

A New Megastigmane Glycoside and Anti-Inflammatory Bibenzyls from the Stems of *Dendrobium henanense*

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Abstract: A new megastigmane glycoside, namely (9S)-O-β-D-glucopyranosyl-2,5-megastigmen-4-one (**1**), along with seven known bibenzyls (**2-8**) were isolated from the aerial stems of *Dendrobium henanense*. Their structures were elucidated by spectroscopic and mass-spectrometric analyses, including 1D-, 2D-NMR and HR-MS. The anti-inflammatory activities of compounds **1-8** evaluated its anti-inflammatory activity by inhibiting the production of inflammatory cytokines such as NO, TNF-α and IL-6 in LPS-stimulated RAW264.7 macrophages. Compounds **3, 4, 6-8** exhibited moderated anti-inflammatory activity with IC₅₀ values of below 100 μM.

Keywords: *Dendrobium henanense*; megastigmane glycoside; anti-inflammatory; RAW264.7 macrophages. © 2023 ACG Publications. All rights reserved.

1. Plant Source

The aerial stems of *D. henanense* were donated by Changchong Traditional Chinese Medicine Development Co. Ltd, Huoshan County, Anhui province, in September 2019, and identified by Prof. Shoujin Liu, Anhui University of Traditional Chinese Medicine. A voucher specimen (ACM2019091801) was stored at the Herbarium Anhui College of Traditional Chinese Medicine.

2. Previous Studies

Dendrobium henanense is a perennial herb of the Orchidaceae family, commonly known as "little dendrobium" (Henan) [1]. It was a new species published by Alex Lu, titler of the new species, in 1990 [2]. It is mainly distributed in Henan Province, such as Xiaoqinling mountain of Lingbao

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County, Taiping town of Xixia County, Buckwheat Mountain of Nanzhao County and others. *D. henanense* grows in the high-altitude area of 680-1260 meters and has strong cold resistance. At present, *D. henanense* is a relatively expensive medicinal material due to the scarcity of plants [3-4]. So far, little has been reported about its chemical compositions and pharmacological activities.

3. Present Study

The crude powder of *D. henanense* (6.3 kg) was soaked with 70% acetone (50 L×3) at room temperature. The solution was filtrated and evaporated under reduced pressure to give a concentrated liquid (6.0 L), which was partitioned with petroleum ether (PE), ethyl acetate (EtOAc) and *n*-BuOH to give each organic fraction, respectively.

The ethyl acetate fraction (140 g) was subjected to silica gel CC using a CH₂Cl₂-MeOH gradient elution from 100: 0 to 80: 20 (v/v) to give *Fr.1*–*Fr.5*. Subfraction *Fr.2* (17 g) was subjected to a MCI gel CC with MeOH-H₂O gradient elution from 80: 20 to 100: 0 (v/v) to get *Fr.2-1*- *Fr.2-4*. *Fr.2-2* was further purified by Sephadex LH-20 (CH₂Cl₂-MeOH 1:1), to give compound **2** (3.28 mg). *Fr.3* (48 g) was separated by MCI gel CC using a MeOH-H₂O gradient elution from 60: 40 to 95: 5 (v/v) to obtain *Fr.3.1* – *Fr.3.5*. *Fr.3.2* was further purified by silica gel CC eluting with suitable solvents systems (CH₂Cl₂-MeOH), and then separated by Sephadex LH-20 and then prep-HPLC on a YMC-Pack-ODS-A (10.0 mm × 250 mm, S-5 μm, 12 nm) to afford **3** (8 mg) and **4** (10 mg). *Fr.3.3* was further separated on a MPLC to obtain *Fr.3.3.1*–*Fr.3.3.3*. *Fr.2.3.2* was passed through silica gel CC to obtain fractions *Fr.3.3.2.1*–*Fr.3.3.2.2*. And then *Fr.3.3.2.1* was further purified by Sephadex LH-20 to acquire compound **5** (18 mg). *Fr.3.3.2.2* was further purified by silica gel CC eluting with CH₂Cl₂-MeOH (95:5) to obtain *Fr.3.3.2.2.1*–*Fr.3.3.2.2.2*, then the first fraction was subjected to a HPLC-preparation to afford **6** (3.8 mg, *t_R* = 48 min, MeOH-H₂O = 60: 40, 2.5 mL·min⁻¹, λ = 254/280 nm), and the other part was purified on Sephadex LH-20 (MeOH) to obtain compound **7** (39 mg). *Fr.3.5* was preliminarily divided by a MPLC to acquire *Fr.3.5.1* and *Fr.3.5.2*. *Fr.3.5.2* (233 mg) was performed on Sephadex LH-20 (CH₂Cl₂-MeOH), silica gel CC with PE-EtOAc (2:1) and then purified by Sephadex LH-20 (MeOH) to obtain **8** (6.2 mg). *Fr.4* (12 g) was subjected to silica gel CC (CH₂Cl₂-MeOH) to get three fractions (*Fr.4.1*- *Fr.4.3*), *Fr.4.2* was purified on Sephadex LH-20 (MeOH) and HPLC-preparation to acquire compound **1** (8 mg, *t_R* = 15 min, MeOH-H₂O = 35: 65, 3.0 mL·min⁻¹, λ = 270 nm).

