Two New 11-Hydroxy-Substituted Gelsedine-Type Indole Alkaloids from the Stems of *Gelsemium elegans*

by **Bin-Feng Zhang**[a])[b]), **Gui-Xin Chou***[a])[b]), and **Zheng-Tao Wang***[a])[b])

[a]) Key Laboratory of Standardization of Chinese Medicines, Ministry of Education, Institute of Chinese Materia Medica of Shanghai University of Traditional Chinese Medicine, Cai Lun Road 1200, Zhangjiang, Shanghai 201203, China (phone: +86-21-50271706, +86-21-51322507; fax: +86-21-50271708, +86-21-51322519; e-mail: chouguixin@hotmail.com, wangzht@hotmail.com)

[b]) Shanghai R&D Center for Standardization of Chinese Medicines, Shanghai 201203, China

Two new 11-hydroxy-substituted gelsedine-type indole alkaloids, named 11,14-dihydroxygelsenicine (**1**) and 11-hydroxygelsenicine (**2**), together with six known alkaloids, *i.e.*, koumine, gelsemine, 14-hydroxygelsenicine, 11-hydroxyhumantenine, gelsenicine, and (19*Z*)-akuammidine, were isolated from the EtOH extract of the stems of *Gelsemium elegans* BENTH. Their structures were determined mainly by means of spectroscopic analyses including HR-ESI-MS and 2D-NMR (HSQC, HMBC, ^{1}H,^{1}H-COSY). The configuration of **1** was confirmed by X-ray-diffraction analysis.

Introduction. – *Gelsemium* (Loganiaceae) is a small genus of three species, *G. elegans* (GARDN. & CHAMP.) BENTH., *G. sempervirens* (L.) JAUME ST.-HILAIRE, and *G. rankinii* SMALL. *G. elegans* is distributed in Southeast Asia, and the other two species are native to North America [1]. *G. elegans*, which is known as 'Gou-Wen' or 'Duan-Chang-Cao' in China, is very toxic and has been used traditionally for the treatment of pain, spasticity, and skin ulcers in Chinese folk medicine [2]. The genus *Gelsemium* is a rich source of indole alkaloids. Pharmacological investigations on the crude and purified alkaloids of this plant have demonstrated promising antitumor [3], analgesic, and anti-inflammatory activities [4]. Presently, more than seventy Gelsemium alkaloids are known, which are classified into six types: sarpagine, koumine, humantenine, gelsedine, gelsemine, and yohimbane. Of these, some gelsedine-type alkaloids showed potent cytotoxic activity against A431 epidermoid carcinoma cells [5].

In our present study, the two new 11-hydroxy-substituted gelsedine-type indole alkaloids, **1** and **2** (*Fig. 1*), together with six known indole alkaloids, *i.e.*, koumine [6], gelsemine [7], 14-hydroxygelsenicine [8], 11-hydroxyhumantenine [9], gelsenicine [8], and (19*Z*)-akuammidine [8], were isolated from the stems of *G. elegans*.

Results and Discussion. – Compound **1** was obtained as colorless cubic crystals. The molecular formula was established to be $C_{19}H_{22}N_2O_5$ from the HR-MS data (*m*/*z* 359.1602 ($[M+H]^+$)). The UV and NMR spectra exhibited the characteristic *N*-methoxyoxindole chromophore. The ^{1}H- and ^{13}C-NMR spectral signals in the aromatic region indicated an *ABX* system (δ(H) 7.71 (*d*, $J=8.2$, H–C(9)); 6.98 (*dd*, $J=8.2$, 2.2, H–C(10)); 6.89 (*d*, $J=2.2$, H–C(12))), suggesting the C(11) position being

