Steroidal Alkaloids from the Roots and Rhizomes of *Vertrum nigrum* L.

by **Yue Cong**[a]), **William Jia**[b]), **Jing Chen**[a]), **Shuang Song**[a]), **Jin-Hui Wang***[a]), and **Yu-Hui Yang**[c])

[a]) School of Traditional Chinese Materia Medica 49#, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, China (phone and fax: +86-24-23986479; e-mail: Wjh.1972@yahoo.com.cn)

[b]) Brain Research Centre, University of British Columbia, 2211 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada

[c]) Jinzhou Medical Colledge, Jinzhou 121001, China

Two new steroidal alkaloids, neoverapatuline (**1**) and (1*β*,3*α*,5*β*)-1,3-dihydroxyjervanin-12-en-11-one (**2**), together with the four known compounds, veratramine (**3**), rubijervine (**4**), veratrosine (**5**), and veratroylzygadenine (**6**), were isolated from the roots and rhizomes of *Veratrum nigrum* L. Their structures were established through combined analyses of physicochemical properties and spectroscopic evidence. All compounds **1**–**6** were tested for their cytotoxicities *in vitro* against the human glioma cell line SF188.

Introduction. – *Veratrum nigrum* L., which belongs to the family liliaceae, is a famous traditional medicinal plant in China, which is used for treatment of hypertension, blood-stroke, excessive phlegm, epilepsy, *etc.* [1][2]. Our present study on the constituents of the roots and rhizomes of *Veratrum nigrum* L. led to the isolation of the two new steroidal alkaloids **1** and **2** and of the four known steroidal alkaloids **3**–**6**. This paper reports the isolation and structure elucidation of the two new alkaloids, as well as the cytotoxicities of the isolated alkaloids against the human glioma cell line SF188.

Results and Discussion. – Compound **1** was obtained as a white powder. Its molecular formula $C_{29}H_{43}NO_5$ was determined by the pseudomolecular-ion peaks at *m*/*z* 486 ($[M+H]^+$) in the ESI-MS and 486.2321 ($[M+H]^+$) in the HR-MS. The structure of **1**, named neoverapatuline, was established as *N*-(methoxycarbonyl)-11-isoveratrobasine by analysis of ^{1}H- and ^{13}C-NMR, HSQC, HMBC, and NOESY data (*Table* and *Fig. 1*).

The ^{1}H-NMR spectrum of compound **1** exhibited signals of five Me groups at δ(H) 1.91 (*s*, Me(18)), 1.23 (*s*, Me(19)), 1.01 (*d*, J = 7.2, Me(27)), 0.95 (*d*, J = 7.2, Me(21)), and 3.71 (*s*, MeO), and of three oxygenated CH groups at δ(H) 4.77 (*d*, J = 6.3, H–C(11)), 3.50–3.54 (*m*, H–C(3)), and 3.53–3.56 (*m*, H–C(23)), as well as of one olefinic proton at δ(H) 5.27 (br. *s*, H–C(6))[1]. The ^{13}C-NMR spectrum ($CDCl_3$) displayed 29 C-signals, including those of 4 olefinic C-atoms at δ(C) 147.5 (C(12)), 142.3 (C(5)), 132.6 (C(13)), and 120.8 (C(6)), of a carbonyl group at δ(C) 158.6 (N–COO), and of four oxygenated C-atoms at δ(C) 71.5 (C(3)), 72.5 (C(11)), 85.0 (C(17)), and 73.0 (C(23)). All Me and olefinic protons were used as starting points to assign the other protons and C-atoms by analysis of HMBC and HMQC correlations. In the HMBC plot, δ(H) 4.77 (H–C(11)) showed cross-peaks with δ(C) 37.3 (C(8)), 37.2