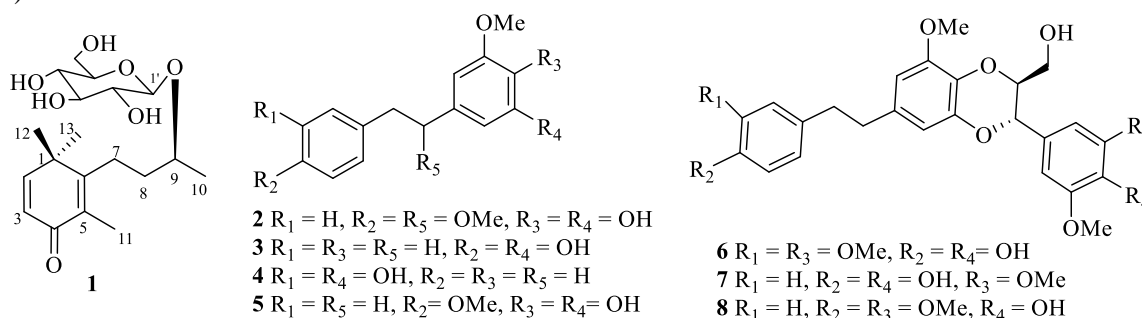


Figure 1. Structure of compounds **1-8** isolated from *D. henanense*

(*9S*)-*O*-β-*D*-glucopyranosyl-2,5-megastigmen-4-one (**1**): White needle crystals, $[\alpha]_D^{25} -11.2$ (*c* 0.145, MeOH); UV(MeOH) λ_{max} (log ϵ) = 237 (3.95) nm, 266 (3.70) nm; CD [CH₃CN, nm ($\Delta\epsilon$): 225(-2.66), 250(+3.02), 329(-0.20). IR (KBr): ν_{max} 3416, 3042, 2965, 2926, 2876, 1730, 1657, 1619, 1458, 1380, 1076, 1034, 1023 cm⁻¹; ESI-MS *m/z* 369 [M - H]⁻, 405 [M + Cl]⁻, 415 [M + COOH]⁻, 739 [2M - H]⁻; HRESIMS *m/z* 369.1924 [M - H]⁻ (calcd for C₁₉H₂₉O₇, 369.1919). ¹H-NMR (400 MHz, MeOD) and ¹³C-NMR (150 MHz, MeOD) data, see Table 1.

Compound **1** was obtained as white amorphous powder. Its quasi-molecular ion at *m/z* 369.1924 [M - H]⁻ suggested a molecular formula of C₁₉H₃₀O₇ (calcd for C₁₉H₂₉O₇, 369.1919) with five

degrees of unsaturation. FT-IR spectrum showed the absorption bands at 3042, 1730, 1659 and 1619 cm^{-1} ascribable to an olefin, carbonyl functions, a broad band at 3416 cm^{-1} suggestive of hydroxyl groups, and a pyranose characteristic bands at 1076, 1034 and 1023 cm^{-1} [5]. The ^1H -NMR spectrum (400 MHz, CD_3OD) (Table 1) presented a double methyl signals at δ 1.30 (3H, d, $J = 6.4$ Hz), three singlet methyl signals at δ 1.89 (3H, s) and 1.29 (3H \times 2, s); an AB-system signals at δ 6.12 (1H, d, $J = 9.8$ Hz) and 6.92 (1H, d, $J = 9.8$ Hz); and an anomeric proton signal at δ 4.37 (1H, d, $J = 7.8$ Hz). The ^{13}C -NMR spectrum (Table 1) showed 19 carbon signals, including four methyl groups at δ 26.2 (C-12), 26.1(C-13), 22.1(C-10) and 11.7 (C-11); two methylene at δ 27.5 (C-7) and 36.4 (C-8), one oxygen-bearing methine at δ 77.9, two sets of olefinic carbon resonances at δ 126.2, 132.6, 160.5 and 166.0, as well as a conjugated ketone carbonyl at δ 188.8; In addition, a group of hexose resonances at δ 104.8, 78.6, 78.1, 75.6, 71.9 and 63.0 were observed. The above NMR data were similar to those of (9*R*)-*O*- β -D-glucopyranosyloxy-2,5-megastigmen-4-one [6]. The ^1H - ^1H COSY spectra (Figure 2) showed correlations of H-7/H-8/H-9/H10 and H-2/H-3. The expected correlations in the HMBC spectra

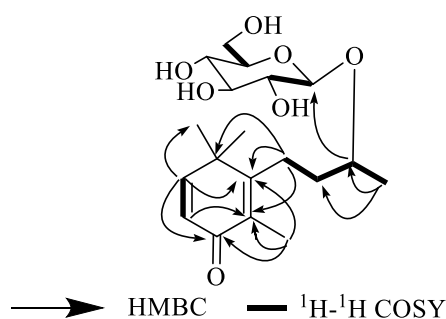


Figure 2. The selected HMBC and ^1H - ^1H COSY correlations of compound 1

Table 1. ^1H - and ^{13}C -NMR data for compound 1 compared with compound in reference in CD_3OD