© 2009 Verlag Helvetica Chimica Acta AG, Zürich

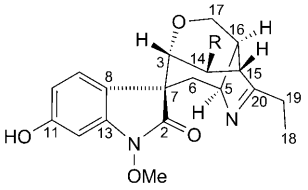

Fig. 1. *Structures of the two new alkaloids* **1** *and* **2**

substituted. The ^{1}H-NMR spectrum showed one N–OMe group (δ(H) 3.87 (*s*)), along with five CH H-atoms (one CH bearing a OH group at δ(H) 5.00 (br. *s*, H–C(14)); one CH–(N=C) at δ(H) 4.57 (br. *s*, H–C(5)); one CH–O at δ(H) 4.25 (br. *s*, H–C(3)); one CH at δ(H) 3.21 (*d*, J = 8.4, H–C(15)); one CH at δ(H) 2.59–2.68 (overlapped, *m*, H–C(16))), three CH_2 groups (δ(H) 4.80 (*dd*, J = 10.5, 3.3) and 4.45 (*d*, J = 10.3) (CH_2(17)); δ(H) 2.97–3.04 (*m*) and 2.59–2.68 (overlapped, *m*, CH_2(19)); δ(H) 2.59–2.68 (overlapped, *m*) and 2.47 (*dd*, J = 15.3, 2.0) (CH_2(6))), and one Me group (δ(H) 1.52 (*t*, J = 7.3, Me(18))). According to the ^{13}C-NMR spectrum and HSQC, **1** contained one N–OMe, one Me, three CH_2 (including one O-bearing CH_2 unit (δ(C) 61.92)), eight CH (including three aromatic C-atoms (δ(C) 126.51, 110.51, 96.11), two O-bearing CH (δ(C) 81.04, 66.31), and one CH–(N=C) (δ(C) 72.72)), and six quaternary C-atoms (including three aromatic C-atoms (δ(C) 159.66, 123.41, 139.99), one C=N C-atom (δ(C) 184.11, C(20)), and one C=O C-atom (δ(C) 172.44, C(2))). The ^{1}H- and ^{13}C-NMR data (*Table*) of **1** were similar to those of the known alkaloid 11-methoxy-14-hydroxygelsenicine [10], except for demethylation at C(11). The HMBC spectrum showed correlations between Me(18) and C-atoms with signals at δ(C) 26.41 (C(19)) and 184.11 (C(20)) (*Fig. 2*). From the above data, compound **1** was deduced to be 11,14-dihydroxygelsenicine. The configuration of the OH group at C(14) was shown to be β-oriented by the coupling constant (J(3,14) = 0). X-Ray crystallographic analysis of **1** (*Fig. 3*) confirmed the configuration.

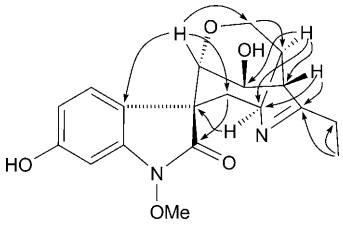

Fig. 2. *Selected HMBC* (H → C) *of* **1**

Compound **2** was obtained as a white powder. The molecular formula was established to be $C_{19}H_{22}N_2O_4$ from the HR-MS (*m*/*z* 343.1652 ([M + H]$^+$)). The UV and NMR data of **2** revealed the existence of an oxindole nucleus. Compound **2** is an analogue of **1**, they possess similar ^{1}H- and ^{13}C-NMR data, except that a CH_2 group (δ(H) 2.35–2.31 (*m*) and 2.05–1.96 (*m*) (CH_2(14)), δ(C) 28.16 (C(14))) was observed, instead of a H–C(14) and a OH group at C(14). From these data, in combination with ^{1}H,^{1}H-COSY and HMBC experiments (*Fig. 4*), the structure of **2** was identified as 11-hydroxygelsenicine.

