Research Article

A New Pentacyclic Triterpene from *Humata tyermanni* Moore with the Inhibitory Activities against LPS-Induced NO Production in RAW264.7 Macrophages

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Received 8 April 2012; Accepted 23 May 2012

Academic Editor: Mohammad El-Shahawi

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One new pentacyclic triterpene, hopane-22(29)-en-24ol (**2**), with five known hopane-type pentacyclic triterpene compounds: hop-22(29)-ene (**1**), adiantone (**3**), 22-hydroxyhopane (**4**), 6 α , 22-dihydroxyhopane (**5**), and 17(21)-hopene (**6**) were isolated from *Humata tyermanni* Moore. The structure of compound **2** was elucidated on the basis of its 1D and 2D NMR spectral analysis. All these compounds were evaluated for their inhibitory activities of LPS-induced nitric oxide (NO) production in RAW264.7 macrophages.

1. Introduction

Humata tyermanni Moore, a plant belonging to the family of Davalliaceae, is widely distributed in the south part of China [1, 2]. People of Yao minority usually use it (Bai Mao She) as their traditional herbal medicines, to cure rheumatism, injuries from falls, pulmonary abscess, and so on. In this paper, we described the isolation and structure elucidation of one new pentacyclic triterpene, hopane-22(29)-en-24ol. Its structure was elucidated on the basis of 1D and 2D NMR spectral techniques. In addition, the inhibitory activities of this new compound, together with other five known pentacyclic triterpenes against LPS-induced NO production in RAW264.7 macrophages, were also reported.

2. Experimental

2.1. Plant Materials

The dry stems of *H. tyermanni* were bought from traditional Chinese medicine market in Qingping of Guangzhou, in March, 2010. The plant materials were authenticated by

Cai Yuewen pharmacist of traditional Chinese medicine, Guangdong Food, and Drug Vocational College. The voucher specimen (no. 2009ZL1) was deposited in the Laboratory of Natural Products Chemistry, Jinan University, Guangdong province.

2.2. Extraction and Isolation. The dry stems of *H. tyermanni* (20 kg) were extracted with 95% EtOH at room temperature for three times. The extract was partitioned between H₂O and petroleum ether, ethyl acetate in turn. The petroleum ether extract (124 g) was separated through silica gel (200–300 mesh) column chromatography (CC), eluted with petroleum ether/acetone gradient system to yield 17 fractions (from Fr.1 to Fr.17). Recrystallization of Fr.1 yielded compound **1** (150 mg). Fr.4 was purified with silica gel CC (petroleum ether: ethyl acetate = 25:1) and yielded compound **3** (28 mg) and **6** (65 mg). Fr.5 was further purified with silica gel CC (petroleum ether: ethyl acetate = 19:1) and recrystallized to give compound **2** (31 mg). Fr.8 was further purified with silica gel CC (petroleum ether: ethyl acetate = 25:1) and recrystallized to give compound **5** (22 mg).

The ethyl acetate extract was separated through silica gel CC (200–300 mesh), eluted with petroleum ether/acetone gradient system and yielded 10 fractions. Fr.5 was purified with silica gel CC and recrystallized (petroleum ether : ethyl acetate = 15 : 1) to yield compound **4** (13 mg).

2.3. In Vitro Anti-Inflammatory Assays and Cytotoxicity Testing. These experiments were carried out as previously described [3, 4]. Briefly, RAW264.7 cells grown on 25 cm² culture flasks were harvested and seeded in 96-well plates at 2×10^5 cells/well for NO production. The plates were pretreated with various concentrations of samples for 30 min and then incubated for 24 h with or without 50 µg/mL of LPS. The nitrite concentration in the culture supernatant was measured by the Griess reaction. Cell viability was measured by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Sigma-Aldrich).