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

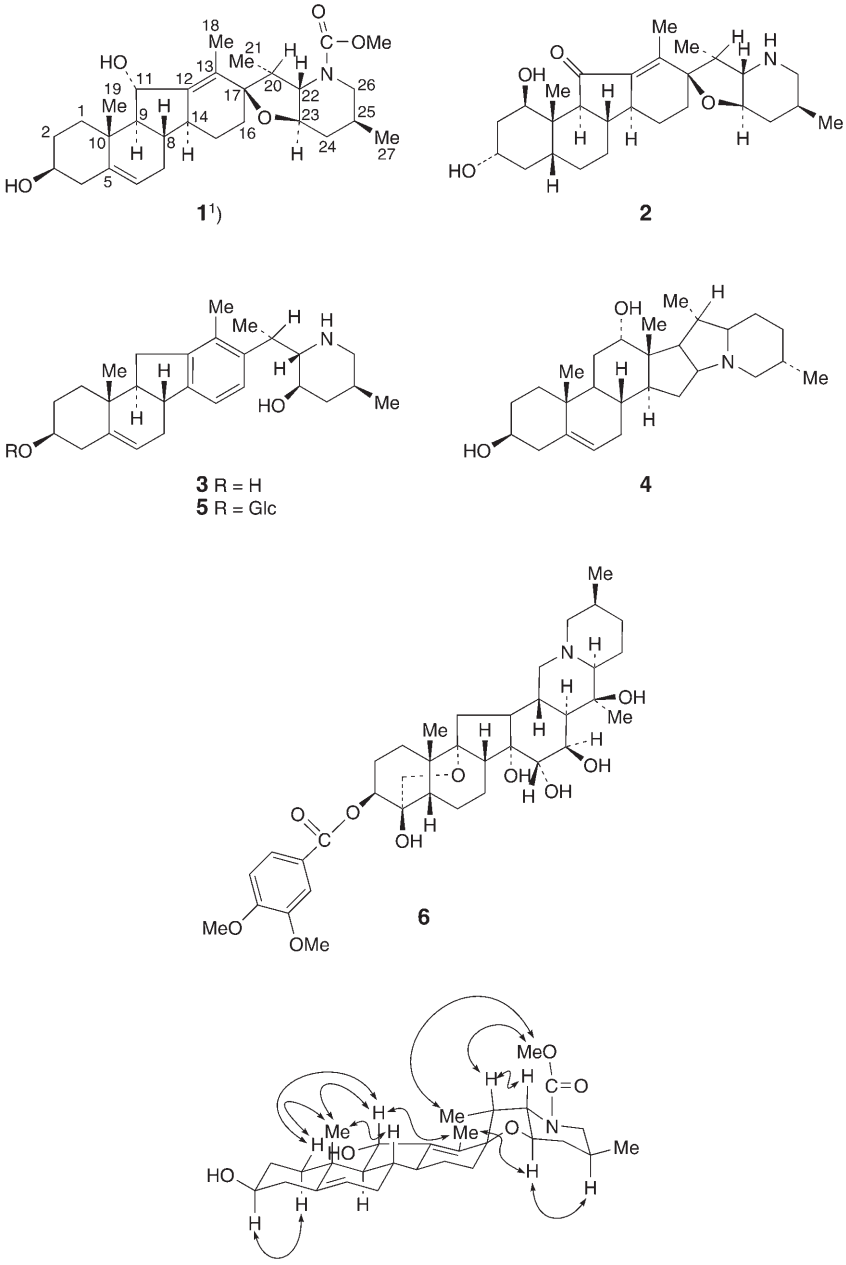

Fig. 1. *Key NOE correlations observed in the NOESY experiment of compound* **1**

(C(10)), 49.3 (C(14)), 132.6 (C(13)), and 147.5 (C(12)). In addition, the long-range correlations between δ(H) 3.71(MeO) and 3.13 (*dd*, $J = 7.2$, 7.8, H–C(22)) and δ(C) 158.6 (N–COO) were observed. These results established the location of the OH group at C(11) and of the methoxycarbonyl group at the N-

Table. *^1H- and ^{13}C-NMR Data of Compounds* **1** (in $CDCl_3$) *and* **2** (in $CDCl_3/CD_3OD$)[1]). At 300 (^1H) or 75 MHz (^{13}C). δ in ppm, J in Hz.

