Two New Steroidal Alkaloids from *Veratrum nigrum* L.

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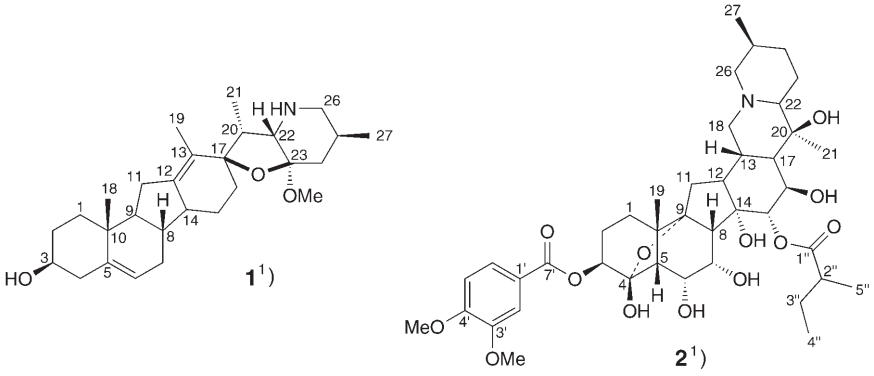
Phytochemical studies on *Veratrum nigrum* L., collected in Shanxi, P. R. China, resulted in the isolation of two new steroidal alkaloids, 23-methoxycyclopamine (**1**) and 15-*O*-(2-methylbutanoyl)-3-*O*-veratroylprotoverine (**2**). The structures of the two new compounds were established by means of extensive NMR spectroscopic studies.

Introduction. – In China, *Veratrum nigrum* L. with the Chinese name 'Li-lu', is one of the 14 species of the genus *Veratrum* (Liliaceae) [1]. The roots and rhizomes of several *Veratrum* species, including *Veratrum nigrum* L., have been used to treat aphasia arising from apoplexy, wind-type dysentery, jaundice, scabies, and chronic malaria for centuries. Previous investigations on chemical constituents of the genus *Veratrum* have reported about 100 steroidal alkaloids [2][3]. In this paper, we report on two new steroidal alkaloids isolated from *Veratrum nigrum* L., named 23-methoxycyclopamine[1]) (**1**) and 15-*O*-(2-methylbutanoyl)-3-*O*-veratroylprotoverine[1]) (**2**), which belong to the rarely occurring class of steroidal alkaloids with a unique skeleton.



1[1]) **2**[1])

Results and Discussion. – Compound **1** was obtained as a yellow amorphous powder, and showed a positive reaction with *Dragendorff*'s reagent. The HR-ESI-MS (positive mode) gave the molecular formula $C_{28}H_{44}NO_3$ ($[M+H]^+$ at m/z 442.3324).

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

The EI-MS of **1** showed a significant fragment-ion peak at *m/z* 424 ($[M-H_2O]^+$). The IR spectrum exhibited absorptions for an OH group (3360 and 1060 cm^{-1}) and C=C bonds (1624 cm^{-1}). Considering the steroidal alkaloids previously reported from the genus *Veratrum*, and after analysis of the ^{13}C- and ^1H-NMR (*Table*), HMBC, and NOESY data, **1** was determined to be an analog of the known compound cyclopamine with the skeleton of a jervine alkaloid [4–9], and its structure was established as 23-methoxycyclopamine.