3. Results and Discussion

3.1. Identification and Structure Elucidation of Compound 2

Compound **2** was obtained as colorless acicular crystal (CHCl₃-MeOH), m.p. 206–208°C. Its Liebermann-Burchard reaction was positive, with a molecular formula of C₃₀H₅₀O based on ¹³CNMR and positive HREIMS data at 426.3855. The IR (KBr) spectra showed the absorptions for hydroxyl group (3423 cm⁻¹) and double bond (1635 cm⁻¹). The ¹³C NMR spectra revealed 30 carbon signals which were sorted by DEPT experiment as six methyls, thirteen methylenes, five methines, six quaternary carbons, and two olefinic carbons (one = CH₂ and one quaternary).

The ¹H NMR and ¹³CNMR spectra of compound **2** showed signals for five tertiary methyls (δ_H 0.72, 0.81, 0.95, 0.95, 0.96), one vinylic methyl (δ_H 1.75), and two protons of an isopropenyl moiety (δ_H 4.77). One vinylic methyl at δ 1.75 (3H, s, Me-30), two protons of an isopropenyl moiety at δ 4.78. These showed closely similar ¹³CNMR chemical shifts with the reported hopane triterpene [5], except for the side chain carbons, indicating that this compound also possesses a hopane skeleton. The conclusion was confirmed by the HMBC and NOE correlations from compound **2**: in the HMBC spectra, the H₂-29 showed correlations with the carbon at δ 25.0 (C-30) and δ 46.5 (C-21). The chemical shift of two protons of terminal alkenes of H-29 at δ 4.78 also supported the hopane skeleton. According to the literature [6], if the skeleton was lupane, the chemical shift of H-29 should be at around 4.57 and 4.69, so the skeleton was hopane rather than lupane.

Additionally, further signals were observed at the presence of a hydroxymethylene group {δ_H: 3.44 (1H, d, *J* = 10.8 Hz), 3.73 (1H, d, *J* = 10.8 Hz); δ_C: 65.5}. It was proved by HSQC spectrum that δ_C 65.5 was both connected with δ_H 3.44 and 3.73. While δ_H 3.44 and 3.73 showed long-rang connection with δ_C 26.8, 35.6, 38.6, and 56.8, it was speculated to be a hydroxyl group linked to C-23 or C-24. The key HMBC correlations were shown in Figure 1. The ¹³C

FIGURE 1: Key HMBC correlations of compound **2**.

FIGURE 2: The key NOE correlations of compound **2**.

and ¹H NMR data and key HMBC correlations were shown in Table 1.

In the NOESY spectrum, H-25 showed NOE correlations with H-24 and H-26, but has no NOE correlations with H-23 or H-27. H-29 showed NOE correlations with H-27, H-28, and H-30. The key NOE correlations were shown in Figure 2. It was reported that when the allyl was linked to C-21 in α-orientation, H-29 was a single signal [5]. So C-24, C-25, and C-26 were determined to be in β-oriented while C-23, C-27, C-28, C-29, and C-30 were determined to be in α-oriented. Therefore, the structure of compound **2** was determined to be hopane-22(29)-en-24ol.

According to the literature [7], compounds **1** and **3** were identified as hop-22(29)-ene and adiantone, respectively. By comparison with literature data [8, 9], the compounds **4**, **5**, and **6** were identified as 22-hydroxyhopane, 6α, 22-dihydroxyhopane, and 17(21)-hopene, respectively.

3.2. The Cytotoxicity of Compounds 1–6 in RAW264.7 Cells. We determined the cytotoxicity of the six pentacyclic triterpenes on RAW264.7 cells by MTT assay (Figure 3). Cell viability was not affected 24 hours after the treatment with the six pentacyclic triterpenes at 200 µM. Therefore, concentrations up to 200 µM were selected for subsequent experiments.

Cells were incubated in the presence of the six compounds, respectively. Cell viability was measured by the MTT assay. Data are expressed as the means ± S.D. of the six different compounds. The six compounds at 200 µM were not cytotoxic.

TABLE 1: ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for compound **2** in CDCl_3 .