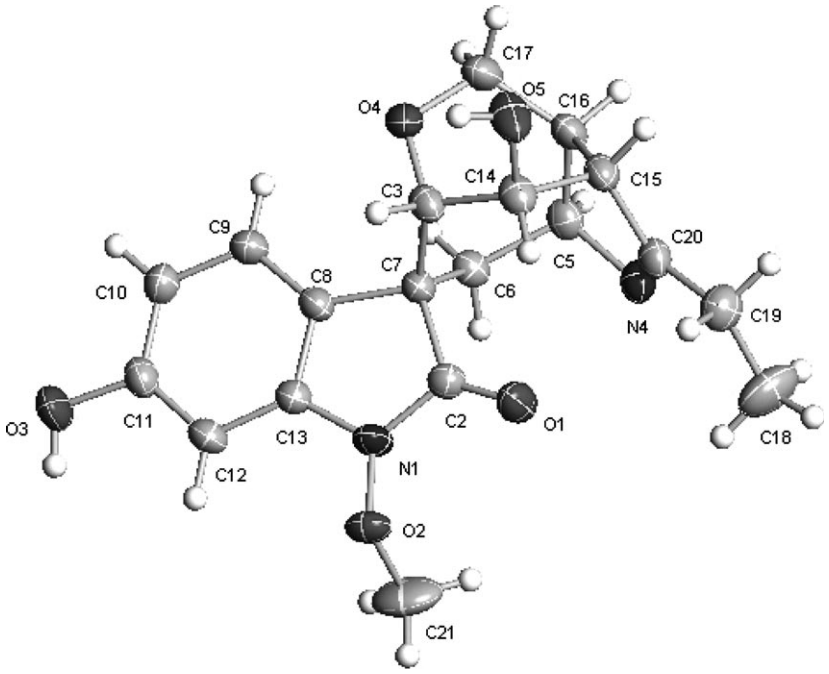

Fig. 3. *Single-crystal X-ray structure of* **1**

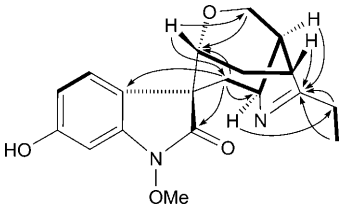

Fig. 4. *^{1}H,^{1}H-COSY* (—) *and selected HMBC* (H → C) *correlations of* **2**

The circular dichroism (CD) spectra of the two new compounds (**1** and **2**) were similar to those of some known gelsedine type indole alkaloids in the literature [10], the absolute configurations of which had already been deduced, indicating that **1** and **2** possessed absolute configurations as depicted in *Fig. 1*.

Both alkaloids **1** and **2** carry a OH group at C(11), which is the first time encountered in gelsedine-type alkaloids from *Gelsemium*.

Experimental Part

General. TLC: *HSGF$_{254}$* SiO_2 plates (*Yantai Jiangyou Silica Gel Development Co., Ltd.*, P. R. China). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh or 38 μm; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China), *ODS* (*SepaxGP-C18*, 40–60 μm, *Sepax Technologies Inc.*) and *Sephadex LH-20* (*GE-Healthcare Bio-Sciences AB*, Uppsala, Sweden) as packing materials. M.p.: *Büchi Melting-Point-B-540* apparatus; uncorrected. Optical rotations: *Krüss P800-T* polarimeter. UV and CD Spectra: *Jasco J-180* spectrometer (*Japan*); in nm. IR Spectra: *Nicolet 380* spectrometer from *Thermo*