	1			**2**		
	^1H	^{13}C	HMBC	^1H	^{13}C	HMBC
CH_2(1) or H−C(1)	2.09 *dt*, (J = 13.2, 3.2), 1.29 (*ddd*, J = 13.2, 13.6, 3.2)	37.1	C(2), C(3), C(10), C(19)	4.79 (br. *s*)	71.9	C(2), C(3), C(5), C(10), C(19)
CH_2(2)	1.89–1.92, 1.56–1.59 (2*m*)	32.8	C(1), C(3), C(10)	1.91–1.97, 1.58–1.62 (2*m*)	37.9	C(1), C(3), C(10)
H−C(3)	3.50–3.54 (*m*)	71.5	C(1), C(4)	4.01–4.07 (*m*)	66.0	C(1), C(2), C(4), C(5)
CH_2(4)	2.24–2.30 (*m*), 2.33 (*dd*, J = 12.5, 3.6)	40.7	C(3), C(5), C(6), C(10)	1.58–1.64, 1.56–1.60 (2*m*)	36.6	C(3), C(5), C(6), C(10)
C(5) or H−C(5)		142.3		1.83–1.88 (*m*)	36.7	C(6), C(9), C(10)
H−C(6) or CH_2(6)	5.27 (br. *s*)	120.8	C(4), C(7), C(10)	1.73–1.81 (*m*), 1.42 (br. *d*, J = 12.0)	27.9	C(4), C(7), C(10)
CH_2(7)	2.34–2.40, 1.70–1.76 (2*m*)	31.6	C(9)	1.85–1.87, 1.47–1.52 (2*m*)	25.4	C(8), C(9)
H−C(8)	1.72–1.80 (*m*)	37.3	C(9), C(10), C(14), C(15)	1.48–1.54 (*m*)	42.8	C(9), C(10), C(14), C(15)
H−C(9)	1.12–1.15 (*m*)	57.3	C(1), C(7), C(10), C(11), C(19)	2.12 (br. *s*)	54.9	C(1), C(7), C(8), C(10), C(11), C(19)
C(10)		37.2			40.6	
H−(11) or C(11)	4.77 (*d*, J = 6.3)	72.5	C(8), C(10), C(12), C(13), C(14)		208.3	C(8), C(10), C(12), C(13), C(14)
C(12)		147.5			137.6	
C(13)		132.6			146.0	
H−C(14)	1.58–1.60 (*m*)	49.3	C(13), C(15)	2.00–2.10 (*m*)	45.1	C(8), C(13), C(15)
CH_2(15)	1.84–1.87, 1.31–1.40 (2*m*)	24.9	C(14), C(17)	1.96–2.00, 1.25–1.31 (2*m*)	24.5	C(14), C(17)
CH_2(16)	1.82–1.85, 1.55–1.60 (2*m*)	31.2	C(14), C(15)	1.94–1.98, 1.54–1.60 (2*m*)	31.5	C(14), C(15), C(17)
C(17)		85.0			86.7	
Me(18)	1.91 (*s*)	14.1	C(12), C(13), C(17)	2.14 (*s*)	12.4	C(12), C(13), C(17)
Me(19)	1.23 (*s*)	21.9	C(1), C(5), C(9), C(10)	1.02 (*s*)	17.7	C(1), C(5), C(9), C(10)
H−C(20)	2.94 (*quint.*, J = 7.2)	41.1	C(13), C(21), C(22), C(23)	2.55 (*quint.*, J = 7.8)	40.6	C(13), C(21), C(22), C(23)
Me(21)	0.95 (*d*, J = 7.2)	10.5	C(17), C(20), C(22)	0.98 (*d*, J = 6.6)	11.0	C(17), C(20), C(22)
H−C(22)	3.13 (*dd*, J = 7.2, 7.8)	63.2	C(20), C(21), C(23), C(24), N−COO	2.73 (*t*, J = 9.0)	66.7	C(20), C(21), C(23), C(24)
H−C(23)	3.53–3.56 (*m*)	73.0	C(20), C(22)	3.34–3.40 (*m*)	76.3	C(20), C(22), C(24)
CH_2(24)	2.15–2.20 (*m*), 1.05 (*q*, J = 9.6)	35.5	C(22), C(23), C(25), C(26), C(27)	2.20 (*dt*, J = 11.4, 3.2), 1.23 (*q*, J = 11.4)	39.1	C(22), C(23), C(25), C(26), C(27)
H−C(25)	1.83–1.88 (*m*)	28.5	C(27)	1.66–1.73 (*m*)	31.4	C(24), C(27)
CH_2(26)	2.79 (*dd*, J = 13.2, 8.5), 3.68 (*dd*, J = 13.2, 4.2)	51.6	C(22), C(24), C(25), C(27)	3.07 (*dd*, J = 13.2, 4.2), 2.33 (*t*, J = 12.0)	54.5	C(22), C(24), C(25), C(27)
Me(27)	1.01 (*d*, J = 7.2)	20.0	C(24), C(25), C(26)	1.00 (*d*, J = 7.2)	18.9	C(24), C(25), C(26)
N−COO		158.6				
MeO	3.71 (*s*)	52.6	N−COO			

