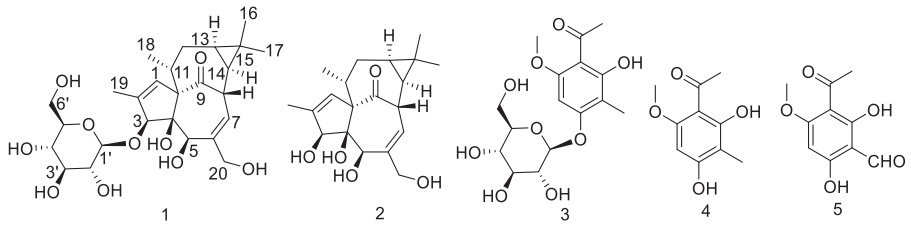

Figure 1. The structures of compounds **1**–**5**

The air-dried roots of *E. fischeriana* Steud. (3 kg) were powdered and extracted with MeOH (3 × 30 L) at room temperature. The extracting solution was distilled under reduced pressure to give crude extract 205 g. Then, the extract was dispersed in water and partitioned with EtOAc and *n*-butanol, sequentially. The *n*-butanol fraction (53.4 g) was applied to a macroporous resin (HP-20) column, eluted with gradient mixtures of H_2O/EtOH (100/0, 70/30, 50/50, 20/80, 0/100, v/v) to give five fractions (A–E). Fr.C (8.0 g) was chromatographically separated on reversed-phase silica gel (MeOH/H_2O, from 30/70 to 100/0, v/v) and Sephadex LH-20 ($CHCl_3$/MeOH, 1/1, v/v) to give compound **3** (10 mg). Fr. D (9.7 g) was chromatographed over a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1, v/v) to yield subfractions D1–D3. Fraction D1 was chromatographed through a silica gel column (CH_2Cl_2/MeOH, 10/1, v/v) to afford compound **1** (9 mg). Fr. D2 was chromatographed by a silica gel column (CH_2Cl_2/acetone, 5/1, v/v), leading to the isolation of compound **2** (5 mg). Fraction D3 was purified by a RP-C18 semipreparative HPLC chromatograph (H_2O/CH_3CN, 25/75, v/v) to yield compounds **4** (7 mg, $t_R = 7.1$ min) and **5** (6 mg, $t_R = 10.0$ min).

Equipment: A Bruker Avance NEO 600 was used to obtain nuclear magnetic resonance (NMR) spectroscopic data. High-resolution electrospray ionization mass spectrometry (HRESIMS) data were acquired using a Bruker Daltonics APEX II mass spectrometer (Bruker, Karlsruhe, Germany). Optical rotation was recorded at room temperature on an Autopol IV polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). The infrared (IR) data were acquired on a Bruker Tensor 27 FT-IR spectrometer (Bruker, Karlsruhe, Germany) with KBr disks. X-ray crystallography data were recorded on a Rigaku XtaLAB Synergy-DW diffractometer with Cu Kα radiation. Column chromatography (CC) was performed on silica gel (Qingdao Marine Chemical Ltd., Qingdao, China), Sephadex LH-20 (Amersham Pharmacia Biotech), and macroporous resin HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan). Purification of compounds was performed on semipreparative HPLC chromatograph (Waters 1525) with a SunFire C18 OBD™ column (10 μm, 250 × 10 mm, flow rate: 2 mL/min).

*Ingenol 3-O-β-D-glucopyranoside (**1**)*: colorless crystal; $[\alpha]_D^{21}$ −40 (c 0.2, MeOH). ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) data, see Table 1; HRESIMS *m/z* 533.2361 $[M + Na]^+$ (calcd for $C_{26}H_{38}O_{10}Na$, 533.2357). Crystal data, see Table S2 (Supporting Information). Deposition number: CCDC22407997.

Compound **1** was obtained as colorless crystals and provided a $[M + Na]^+$ ion peak at *m/z* 533.2361 (calcd. for $C_{26}H_{38}O_{10}Na$, 533.2357) in its HRESIMS spectrum, consistent with a molecular formula of $C_{26}H_{38}O_{10}$ that required eight degrees of unsaturation. Its 1D NMR data (Table 1) complemented by DEPT and HSQC spectra revealed the presence of four methyls, three methylenes (two oxygenated), 12 methines (seven oxygenated), an oxygenated tertiary carbon, a quaternary carbon, four olefinic carbons, and a ketone carbonyl. In addition, the presence of a β-glucose was easily deduced from the 1D NMR resonances of an anomeric proton at δ_H 4.31 (d, $J = 8.3$ Hz, 1H) and resonances at δ_H 3.0–4.0 (6H), along with chemical shifts at δ_C 103.2 (C-1′), 73.6 (C-2′), 77.2 (C-3′), 70.0 (C-4′), 76.7 (C-5′), and 61.1 (C-6′). The two double bonds, ketone carbonyl, and sugar moiety accounted for four out of eight degrees of unsaturation, the remaining ones indicated that compound **1** was a tetracyclic diterpenoid. The relatively upfield signals of two methine protons (δ_H 0.61, dd, $J = 15.0$, 8.4 Hz; 0.76, dd, $J = 11.9$, 8.4 Hz) in its ^{1}H NMR spectrum and the HMBC cross peaks of H-13/C-15 and C-17 and H-14/C-7, C-9, C-15, and C-17 (Figure 1) delineated a 1,1-dimethylsubstituted cyclopropane moiety, suggesting that compound **1** was an ingenane-type diterpenoid. Comparison of its NMR spectroscopic data with those of ingenol (**2**) and the known ingenane glucoside, 20-deoxy-16-hydroxyingenol-3-β-D-glucopyranoside [12], indicated