The ^{13}C-NMR spectrum of **1** showed signals due to 4 Me, 9 CH_2, and 8 CH groups, 6 quaternary C-atoms, and an MeO group, which included two C=C bonds (δ(C) 143.10 (C(5)), 123.05 (C(6)), 127.23 (C(12)), and 144.53 (C(13))) and three O-bearing sp^3 C-atoms. The ^1H-NMR spectrum indicated proton resonances for four Me groups at δ 0.85 (*d*, *J* = 4.0 Hz, Me(21)), 0.87 (*d*, *J* = 2.8 Hz, Me(27)), 0.96 (*s*, Me(18)), and 1.80 (*s*, Me(19)), and the ^{13}C-NMR exhibited the signals of two C-atoms attached to an N-atom at δ(C) 65.65 (C(22)) and 53.30 (C(26)), which are the typical signals of a C_{27} hexacyclic jervine-type steroid alkaloid. The OH group was positioned at C(3) (δ(C) 73.07) due to the HMBC cross-peaks δ(H) 3.55 (br. *s*, H–C(3))/C(2) (δ(C) 32.64) and C(4) (δ(C) 43.40). A C=C bond was located between C(5) and C(6) by the long-range correlations of the olefinic proton at δ 5.37 (*t*, *J* = 2.5 Hz, H–C(6)) with C(5), C(7) (δ(C) 43.12), C(10) (δ(C) 37.91), and C(4), while the other C=C bond was located between C(12) and C(13), as deduced by the HMBC cross-peaks of δ 2.02–2.07 (*m*, H_α–C(11)), 1.90 (*d*, *J* = 2.1 Hz, H–C(14)), and 2.67 (*d*, *J* = 7.3 Hz, H–C(20)) with the olefinic C(12) and C(13). The MeO group showed an HMBC cross-peak with C(23) (δ(C) 106.33). The O-bearing quaternary C-atom at δ(C) 90.16 was attributed to C(17) by its HMBC cross-peak with δ(H) 1.21–1.26 (*m*, H_α–C(16)), 1.70–1.75 (*m*, H_β–C(16)), H–C(20), and Me(21). From the above analysis, and considering the chemical shifts of C(17) and C(23), it could be proposed that C(17) and C(23) were linked to an O-atom and formed an epoxy moiety, and MeO also was attached to C(23). The configurational relationship among the substituents, *i.e.*, of OH–C(3), Me(19), Me(21), Me(27), and the epoxy bridge, was established to be identical with that of the known alkaloid cyclopamine by NOESY analysis and also by comparison of the chemical shifts and coupling constants of **1** with those of cyclopamine. The relative configuration α of MeO–C(23) was deduced by the NOESY correlations MeO/H_α–C(24) (δ 2.38 (*m*)) and H_β–C(15) (δ 1.84 (*m*)).

Compound **2** was obtained as a white amorphous powder, and exhibited a positive reaction with *Dragendorff*'s reagent. The molecular formula of **2** was deduced as $C_{41}H_{59}NO_{13}$ by HR-ESI-MS ($[M+H]^+$ at *m/z* 774.4067) and the analysis of NMR data. The EI-MS of **2** gave a significant fragment-ion peak at *m/z* 756 ($[M-H_2O]^+$), implying the presence of OH groups in **2** [10–12]. The IR spectrum exhibited absorptions for OH groups (3480 and 1160 cm^{-1}) and a benzene moiety (1610 and 820 cm^{-1}). Considering the steroidal alkaloids previously reported from the genus *Veratrum*, and after analysis of the ^{13}C- and ^1H-NMR (*Table*), HMBC, and NOESY data, **2** was determined to be an analog of the known compound (+)-verabenzoamine with the skeleton of a cervine alkaloid [4–6][13–17], and its structure was established as 15-*O*-(2-methylbutanoyl)-3-*O*-veratroylprotoverine.

The ^{13}C-NMR spectrum of **2** showed signals of 7 Me, 8 CH_2, and 16 CH groups and of 10 quaternary C-atoms, including nine O-bearing sp^3 C-atoms. The ^1H-NMR spectrum showed resonances for three Me groups at δ 1.22 (*s*, Me(19)), 1.04 (*s*, Me(21)), and 1.17 (*d*, *J* = 7.0 Hz, Me(27)), and the ^{13}C-NMR exhibited the signals of three C-atoms attached to an N-atom at δ 61.18 (C(18)), 70.01 (C(22)), and 61.53 (C(26)), which are the typical signals of a C_{27} cervine-type steroidal alkaloid. The OH groups were located at C(6) (δ(C) 69.62), C(7) (δ(C) 69.38), and C(16) (δ(C) 70.50), due to the HMBC cross-peaks δ(H) 4.33 (*s*, H–C(6))/C(10) (δ(C) 46.97) and C(4) (δ(C) 106.53), δ(H) 4.67 (*s*, H–C(7))/C(6) and C(8)