atom. The above conclusions and NMR experiments (^{1}H- and ^{13}C-NMR, HMBC, and HMQC) suggested that the structure of **1** is identical to that of verapatuline [3], except for the C(11)=O group of verapatuline which is replaced by a CH(11)–OH moiety in **1**. The relative configuration of compound **1** was established by a NOESY experiment (*Fig. 1*).

Compound **2** was obtained as white powder. Its molecular formula $C_{27}H_{41}NO_4$ was determined by pseudomolecular-ion peaks at *m*/*z* 444 ($[M+H]^+$) in the ESI-MS and 444.2136 ($[M+H]^+$) in the HR-MS. The structure of **2** was established as (1β,3α,5β)-1,3-dihydroxyjervanin-12-en-11-one by analysis of ^{1}H- and ^{13}C-NMR, HSQC, HMBC, and NOESY data (*Table* and *Fig. 2*).

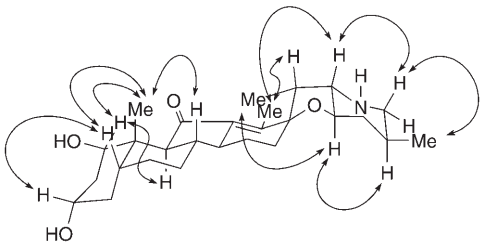

Fig. 2. *Key NOE correlations observed in the NOESY experiment of compound* **2**

The ^{1}H-NMR spectrum of compound **2** exhibited signals of four Me groups at δ(H) 2.14 (*s*, Me(18)), 1.02 (*s*, Me(19)), 1.00 (*d*, *J* = 7.2, Me(27)), and 0.98 (*d*, *J* = 6.6, Me(21)) and of three oxygenated CH groups at δ(H) 4.79 (br. *s*, H–C(1)), 4.01–4.07 (*m*, H–C(3)), and 3.34–3.40 (*m*, H–C(23)). The ^{13}C-NMR spectrum showed 27 C-signals, which included those of 2 olefinic C-atoms at δ(C) 137.6 (C(12)) and 146.0 (C(13)), of a carbonyl group at δ(C) 208.3 (C(11)), and of four oxygenated C-atoms at δ(C) 71.9 (C(1)), 66.0 (C(3)), 86.7 (C(17)), and 76.3 (C(23)). Analysis of HMBC and HMQC correlations, suggested a close resemblance of **2** to 1-hydroxy-5,6-dihydrojervine [4]. The NOESY experiment revealed correlations between δ(H) 1.02 (*s*, Me(19)) and 1.83–1.88 (*m*, H–C(5)), between δ(H) 1.02 (*s*, Me(19)) and 1.48–1.54 (*m*, H–C(8)), between δ(H) 4.79 (br. *s*, H–C(1)) and 2.12 (br. *s*, H–C(9)), and between δ(H) 4.01–4.07 (*m*, H–C(3)) and 1.83–1.88 (*m*, H–C(5)) (*Fig. 2*), implying the β-position of OH–C(1) (axial orientation), the α-position of OH–C(3) (equatorial orientation), and the β-position of H–C(5).

