SHORT REPORT

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

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Abstract: A new megastigmane glycoside, namely (9*S*)-*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 IC_{50} values of below 100 *μ*M.

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 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.

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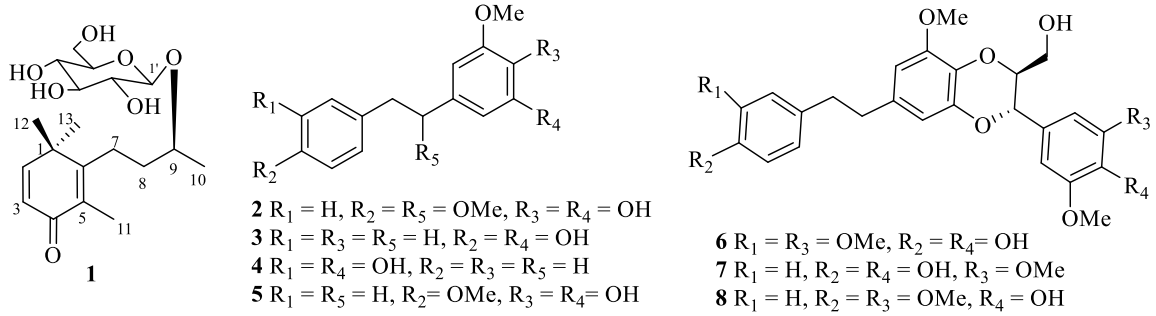
3. Present Study

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 CH_2Cl_2–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-H_2O 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 (CH_2Cl_2-MeOH 1:1), to give compound **2** (3.28 mg). *Fr.3* (48 g) was separated by MCI gel CC using a MeOH-H_2O 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 (CH_2Cl_2-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 CH_2Cl_2-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-H_2O = 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 (CH_2Cl_2-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 (CH_2Cl_2-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-H_2O = 35: 65, 3.0 mL·min^{-1}, λ = 270 nm).



2 R_1 = H, R_2 = R_5 = OMe, R_3 = R_4 = OH
3 R_1 = R_3 = R_5 = H, R_2 = R_4 = OH
4 R_1 = R_4 = OH, R_2 = R_3 = R_5 = H
5 R_1 = R_5 = H, R_2= OMe, R_3 = R_4= OH

6 R_1 = R_3 = OMe, R_2 = R_4= OH
7 R_1 = H, R_2 = R_4 = OH, R_3 = OMe
8 R_1 = H, R_2 = R_3 = OMe, R_4 = OH

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 [CH_3CN, nm (Δε)]: 225(-2.66), 250 (+3.02), 329 (-0.20). IR (KBr): υ_{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 $C_{19}H_{29}O_7$, 369.1919). ^{1}H-NMR (400 MHz, MeOD) and ^{13}C-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 $C_{19}H_{30}O_7$ (calcd for $C_{19}H_{29}O_7$, 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 ^{1}H-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 × 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 ^{1}H-^{1}H 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

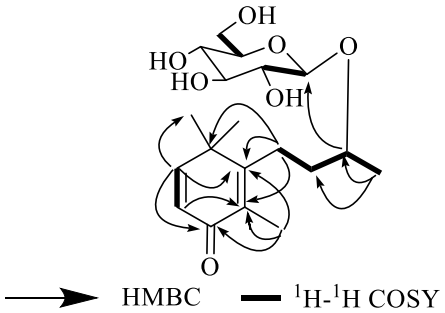


Figure 2. The selected HMBC and ^{1}H-^{1}H COSY correlations of compound **1**

Table 1. ^{1}H- 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)

[a]Data 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 CD_3OD [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 IC_{50} 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 IC_{50} 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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References

[1] X. S. Bao, Q. S. Shun and X. S. Wang (2012). *Dendrobium huoshanense*, Shanghai Jiao Tong University Press, Shanghai.

[2] J. L. Lu and L. X. Gao (1990). A new species of Dendrobium from Henan, China, *Bull. Bot. Res.* **10**, 29-31.

[3] J. L. Lu and P. J. Wang (1990). Rare and endangered plants in Henan Province, Henan University Press. Kaifeng.

[4] W. D. Feng (2017). Atlas of rare plants in Henan, Henan Science and Technology Press, Zhengzhou.

[5] J. X. Xie (1987). Applications of IR Spectroscopy in organic and medicinal chemistry, Science Press. Beijing.

[6] J. M. Xu, B.C. Hu, L. Yuan, Y. L. Wu, S. S. Luan, T. Yuan, D. Wang and L. X. Chen (2019). Labdanes and megastigmanes from *Vitex negundo var. heterophylla*. *Fitoterapia* **137**, 104265.

[7] F. Q. Xu, N. Wang, W. W. Fan, C. T. Zi, H. S. Zhao, J. M. Hu, J. Zhou (2016). Protective effects of cycloartane triterpenoides from *Passiflora edulis* Sims against glutamate-induced neurotoxicity in PC12 cell, *Fitoterapia* **115**, 122–127.

[8] K. Matsunami, H. Otsuka and Y. Takeda (2010). Structural revisions of Blumenol C glucoside and Byzantionoside B, *Chem. Pharm. Bull*, **58**, 438–441.

[9] Y. Li, C. L. Wang, S. X. Guo, J. S. Yang and P. G. Xiao (2008). Two new compounds from *Dendrobium candidum,. Chem. Pharm. Bull*. **56**, 1477–479.

[10] M. Wang, C. F. Zhang, Z. T. Wang and M. Zhang (2007). Studies on constituents of *Dendrobium gratiosissimum, Chin. J. Chin. Mater. Pharm*. **24**, 701–703.

[11] H. S. Zhao, F. Q. Xu, X. X. Chen, J. M. Hu, F. J. Zeng, D. Y. Peng and D. L. Wu (2021). Chemical constituents of *Dendrobium huoshanense* C. Z. Tang et S. J. Chen, *Nat. Prod. Res. Dev.* **33**, 1491–1498.

[12] R. M. Letcher, L. R. M. Nhamo and I. T. Gumiro (1972). Chemical constituents of the *Combretaceae*. Part II. Substituted phenanthrenes and 9,10-dihydrophenanthrenes and a substituted bibenzyl from the heartwood of *Combretum mole*, *J. Chem. Soc. Perkin Trans*. **1**, 206–210.

[13] L. Yang, S. J. Liu, H. R. Luo, J. Cui, J. Zhou, X. J. Wang, J. Sheng and J. M. Hu (2014). Two new dendrocandins with neurite outgrowth-promoting activity from *Dendrobium officinale*, *J. Asian Nat. Prod. Res*. **17** (**2**), 125–131.

[14] J. Tang, P. Diao, X. Shu, L. Li and L. Xiong (2019). Quercetin and quercitrin attenuates the inflammatory response and oxidative stress in LPS-induced RAW264.7 cells: in vitro assessment and a theoretical mode, *BioMed. Res. Int*. 7039802, doi: 10.1155/2019/7039802.

[15] N. H. Dang, L. N. Thanh, D. H. Giang and N. T. Dat (2022). Anti-inflammatory components from the fruits of *Amomum aromaticum*, *Rec. Nat. Prod*. **16**, 236–241.

[16] F. Aktan (2004). iNOS-mediated nitric oxide production and its regulation, *Life Sci*. **75**, 639–653.

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