Table. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz, resp.; $CDCl_3$) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	39.43	1.17–1.23, 1.72–1.78 (2*m*)	31.98	1.21–1.27, 1.28–1.34 (2*m*)
CH_2(2)	32.64	1.50–1.55, 1.81–1.86 (2*m*)	26.86	1.44–1.49, 1.59–1.64 (2*m*)
H–(3)	73.07	3.55 (br. *s*, 1 H)	76.00	5.10 (*d*, *J* = 4.0)
CH_2(4) or C(4)	43.40	2.09–2.15, 2.21–2.27 (2*m*)	106.53	–
C(5) or H–C(5)	143.10	–	51.62	2.58 (*d*, *J* = 2.0)
H–C(6)	123.05	5.37 (*t*, *J* = 2.5)	69.62	4.33 (*s*)
CH_2(7) or H–C(7)	43.12	2.24–2.29, 2.35–2.40 (2*m*)	69.38	4.67 (*s*)
H–(8)	43.75	1.14–1.19 (*m*)	48.39	2.84 (*d*, *J* = 4.0)
H–C(9) or C(9)	53.62	1.44–1.49 (*m*)	93.94	–
C(10)	37.91	–	46.97	–
CH_2(11)	29.35	2.02–2.07, 2.20–2.25 (2*m*)	32.83	1.66–1.71, 2.26–2.31 (2*m*)
C(12) or H–C(12)	127.23	–	47.63	1.75–1.80 (*m*)
C(13) or H–C(13)	144.53	–	31.51	1.22–1.27 (*m*)
H–C(14) or C(14)	49.88	1.90 (*d*, *J* = 2.1)	80.61	–
CH_2(15) or H–C(15)	25.67	1.24–1.29, 1.82–1.87 (2*m*)	70.78	5.35 (*d*, *J* = 3.0)
CH_2(16) or H–C(16)	30.95	1.21–1.26, 1.70–1.75 (2*m*)	70.50	4.32 (*d*, *J* = 3.0)
C(17) or H–C(17)	90.16	–	45.58	1.40–1.45 (*m*)
Me(18) or CH_2(18)	19.71	0.96 (*s*)	61.18	1.99–2.04, 3.08–3.13 (2*m*)
Me(19)	16.74	1.80 (*s*)	18.42	1.22 (*s*)
H–C(20) or C(20)	48.69	2.67 (*d*, *J* = 7.3)	73.00	–
Me(21)	13.44	0.85 (*d*, *J* = 4.0)	18.82	1.04 (*s*)
H–C(22)	65.65	3.07 (*d*, *J* = 6.1)	70.01	2.05 (*d*, *J* = 6.4)
C(23) or CH_2(23)	106.33	–	24.94	1.64, 1.39 (2*d*, *J* = 6.4)
CH_2(24)	40.29	1.16–1.20, 2.35–2.40 (2*m*)	29.70	1.26–1.31, 1.35–1.40 (2*m*)
H–C(25)	30.52	1.74–1.79 (*m*)	30.38	1.29–1.34 (*m*)
CH_2(26)	53.30	2.96, 2.25 (2*d*, *J* = 11.8)	61.53	2.52–2.57, 2.94–2.99 (2*m*)
Me(27)	20.26	0.87 (*d*, *J* = 2.8)	16.79	1.17 (*d*, *J* = 7.0)
MeO–C(23)	48.46	3.24 (*s*)	–	
C(1′)			122.96	–
H–C(2′)			112.96	7.56 (*s*)
C(3′)			149.11	–
C(4′)			153.67	–
H–C(5′)			110.80	6.89 (*d*, *J* = 8.4)
H–C(6′)			123.92	7.69 (*d*, *J* = 8.4)
C(7′)=O			166.36	–
*Me*O–C(3′)			56.16	3.92 (*s*)
*Me*O–C(4′)			56.26	3.93 (*s*)
C(1″)=O			175.96	–
H–C(2″)			41.36	2.42 (*d*, *J* = 6.8)
CH_2(3″)			17.10	1.10 (*d*, *J* = 6.8)
Me(4″)			11.61	0.92 (*d*, *J* = 6.8)
Me(5″)			27.38	1.82–1.87 (*m*)