No	^1H -NMR	^{13}C -NMR	HMBC (H $\rightarrow$ C)
1	0.82 (m), 1.67 (m)	40.4	—
2	1.38 (m), 1.51 (m)	18.3	—
3	0.93 (m), 1.75 (m)	35.6	—
4	—	38.6	—
5	0.87 (dd, $J = 12.4, 1.6$)	56.8	—
6	1.30 (m), 1.59 (m)	18.9	—
7	1.21 (m), 1.45 (m)	33.6	—
8	—	42.0	—
9	1.26 (1H, d, $J = 12.4$)	50.1	—
10	—	37.0	—
11	1.27 (m), 1.52 (m)	21.1	—
12	1.37 (m), 1.43 (m)	24.0	—
13	1.35 (1H, d, $J = 2.8$)	49.0	—
14	—	41.9	—
15	1.22 (m), 1.39 (m)	33.7	—
16	1.41 (m), 1.63 (m)	21.6	—
17	1.39 (m)	54.9	—
18	—	44.8	—
19	1.02 (1H, d, $J = 7.2$), 1.60 (1H, dd, $J = 7.2, 3.2$)	41.9	—
20	1.82 (m), 1.85 (m)	27.4	—
21	2.6 (1H, td, $J = 10.4, 7.2$)	46.5	—
22	—	148.8	—
23	0.96 (3H, s)	26.8	$\text{C}_3, \text{C}_4, \text{C}_5, \text{C}_{24}$
24	3.7 (1H, d, $J = 10.8$), 3.4 (1H, d, $J = 10.8$)	65.5	$\text{C}_3, \text{C}_4, \text{C}_5, \text{C}_{23}$
25	0.81 (3H, s)	16.4	$\text{C}_1, \text{C}_5, \text{C}_9, \text{C}_{10}$
26	0.95 (3H, s)	16.6	$\text{C}_7, \text{C}_8, \text{C}_9, \text{C}_{10}$
27	0.95 (3H, s)	16.7	$\text{C}_{14}, \text{C}_{15}$
28	0.72 (3H, s)	16.1	$\text{C}_{13}, \text{C}_{17}, \text{C}_{18}, \text{C}_{19}$
29	4.77 (2H, s)	110.3	$\text{C}_{21}, \text{C}_{22}, \text{C}_{30}$
30	1.75 (3H, s)	25.0	$\text{C}_{21}, \text{C}_{22}, \text{C}_{29}$

3.3. Assay for Inhibitory Activities against NO Production. The potent anti-inflammation of some triterpenes had received considerable attention, such as triterpene alcohols from the flowers of compositae [10]. Therefore, it was meaningful to investigate the anti-inflammatory effects of these pentacyclic triterpene isolated from *H. tyermanni*. In this study, compounds **1–6** were tested for inhibitory activities against LPS-induced NO production to RAW264.7 at a concentration range from 12.5 μM to 200 μM (Figure 4). The IC_{50} values were in the range of 50–100 μM except for compounds **1** and **6**, which were attributed to the absence of the hydroxyl group. Four compounds (compounds **2**, **3**, **4**, and **5**) with oxygen as hydroxyl or carbonyl groups exhibited good inhibitory activities against LPS-induced NO production in RAW264.7 macrophages. Interestingly, the compound **2** with a 24-OH

exhibited better inhibitory activity than the compound **1** without a hydroxyl. So, it was inferred that the 24-OH may play an important role in inhibiting NO production in RAW264.7 macrophages. Although there were not enough pentacyclic triterpenes to discuss the structure-activity relationship, our data offered a reference that pentacyclic triterpenes were an important source of new anti-inflammatory agents.

4. Conclusion

In conclusion, One new pentacyclic triterpene from *H. tyermanni*, hopane-22(29)-en-24ol, was isolated and elucidated on the basis of 1D and 2D NMR spectral techniques. In addition, it exhibited stronger inhibitory activity than that of

FIGURE 3: Cytotoxicity of compounds 1–6 in RAW264.7 cells.

FIGURE 4: Inhibitory effects of the six pentacyclic triterpene compounds 1–6 isolated from *H. tyermanni* Moore LPS-induced NO production in RAW264.7 macrophages ($n = 4$). The NO level was assayed by Griess reagent.

the other five known compounds against LPS-induced NO production in RAW264.7 macrophages.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 20772047, 21310104) and the Natural Science Foundation of Guangdong Province (no. 32210018).

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