Position	1 ^a		compound in reference ^b	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	42.5	-	42.4	-
2	160.5	6.92 (1H, d, 9.8 Hz)	160.4	6.94 (1H, d, 10.5 Hz)
3	126.2	6.12 (1H, d, 9.8 Hz)	126.2	6.14 (1H, d, 10.5 Hz)
4	188.8	-	188.8	-
5	132.6	-	132.5	-
6	166.0	-	166.3	-
7	27.5	2.66 (1H, m) 2.44 (1H, m)	27.9	2.68 (1H, m) 2.44 (1H, m)
8	36.4	1.68 (1H, m) 1.64 (1H, m)	37.1	1.68 (2H, m)
9	77.9	3.91 (1H, m)	75.8	3.99 (1H, m)
10	22.1	1.30 (3H, d, 6.4 Hz)	20.0	1.24 (3H, d, 6.2 Hz)
11	11.7	1.89 (3H, s)	11.7	1.88 (3H, s)
12	26.2	1.28 (3H, s)	26.1	1.27 (3H, s)
13	26.1	1.28 (3H, s)	26.1	1.27 (3H, s)
1'	104.8	4.37 (1H, d, 7.8 Hz)	102.4	4.36 (1H, d, 7.7 Hz)
2'	75.6	3.17 (1H, t, 8.8 Hz)	75.3	3.18 (1H, m)
3'	78.6	3.33 (1H, t, 8.4 Hz)	78.3	3.37 (1H, m)
4'	71.9	3.30 (1H, m)	72.0	3.28 (1H, m)
5'	78.1	3.26 (1H, m)	78.1	3.27 (1H, m)
6'	63.0	3.87 (1H, d, 12.0 Hz) 3.67 (1H, dd, 12.0, 4.4 Hz)	63.1	3.87 (1H, dd, 11.8, 1.5 Hz) 3.67 (1H, dd, 11.8, 5.3 Hz)

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^aData were measured on δ_{H} 400 MHz and δ_{C} 150 MHz, J in Hz. Assignments were based on 2D-NMR experiments. ^b The corrected data were displayed based on its supplement materials.

(Figure 2) as follows: from the H-2 to C-4, C-6, C-12 and C-13; H-3 to C-1 and C-5; H-7 to C-5, C-6 and C-1; H-11 to C-4, C-6 and C-5. Furthermore, the D-glucopyranosyl was confirmed by the results of acid hydrolysis [7], and the correlations of H-9 (1H, sextet, $J = 6.4$ Hz) to C-1' indicated the hexose unit was attached to C-9 via a β -linkage (H-1', $J = 7.8$ Hz). The absolute configuration of C-9 was assigned as "S" based on the chemical shift values of C-9 (δ 77.9), C-10 (δ 22.0) and C-1' (δ 104.8) in CD₃OD [8]. In addition, the experimental ECD spectrum of compound **1** showed a negative Cotton effect at 225 nm and a positive Cotton effect at 250 nm. The calculated ECD of (9R) -1a showed opposite Cotton effects at 225 and 250 nm. Therefore, the absolute configuration of C-9 was confirmed by the calculated and experimental ECD spectra. (Figure S19).

The known isolates were identified as dendrocandin A (**2**) [9], 3, 4'-dihydroxy-5-methoxy bibenzyl (**3**) [10], 3,3'- dihydroxy-5-methoxy bibenzyl (**4**) [11], 4',5-dihydroxy-3,4-dimethoxybibenzyl (**5**) [12], dendrocandin T (**6**) [13], Dendrocandin U (**7**) [13] and Dendrocandin B (**8**) [9] based on comparison with NMR and MS data in the references.

Compounds **1-8** were showed no or weak cytotoxic activities against RAW264.7 cells at the concentrations of below 100 μM by CCK-8 method [14]. The anti-inflammatory activities of all compounds were evaluated by inhibiting the production of NO, TNF- α and IL-6 in LPS-induced macrophage cells [15-16], and the IC₅₀ value of each compound was calculated on GraphPad Prism software, version 4.00 (GraphPad Software Inc., San Diego, CA, USA). All of the experiments were performed in triplicate. Compounds **3**, **4**, **6-8** exhibited moderated anti-inflammatory activity, while other compounds were inactive (Table 2).

Table 2. The IC₅₀ values of all tested compounds against NO, TNF- α and IL-6 (μM)

No.	NO	TNF- α	IL-6
1	> 100	> 100	> 100
2	> 100	> 100	> 100
3	61.82±0.99	52.71±0.86	59.45±0.63
4	89.83±0.45	80.85±0.82	88.89±0.62
5	> 100	> 100	> 100
6	59.03±0.82	48.79±0.93	66.50±0.59
7	46.77±0.97	37.22±0.82	56.25±0.73
8	77.61±0.05	67.51±0.88	82.20±0.99
Dexamethason	11.01±0.84	8.78±1.0	21.94±0.98

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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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