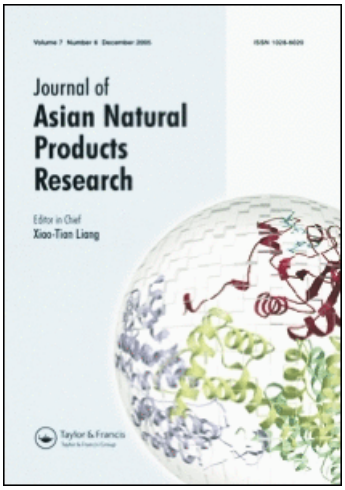

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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[a] Shenyang Northern Hospital, Shenyang, China [b] Shenyang Pharmaceutical University, Shenyang, China

Online publication date: 21 April 2010

To cite this Article Zhao, Qing-Chun , Hua, Wei , Zhang, Lin , Guo, Tao , Zhao, Ming-Hong , Yan, Ming and Wu, Li-Jun(2010) 'Two new alkaloids from *Gelsemium elegans*', Journal of Asian Natural Products Research, 12: 4, 273 — 277
To link to this Article: DOI: 10.1080/10286021003689809
URL: http://dx.doi.org/10.1080/10286021003689809

Journal of Asian Natural Products Research
Vol. 12, No. 4, April 2010, 273–277

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ORIGINAL ARTICLE

Two new alkaloids from *Gelsemium elegans*

Qing-Chun Zhao[a]*, Wei Hua[b], Lin Zhang[b], Tao Guo[a], Ming-Hong Zhao[a], Ming Yan[a] and Li-Jun Wu[b]

[a]*Shenyang Northern Hospital, Shenyang 110016, China;* [b]*Shenyang Pharmaceutical University, Shenyang 110016, China*

(*Received 13 November 2009; final version received 19 January 2010*)

Two new alkaloids, named gelsenine (**1**) and 11-methoxyhumantenmine (**2**), were isolated from the whole plant of *Gelsemium elegans*. The structures were elucidated on the basis of 1D NMR, 2D NMR, and MS methods.

Keywords: *Gelsemium elegans*; gelsenine; 11-methoxyhumantenmine; oxindol alkaloid

1. Introduction

Gelsemium elegans Benth. is a well-known toxic plant in southeastern Asia, which is used in Chinese folk medicine as an analgesic, antispasmodic, and antitumor agent [1]. The continuing interest in the chemistry of *Gelsemium* alkaloids comes largely from the potent biological activity and special chemical structure [2–10]. As part of our systematic studies on the chemical constituents of Chinese medicinal plants, we initiated a chemical study on *G. elegans*. By the general isolation methods, two new alkaloids, named gelsenine (**1**) and 11-methoxyhumantenmine (**2**) (Figure 1), were isolated from the whole plant. On the basis of a spectroscopic analysis, these chemical structures were determined. In this paper, the isolation and structural elucidation of these compounds are described.

2. Results and discussion

Compound **1** was obtained as a pale yellow amorphous powder. The molecular formula $C_{20}H_{26}N_2O_4$ was confirmed by HR-MS at *m/z* 359.1977 $[M+H]^+$. It gave a brown spot in normal-phase TLC ($CHCl_3$–MeOH, 90:10) with Dragendorff–Wagner (1:1) reagent. The UV absorption maxima of **1** at 209 ($\log \varepsilon$ 4.25) and 257 nm ($\log \varepsilon$ 3.80) revealed an oxindole chromophore [3]. In the IR spectrum, **1** showed absorption bands at 3423 cm^{-1} (NH group), 1705 cm^{-1} (carbonyl group), 1616 and 1463 cm^{-1} (aromatic ring).