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)[a])	δ(C)[b])	δ(H)[c])	δ(C)[d])
C(2)		172.44		173.06
CH(3)	4.25 (br. *s*)	81.04	3.75 (br. *d*, *J* = 2.7)	76.64
CH(5)	4.57 (br. *s*)	72.72	4.42 (br. *s*)	73.85
CH_2(6)	2.59–2.68 (*m*)[e]), 2.47 (*dd*, *J* = 15.3, 2.0)	38.49	2.43 (*dd*, *J* = 15.4, 4.8), 2.31–2.35 (*m*)[f])	39.40
C(7)		54.48		56.86
C(8)		123.41		123.88
CH(9)	7.71 (*d*, *J* = 8.2)	126.51	7.55 (*d*, *J* = 8.2)	127.29
CH(10)	6.98 (*dd*, *J* = 8.2, 2.2)	110.51	6.90 (*dd*, *J* = 8.2, 2.2)	111.14
C(11)		159.66		160.40
CH(12)	6.89 (*d*, *J* = 2.2)	96.11	6.85 (*d*, *J* = 2.2)	96.81
C(13)		139.99		140.85
CH(14) or CH_2(14)	5.00 (br. *s*)	66.31	2.31–2.35 (*m*)[f]), 1.96–2.05 (*m*)	28.16
CH(15)	3.21 (*d*, *J* = 8.4)	53.56	2.69 (*t*, *J* = 9.2)	43.82
CH(16)	2.59–2.68 (*m*)[e])	39.78	2.39 (br. *d*, *J* = 2.8)	41.32
CH_2(17)	4.80 (*dd*, *J* = 10.5, 3.3), 4.45 (*d*, *J* = 10.3)	61.92	4.22 (br. *d*, *J* = 2.1)	62.98
Me(18)	1.52 (*t*, *J* = 7.3)	10.49	1.41 (*t*, *J* = 7.3)	11.24
CH_2(19)	2.97–3.04 (*m*), 2.59–2.68 (*m*)[e])	26.41	2.79 (*dq*, *J* = 17.0, 7.3), 2.37–2.38 (*m*)	26.77
C(20)		184.11		183.67
N–OMe	3.87 (*s*)	63.12	3.82 (*s*)	63.94

[a]) Measured at 500 MHz. [b]) Measured at 125 MHz. [c]) Measured at 400 MHz. [d]) Measured at 100 MHz. [e]) Assignment confirmed by HSQC and HMBC experiments. [f]) Assignment confirmed by ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments.

Electron; in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AV-500* or *Bruker AV-400* instrument. HR-MS: *Waters UPLC Premior Q-TOF* spectrometer.

Plant Material. The stems of *G. elegans* were collected in Fu'an Fujian, China by *Rui-Zhi Liu* and identified by Associate Professor *Li-Hong Wu* (Institute of Chinese Materia Medica of Shanghai University of Traditional Chinese Medicine). A voucher specimen (No. GW-061229) was deposited with the laboratory of Shanghai R&D Center for Standardization of Chinese Medicines.

Extraction and Isolation. Air-dried stems (5912 g) of *G. elegans* were extracted with hot 95% EtOH (60 l, 4 × 3 h). After evaporation of the solvent, the residue was suspended in H_2O, acidified with 0.1M HCl to *ca.* pH 1 and defatted with CH_2Cl_2. The acidic layer was basified with aq. ammonia to *ca.* pH 10 and extracted with $CHCl_3$ five times to furnish the total alkaloids (14 g). This extract was subjected to CC (SiO_2, gradient petroleum ether (PE) (60–90°)/AcOEt 0 → 100%, then MeOH, containing 1% Et_2NH): *Frs. 1–168*. *Frs. 131–139* were further submitted to CC: **1**; *Frs. 113–130* to CC: **2** and 11-hydroxyhumantenine; *Frs. 60–67* to CC: koumine; *Frs. 68–81* to CC: gelsemine; *Fr. 94–112* to CC: 14-hydroxygelsenicine and (19*Z*)-akuammidine; and *Frs. 42–53* to CC: gelsenicine. Compounds **1** and **2**, and koumine, gelsemine, and 14-hydroxygelsenicine were further purified by repeated CC (SiO_2, *ODS* (MeOH/H_2O) and *Sephadex LH-20* (MeOH)): **1** (33.7 mg), **2** (10.1 mg), koumine (0.5 g), gelsemine (0.5 g), and 14-hydroxygelsenicine (164.4 mg). 11-Hydroxyhumantenine, gelsenicine, and (19*Z*)-akuammidine were further purified by repeated CC (SiO_2, *Sephadex LH-20* (CH_2Cl_2/MeOH 2 : 1): 11-hydroxyhumantenine (24.0 mg), gelsenicine (23 mg), and (19*Z*)-akuammidine (8.5 mg).