The known compounds **3**–**6** were identified by comparison of their physical and spectral data with those reported in [3–5].

Compounds **1**–**6** were examined for their dose–response effects of cytotoxicity against the human glioma cell line SF188 [6][7]. Cell viability measured using the MTT assay showed that the IC_{50} of compounds **3** and **4** were *ca.* 97.8 μM and 157.4 μM respectively. The IC_{50} of the positive control, (20*S*)-protopanaxadiol, against cell line SF188 was 12.5 μM. On the other hand, compounds **1**, **2**, **5**, and **6** showed no cytotoxicity against the human glioma cell line SF188.

This work was financially supported by the *Program for New Century Excellent Talents in University of Peoples Republic of China* (NO.NCET-04-0289). We thank *Shenyang Jiqi Pharmaceutical Product Ltd. Co.* of China for processing medical material of the roots and rhizomes of *Veratrum nigrum* L.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Group, Co.*). Melting point: *Mel-Temp* capillary melting point apparatus. Optical rotation: *Perkin-Elmer*

241MC polarimeter. NMR Spectra: *Bruker AV-600* and *Bruker ARX-300* spectrometer; $SiMe_4$ as internal standard; δ in ppm, J in Hz. ESI-MS: *Finnigan LCQ* mass spectrometer. HR-MS: *QSTAR LCQ* mass spectrometer.

Plant Material. The roots and rhizomes of *Veratrum nigrum* L. were collected in Hunan province, P. R. China, in 2004 and identified by Prof. *Qishi Sun*, Shenyang Pharmaceutical University. A voucher specimen (No. 20040710) is deposited in the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and Isolation. The dried roots and rhizomes of *Veratrum nigrum* L. (10 kg) were extracted with 95% EtOH (3×50 l) under reflux. The extract was concentrated and then acidified (pH 3) with HCl followed by filtration, and the filtrate was basified (pH 10) with NH_4OH and then extracted with $CHCl_3$, resulting in 50 g of crude alkaloids. This material was subjected to CC (silica gel, gradient petroleum ether/acetone): *Fractions A1–A4*. *Fr. A1*, eluted with petroleum ether/acetone 100:20, was further purified by recrystallization to give **3** (5.1 g). *Fr. A2*, eluted with petroleum ether/acetone 100:30, was subjected to CC (silica gel) to yield **4** (50 mg), eluted with $CHCl_3$/acetone 10:2, and **1** (40 mg), eluted with $CHCl_3$/acetone 5:2. *Fr. A3*, eluted with petroleum ether/acetone 100:45, was subjected to CC (silica gel, $CHCl_3$/acetone 10:5): **6** (35 mg). *Fr. A4*, eluted with petroleum ether/acetone 30:70, was subjected to CC (silica gel, petroleum ether/acetone/MeOH 30:70:8): **2** (25 mg) and **5** (1.1 g).