The ^1H NMR spectrum of **1** showed four aromatic protons due to the ring of the oxindole system, a N_a-methoxy group at δ 4.02 (3H, s), a methine group bearing amine nitrogen at δ 3.78 (br s, H-5), an oxymethine proton at δ 3.55 (d, H-3), oxymethylene protons at δ 4.27 (br s, H-18), oxymethylene protons at δ 3.28 (d) and 3.47 (d, H_2-17), and an ethyl group at δ 0.91 (3H, t, H_3-20) and 1.95 (2H, q, H_2-21). The ^{13}C NMR spectrum of **1** showed 20 carbons, including a methine

*Corresponding author. Email: zhaoqc53@yahoo.com.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286021003689809
http://www.informaworld.com

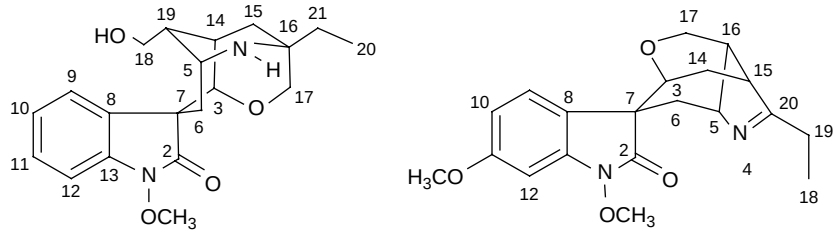

Figure 1. Chemical structures of compounds **1** and **2**.

bearing a nitrogen atom at δ 59.3 (C-5), a quaternary carbon bearing a nitrogen atom at δ 69.8 (C-16), an oxygenated methine at δ 74.8 (C-3), and two oxygenated methylenes at δ 62.7 (C-17) and 63.4 (C-18).

In the HMBC spectrum, correlations of H-3 with C-6, C-8, and C-14, H-6 with C-2, C-8, C-3, and C-7, H-18 with C-5, C-14, and C-19, H-15 with C-3 and C-16, H-14 with C-5 and C-15, H-17 with C-14, C-15, and C-21, H-21 with C-16 and C-17 allowed to deduce the presence of three six-member rings which are connected to each other (Figure 2).

The stereochemistry of **1** was determined by the NOE correlations (Table 1). H-18 showed a clear correlation with H-9, suggesting that this compound has a C-19a-H. This was further confirmed by the NOESY correlation between H-19 and H-15a. The NOE correlation between H-9 and one of the C-6 methylene proton signals at δ 2.18 led to the assignment

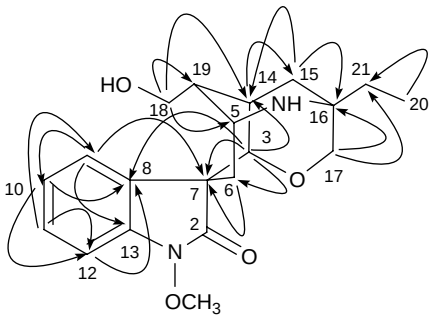

Figure 2. Selected HMBC correlations of compound **1** (H → C).

of this signal as H-6, whereas the remaining signal at δ 2.04 is for H-6β. The NOE correlation between H-20, H-21, and H-17β indicates the probable assignment of the proton NMR resonances of the C-17 methylene protons. The NOE correlation between H-15a and H-19 also indicates the assignment of the NMR proton resonances of C-15 methylene protons. Thus, all of the protons and functional groups were assigned and the complete structure of **1** was elucidated, named gelsenine. The HMBC, $^{1}H-^{1}H$ COSY, and NOESY results of **1** led to assignments for the ^{1}H and ^{13}C signals, as shown in Table 1.

Compound **2** was obtained as a pale yellow amorphous powder. The molecular formula $C_{20}H_{24}N_2O_4$ was confirmed by HR-MS at *m/z* 357.1815 $[MM+H]^+$. It gave a brown spot in normal-phase TLC ($CHCl_3$–MeOH, 90:10) with Dragendorff–Wagner (1:1) reagent. The UV absorption maxima at 257 and 209 nm revealed an oxindole system [3]. The ^{1}H NMR spectrum displayed three aromatic proton signals at δ 7.41 (1H, d, $J = 8.3$ Hz, H-9), 6.56 (1H, dd, $J = 8.3$, 2.4 Hz, H-10), and 6.47 (1H, d, $J = 2.4$ Hz, H-12), forming an ABX system; two methoxyl signals at δ 3.94 (3H, s) and 3.81 (3H, s); one ethyl signal at δ 1.29 (3H, t, $J = 7.4$ Hz, H-18), 2.73 (1H, dq, $J = 17.0$, 7.4 Hz, H-19α), and 2.45 (1H, dq, $J = 17.0$, 7.4 Hz, H-19β). The ^{13}C NMR and HMQC spectra of compound **2** exhibited 20 carbon signals, including one