11,14-Dihydroxygelsenicine (=*(3S,6′R)-2′-Ethyl-3a′,4′,8′,8a′-tetrahydro-6,9′-dihydroxy-1-methoxy-3′H,6′H-spiro[indole-3,7′-[3,6]methanooxepino[4,3-b]pyrrol]-2(1H)-one*; **1**). Colorless cubic crystals. M.p. 183.0–184.5°. $[\alpha]_D^{25} = -175$ ($c = 0.020$, MeOH). UV (MeOH): 216, 286. CD ($c = 0.5$ mg/ml, MeOH, r.t.): 0 (366), −1.2 (306), −9.5 (268), 0 (253), +25.2 (237), 0 (226), −32.2 (212). IR (KBr): 3423, 1716, 1627. ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): *Table*. HMBC: (H → C): H−C(3)/C(17), C(15), C(14), C(8), C(6); H−C(5)/C(17), C(7); CH_2(6) (2.47 (*dd*))/C(16), C(7), C(5), C(3), C(2); CH_2(6) (2.59–2.68 (*m*))/C(16), C(8), C(7), C(5), C(2); H−C(9)/C(13), C(11), C(7); H−C(10)/C(12), C(11), C(8); H−C(12)/C(13), C(11), C(10) C(8); H−C(14)/C(20), C(16); H−C(15)/C(20), C(16), C(14), C(5), C(3); H−C(16)/C(20), C(15), C(14), C(6), C(5); CH_2(17) (4.80 (*dd*))/C(16), C(5), C(3); CH_2(17) (4.45 (*d*))/C(16), C(15), C(5), C(3); Me(18)/C(20), C(19); CH_2(19)/C(20), C(18). HR-MS (pos.): 359.1602 ($[M+H]^+$, $C_{19}H_{23}N_2O_5^+$; calc. 359.1607).

11-Hydroxygelsenicine (=*(3S,6′S)-2′-Ethyl-3a′,4′,8′,8a′-tetrahydro-6-hydroxy-1-methoxy-3′H,6′H-spiro[indole-3,7′-[3,6]methanooxepino[4,3-b]pyrrol]-2(1H)-one*; **2**). White powder. M.p. 223.0–225.0°. $[\alpha]_D^{25} = +50$ ($c = 0.024$, MeOH). UV (MeOH): 216, 286. CD ($c = 0.48$ mg/ml, MeOH, r.t): 0 (322), −1.2 (306), −9.4 (267), 0 (252), +18.7 (237), 0 (227), −29.1 (214). IR (KBr): 3432, 1719, 1626. ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): *Table*. HMBC (H → C): H−C(3)/C(17), C(15), C(6); H−C(5)/C(20); CH_2(6)/C(16), C(8), C(7), C(5), C(3), C(2); H−C(9)/C(13), C(11), C(7); H−C(10)/C(12), C(8); H−C(12)/C(13), C(11), C(10); CH_2(14) (2.31–2.35 (*m*))/C(20), C(16), C(15), C(7), C(3); CH_2(14) (1.96–2.05 (*m*))/C(20), C(7), C(3); H−C(15)/C(20), C(14), C(5), C(3); H−C(16)/C(20), C(15), C(14), C(6), C(5); CH_2(17)/C(16), C(15), C(5), C(3); Me(18)/C(20), C(19); CH_2(19)/C(20), C(18). ^{1}H,^{1}H-COSY: H−C(16)/CH_2(17), H−C(15), H−C(5); H−C(5)/CH_2(6); H−C(14)/H−C(15), H−C(3); H−C(9)/H−C(10); Me(18)/CH_2(19). HR-MS (pos.): 343.1652 ($[M+H]^+$, $C_{19}H_{23}N_2O_4^+$; calc. 343.1658).