Determination of Cell Viability. The human glioma cell line SF188 was grown as a monolayer in *Dulbecco*'s modified *Eagle*'s medium (DMEM) containing 10% fetal bovine serum (FBS). Cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37°. Sensitivity of SF188 cells to compounds **1**–**6** were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-1*H*-tetrazolium bromide (MTT) assay. Briefly, exponentially growing cells were plated into 96-well plates (30000 cells/well). After 24 h, serial dilutions of the drugs were added to the cells, which were incubated for 24 h Then the cells were incubated with 10 µl of MTT (5 mg/ml) at 37° for 5 h. DMSO (100 µl) was added to solubilize the formazan crystals formed, and the optical densities at 570 nm were measured by using a microplate reader.

Neoverapatuline (=*(2′*R,*3*S,*3′*R,*3′a*S,*6′*S,*6a*S,*6b*S,*7′a*R,*11*S,*11a*S,*11b*R*)-1,2,3,4,5′,6,6′,6a,6b,7,7′,7′a,8,11,11a,11b-Hexadecahydro-3,11-dihydroxy-3′,6′,10,11b-tetramethylspiro[9*H*-benzo[a]fluorene-9,2′(*3′H*)-furo[3,2-b]pyridine]-4′(*3′aH*)-carboxylic Acid Methyl Ester*; **1**): White powder ($CHCl_3$). M.p. 155–156°. $[\alpha]_D^{20} = -6.0$ ($c = 0.015$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$): *Table*. ESI-MS (pos.): 486 ($[M+H]^+$). HR-MS: 486.2321 ($[C_{29}H_{43}NO_5+H]^+$; calc. 486.2326).

(1β,3α,5β)-1,3-Dihydroxyjervanin-12-en-11-one (=*(1*R,*2′*R,*3*S,*3′*R,*3′a*S,*4a*R,*6′*S,*6a*S,*6b*S,*7′a*R,*11a*S,*11b*R*)-2,3,3′a,4,4′,4a,5,5′,6,6′,6a,6b,7,7′,7′a,8,11a,11b-Octadecahydro-1,3-dihydroxy-3′,6′,10,11b-tetramethylspiro[9*H*-benzo[a]fluorene-9,2′(*3′H*)-furo[3,2-b]pyridine]-11(*1H*)-one*; **2**): White powder ($CHCl_3$). M.p. 239–240°. $[\alpha]_D^{20} = -57$ ($c = 0.010$, MeOH/$CHCl_3$). ^{1}H-NMR (300 MHz, MeOD/$CDCl_3$) and ^{13}C-NMR (75 MHz, MeOD/$CDCl_3$): *Table*. ESI-MS (pos.): 444 ($[M+H]^+$). HR-MS: 444.2136 ($[C_{27}H_{41}NO_4+H]^+$; calc. 444.2143).

REFERENCES

[1] J. Tang, H. L. Li, H. Q. Huang, W. D. Zhang, *Prog. Pharm. Sci.* **2006**, *30*, 206.
[2] Y. H. Xu, Y. H. Xu, *Oversea Medicine Plant Medicine Section* **2002**, *17*, 185.
[3] Y. Tezuka, T. Kikuchi, W. J. Zhao, J. Chen, Y. T. Guo, *J. Nat. Prod.* **1998**, *61*, 1078.
[4] K. A. E. Sayed, J. D. Mcchesney, A. F. Halim, A. M. Zahloul, I. S. Lee, *Int. J. Pharm.* **1996**, *34*, 161.
[5] S. Kadota, S. Z. Chen, J. X. Li, G. J. Xu, T. Namba, *Phytochemistry* **1995**, *38*, 777.
[6] J. T. Rutka, J. R. Giblin, D. Y. Dougherty, H. C. Liu, J. R. McCulloch, C. W. Bell, R. S. Stern, C. B. Wilson, M. L. Rosenblum, *Acta Neuropathol.* **1987**, *75*, 92.
[7] N. Ishii, D. Maier, A. Merlo, M. Tada, Y. Sawamura, A. C. Diserens, E. G. Vanmeir, *Brain Pathol.* **1999**, *9*, 469.

Received, January 23, 2007