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data of gelsenine (**1**).[a]

Position	δ_H	δ_C	HMBC[b]		COSY	NOESY[c]
			Two-bond	Three-bond		
2		174.7				
3	3.55 (1H, d, $J = 6.0$ Hz)	74.8	14	6, 8, 17	14	9
5	3.78 (1H, br s)	59.3			6a, 19	
6a	2.18 (1H, dd, $J = 16.0$, 3.4 Hz)	33.4	7	2, 3, 8	5, 6β	9, 18
6β	2.04 (1H, d, $J = 16.0$ Hz)		7	3	6a	
7		57.3				
8		131.3				
9	7.40 (1H, d, $J = 7.6$ Hz)	125.3	10	7, 11, 13	10	3, 6α, 18
10	7.14 (1H, t, $J = 7.6$ Hz)	123.9	9	8, 12	9, 11	
11	7.31 (1H, t, $J = 7.6$ Hz)	128.4	12	9	10, 12	
12	6.97 (1H, d, $J = 7.6$ Hz)	107.3		8, 10	11	OCH_3
13		137.9				
14	2.04 (1H, m)	36.2	15	5	3, 15β, 19	18
15a	2.28 (1H, d, $J = 14.0$ Hz)	22.9	14, 16		15β	19, 20, 21
15β	2.11 (1H, over)		14, 16	3	14, 15a	20, 21
16		69.8				
17a	3.28 (1H, d, $J = 10.4$ Hz)	62.7	16	15, 21	17β	
17β	3.47 (1H, d, $J = 10.4$ Hz)		16	15, 21	17a	20,21
18	4.27 (2H, br s)	63.4	19	5, 14	19	6α, 9, 14
19	2.79 (1H, br m)	39.5			5, 14, 18	15α
20	0.91 (3H, t, $J = 7.4$ Hz)	9.4	21	16	21	15α, 15β, 17β
21	1.95 (2H, q, $J = 7.4$ Hz)	23.0	16, 20	17	20	15α, 15β, 17β
—N—O—CH_3	4.02 (3H, s)	63.4				12

Notes: [a]$CDCl_3$ was used as a solvent; chemical shifts (δ) in ppm; coupling constants J in Hz.
[b] Numbers in each column, respectively, indicate the carbons coupled with the proton through two or three bonds.
[c] NOESY spectra were obtained at 600 MHz.

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound **2**.[a]

Position	δ_H (Hz)	δ_C
2		171.7
3	3.71 (dd, 4.5, 2.0)	75.3
5	4.40 (m)	72.1
6a	2.37 (m)	37.8
6β	2.27 (dd, 14.3, 2.3)	
7		55.4
8		124.0
9	7.41 (d, 8.3)	125.4
10	6.56 (dd, 8.3, 2.4)	107.8
11		160.1
12	6.47 (d, 2.4)	93.9
13		139.1
14a	2.36 (m)	26.9
14β	2.14 (ddd, 14.8, 10.0, 4.7)	
15	2.87 (t, 9.2)	42.5
16	2.57 (t, 8.1)	39.7
17	4.27 (2H, m)	62.0
18	1.29 (3H, t, 7.4)	9.9
19a	2.73 (dq, 17.0, 7.4)	25.6
19β	2.45 (dq, 17.0, 7.4)	
20		184.8
—OCH_3	3.81 (3H, s)	55.5
—*N*—OCH_3	3.94 (3H, s)	63.4

Note: [a]$CDCl_3$ was used as a solvent. Chemical shifts (δ) in ppm.

carbonyl carbon, six aromatic carbons, two methoxyl carbons, one methyl carbon, four methylene carbons, four methine carbons, one quaternary carbon, and one imine carbon. The ^{1}H NMR and ^{13}C NMR spectra (see Table 2) were very similar to humantenmine [11] except for the signals in the aromatic region, namely the lack of an aromatic proton signal and the existence of a methoxyl group at δ_H 3.81 and δ_C 55.5. The HMBC correlations (Figure 3) between the proton at δ 3.81 (3H, s) and the carbon at δ_C 160.1 indicated that a methoxyl group was attached to the oxindole ring at C-11. Furthermore, the HMBC correlations between the protons at δ 2.73 (H-19α), 2.45 (H-19β) and the carbons at δ_C 184.8 (C-20), 42.5 (C-15) showed that an ethyl group was attached to the imine group C-20. From the above data, compound **2** was deduced to be 11-methoxyhumantenmine.