X-Ray Crystal-Structure Analysis of **1**[1]). Single crystals suitable for X-ray analysis were obtained from EtOH. A colorless cubic crystal with approximate dimensions of 0.422 mm × 0.369 mm × 0.171 mm was used for analysis. All measurements were recorded on a *Bruker SMART CCD* area-detector diffractometer employing graphite-monochromated MoK_α radiation (λ 0.71073 Å) at 293 K and operating in ϕ-ω mode. Data collection and cell refinement: *Bruker SMART*. Program used to refine structure: SHELXL-97; refinement on F^2, full-matrix least-squares calculations. Crystal data and experimental details: empirical formula $C_{21}H_{30}N_2O_7$, M_r 422.47, orthorhombic, space group $P2_12_12_1$ ($Z = 4$), $a = 8.7326(6)$, $b = 13.0882(9)$, $c = 18.6300(13)$ Å; $\alpha = \beta = \gamma = 90°$; $V = 2129.3(3)$ Å^3, reflections collected/unique 12580/2642 ($R_{int} = 0.0228$), θ range 1.90–27.00°, R ($I > 2\sigma(I)$) $R_1 = 0.0454$, $wR_2 = 0.1298$, R indices (all data) $R_1 = 0.0476$, $wR_2 = 0.1315$, largest peak and hole in difference map: 0.572 and −0.381 e Å^{-3}.

REFERENCES

[1] P. T. Li, A. J. M. Leeuwenberg, in 'Flora of China', Eds. Z. Y. Wu, P. H. Raven, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis 1996, Vol. 15, p. 329.

[2] Editorial Committee of Chinese Materia Medica, the Administration Bureau of Traditional Chinese Medicine, Chinese Materia Medica (Zhonghua Bencao) Shanghai Science & Technology Press: Shanghai 2000, Vol. 6, p. 213–215.

[3] L. L. Zhang, C. Q. Huang, Z. Y. Zhang, Z. R. Wang, J. M. Lin, *J. First Milit. Med. Univ. (Di Yi Jun Yi Da Xue Xue Bao)* **2005**, *25*, 547; D. B. Chi, L. S. Lei, H. Jin, J. X. Pang, Y. P. Jiang, *J. First Milit. Med. Univ. (Di Yi Jun Yi Da Xue Xue Bao)* **2003**, *23*, 911.

[4] C. Rujjanawate, D. Kanjanapothi, A. Panthong, *J. Ethnopharmacol.* **2003**, *89*, 91.

[5] M. Kitajima, T. Nakamura, N. Kogure, M. Ogawa, Y. Mitsuno, K. Ono, S. Yano, N. Aimi, H. Takayama, *J. Nat. Prod.* **2006**, *69*, 715.

---

[1]) CCDC-722444 contains the supplementary crystallographic data for **1**. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The *Cambridge Crystallographic Data Centre*, 12, Union Road, Cambridge CB21EZ, UK; fax: +441223336033.

[6] F. Sun, Q. Y. Xing, X. T. Liang, *J. Nat. Prod.* **1989**, *52*, 1180.
[7] L. Z. Lin, S. Yeh, G. A. Cordell, C. Z. Ni, J. Clardy, *Phytochemistry* **1991**, *30*, 679.
[8] D. Ponglux, S. Wongseripipatana, S. Subhadhirasakul, H. Takayama, M. Yokota, K. Ogata, C. Phisalaphong, N. Aimi, S. I. Sakai, *Tetrahedron* **1988**, *44*, 5075.
[9] L. Z. Lin, G. A. Cordell, C. Z. Ni, J. Clardy, *J. Nat. Prod.* **1989**, *52*, 588.
[10] M. Kitajima, A. Urano, N. Kogure, H. Takayama, N. Aimi, *Chem. Pharm. Bull.* **2003**, *51*, 1211.

Received March 16, 2009