that compound **1** was an ingenol glucoside. The key HMBC cross peak of H-3/C-1′ verified that the β-glucosyl should be linked to C-3. Moreover, compound **1** and the known ingenane glucoside differ in the location of a hydroxyl group which was linked to C-17 rather than to C-20 in compound **1**.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compound **1** in DMSO-d_6 (δ in ppm, J in Hz)

No.	δ_H (J in Hz)	δc, type	No.	δ_H (J in Hz)	δc, type
1	5.75 d (1.3)	129.4 CH	15		23.3 C
2		137.7 C	16	1.03 d	15.2 CH_3
3	4.38 s	88.2 CH	17	1.05 s	28.4 CH_3
4		85.7 C	18	0.84 d (7.1)	17.2 CH_3
5	3.33 s	73.1 CH	19	1.76 d (1.1)	15.2 CH_3
6		143.1 C	20a	3.95 dd (14.3, 5.3)	63.0 CH_2
7	5.83 dd (4.1, 1.7)	120.5 CH	20b	3.86 dd (14.3, 4.6)	
8	4.10 overlapped	42.7 CH	1′	4.31 d (8.3)	103.2 CH
9		206.8 C	2′	3.03 td (8.3, 4.4)	73.6 CH
10		71.7 C	3′	3.10 overlapped	77.2 CH
11	2.34 m	38.1 CH	4′	3.09 overlapped	70.0 CH
12a	2.20 m	30.6 CH_2	5′	3.17, overlapped	76.7 CH
12b	1.68 dt (10.9, 5.7)		6′a	3.69 dd (11.1, 4.7)	61.1 CH2
13	0.61 m	22.7 CH	6′b	3.48 m	
14	0.76 dd (11.9, 8.4)	23.0 CH			

The relative configuration of compound **1** was partially assigned utilizing a NOESY analysis (Figure 2). The NOESY cross-peaks of H-3/H-5 showed the co-facial orientation of H-3 and H-5. While, the cross-peaks of H_3-18/H-13 and H-11/H-8 indicated that H_3-18 and H-13 were co-facial, and H-11 and H-8 were on the opposite side of the ring system. An X-ray diffraction data analysis (Cu Kα radiation) [Flack parameter of 0.08(12)] not only assigned the relative configuration of the whole molecule but also determined the absolute configuration of **1** as (3*S*, 4*S*, 5*R*, 8*S*, 10*S*, 11*R*, 13*R*, 14*R*, 1′*S*, 2*R*′, 3′*S*, 4′*S*, 5′*R*). Therefore, the structure of compound **1** was defined as ingenol 3-O-β-D-glucopyranoside.

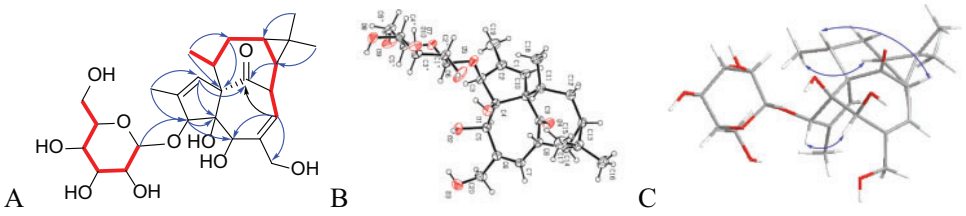

Figure 2. A: Key HMBC (H→C) and ^{1}H-^{1}H COSY (bold lines) correlations of compound **1**. B: ORTEP drawing of compound **1**. C: Key NOESY correlations of compound **1**

Assay for NO Inhibitory Activity: The cytotoxicity of compounds **1** and **3–5** at the concentration of 40 μM against RAW264.7 macrophage cells was determined by an MTT assay followed the previously published protocols [13]. The inhibitory activity of compounds **1** and **3–5** on LPS-induced NO production was determined by a colorimetric assay based on the Griess reaction as previously described [13]. L-NMMA (Sigma), a relatively non-selective inhibitor of all NOS isoforms was used as the positive control (IC_{50} = 20.10 ± 3.43 μM). Compounds **1** and **3–5** showed no toxicity to RAW264.7 macrophages (The viability of cells was over 80%) at the concentration of 40 μM. While, compound **1** demonstrated moderate inhibitory effect on NO production in LPS-stimulated RAW 264.7 macrophage cells, with IC_{50} value of 25.24 ± 3.48 μM (Results are averages of three independent

experiments). Compounds **3**–**5** showed no obvious effects on NO production in LPS-stimulated RAW 264.7 macrophage cells at the concentration of 40 μM.

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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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