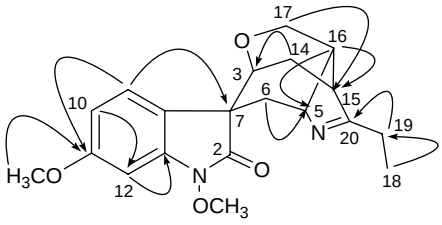

Figure 3. Selected HMBC correlations of compound **2** (H → C).

3. Experimental

3.1 General experimental procedures

UV spectra were recorded on a Shimadzu UV-2501PC spectrometer. Optical rotations were measured on a Perkin-Elmer digital polarimeter. IR spectra were taken on a Perkin-Elmer RX1 FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ using TMS as the internal standard on a Bruker-APX-300, Bruker-APX-400, and Bruker-APX-600 instrument. ESI-MS were obtained on a Bruker Esquire 2000 instrument. HPLC was performed on a JASCO LC-2000 instrument with a HiQ sil C_{18}V (250 × 10 mm, 5 μm) column using a UV detector. Column chromatography was carried out on a glass open column, and Sephadex LH-20 (Amersham Biosciences, Shanghai, China) and silica gel (200–300 mesh; Qingdao Haiyang Chemical Co., Ltd, China) were used as adsorbents.

3.2 Plant material

The specimens of *G. elegans* Benth. were collected from the Fujian Province of China (January 2003), and identified by Prof. Qishi Sun, Shenyang Pharmaceutical University, China. Herbarium voucher specimens (GE-03002) are deposited at the Department of Pharmacy, Shenyang Northern Hospital.

3.3 Extraction and isolation

The powdered whole plant of *G. elegans* was alkalified with 3% NaOH, dried at room temperature, and then extracted with $CHCl_3$. The chloroform extract was dissolved in the acid water, partitioned with $CHCl_3$, and then the water layer was alkalified with saturated NaOH, and extracted with $CHCl_3$ again, to obtain the alkaloids of *G. elegans*. The alkaloids were further subjected to a silica gel column, eluted with a step gradient solvent system of $CHCl_3$–MeOH (99:1 → 9:1, v/v) to obtain nine fractions (1–9). Fraction 8 (0.73 g) was repeatedly chromatographed on HPLC (250 × 10 mm, 5 μm) and eluted with MeOH–H_2O–diethylamine (65:35:0.01, v/v/v) to yield gelsenine (**1**, 4.1 mg) and 11-methoxyhumantenmine (**2**, 10.3 mg).

3.3.1 Compound **1**

A pale yellow amorphous powder, mp 180–182°C, $[\alpha]_D$ − 114 ($c = 0.81$, MeOH), UV (MeOH) λ_{max} (log ε): 208 (3.90), 254 (3.22) nm. IR (KBr) ν_{max} (cm^{-1}): 3422, 1718, 1615, 1463. ^1H and ^{13}C NMR spectral data (see Table 1). HR-FAB-MS: *m/z* 359.1977 $[MM+H]^+$ (calcd for $C_{20}H_{27}N_2O_4$, 359.1971).

3.3.2 Compound **2**

A pale yellow amorphous powder, mp 160–162°C, $[\alpha]_D$ − 74 ($c = 0.93$, MeOH), UV (MeOH) λ_{max} (log ε): 209 (4.25), 257 (3.80) nm. IR (KBr) ν_{max} (cm^{-1}): 3430, 1726, 1629, 1496. ^1H and ^{13}C NMR spectral data (see Table 2). HR-FAB-MS: *m/z* 357.1815 $[MM+H]^+$ (calcd for $C_{20}H_{25}N_2O_4$, 357.1814).

Acknowledgements

We wish to thank Prof. Qi-Shi Sun of Shenyang Pharmaceutical University, for identification of the plant materials. This work was supported by the Scientific Research Foundation for the Liaoning Province Doctoral Scholars (Q.C.Z., No. 20031040), Liaoning, China.

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