(δ(C) 48.39), and δ(H) 4.32 (*d*, *J* = 3.0 Hz, H–C(16))/C(15) (δ(C) 70.78) and C(17) (δ(C) 45.58). The ^{13}C-NMR spectrum showed that two OH groups were located at the quaternary C(14) (δ(C) 80.61) and C(20) (δ(C) 73.00). In the ^{1}H-NMR spectrum, five resonances at δ 7.56 (*s*, H–C(2′)), 6.89 (*d*, *J* = 8.4 Hz, H–C(5′)), 7.69 (*d*, *J* = 8.4 Hz, H–C(6′)), 3.92 (*s*, MeO–C(3′)), and 3.93 (*s*, MeO–C(4′)) implied that

there is a veratroyloxy group in **2**, and this veratroyloxy group was positioned at C(3) (δ(C) 76.00) based on the HMBC cross-peak δ 5.10 (d, J = 4.0 Hz, H–C(3))/C(7′) (δ(C) 166.36). The ^{1}H-resonances at δ 2.42 (d, J = 6.8 Hz, H–C(2″)), 1.10 (d, J = 6.8 Hz, H–C(3″)), 0.92 (d, J = 6.8 Hz, Me(4″)), and 1.82–1.87 (m, Me(5″)) implied that a (2-methylbutanoyl)oxy group was present in **2**. This (2-methylbutanoyl)oxy group showed an HMBC cross-peak δ 5.35 (d, J = 3.0 Hz, H–C(15))/C(1″) (δ(C) 175.96), allowing to locate this group at C(15). The O-bearing quaternary C-atom (δ(C) 93.94) was attributed to C(9) by its HMBC cross-peaks with H–C(8) (δ 2.84 (d, J = 4.0 Hz)), H_α–C(11) (δ 2.26–2.31 (m)), and Me(19) (δ 1.22 (s)). Another O-bearing quaternary C-atom (δ(C) 106.53) was attributed to C(4) due to its HMBC cross-peaks with H–C(5) (δ 2.58 (d, J = 2.0)), H–C(3), and H–C(6) (δ 4.33 (s)). Considering the chemical shifts of C(9) and C(4) and those of similar steroidal alkaloids isolated from the genus *Veratrum*, it could be proposed that C(4) and C(9) were linked by an O-atom forming an epoxy bridge, and an OH group was attached to C(4). This suggested that **2** is a hydroxylated derivative of the known (+)-verabenzoamine and that the relative configuration of **2** is identical with that of (+)-verabenzoamine. This deduction was further confirmed by the NOESY experiment which established the relative configuration α of OH–C(6) (NOEs H–C(6)/H–C(5) and H–C(8)).

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Experimental Part

General. Column chromatography (CC): silica gel (160–200 or 300–400 mesh; *Marine Chemical Factory*, Qingdao, People's Republic of China); *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA); *MCI* gel (high-porous polymer 75–150 μ; *Mitsubishi Chemical*). TLC: silica gel plates, visualization by spraying with 10% H_2SO_4 in EtOH. Optical rotations: *Perkin-Elmer 241* polarimeter. IR Spectra: *Nicolet Compact-40* and *Bruker Vector-22* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer (500 MHz); δ in ppm with $SiMe_4$ as internal standard, J in Hz. MS: *HP5989A* (for EI) and *Micromass Q-Tof* spectrometer (for HR-ESI); in m/z.

Plant Material. Veratrum nigrum L. was collected from Mount Taibai (Shanxi Province, China) in May 2005, and authenticated by Prof. *Hanchen Zheng*, Department of Phytochemistry, Second Military Medical University. The voucher specimens (collection No. 2005287) are kept in the Herbarium of the Second Military Medical University, Shanghai, China.

Extraction and Isolation. The dried whole plants of *Veratrum nigrum* L. (29 kg) were ground to a coarse powder, and extracted twice with 70% EtOH for 12 h each time. The EtOH extract was evaporated: dark residue (1538 g). Of the residue, 5% were set apart for bioassays; all the remaining was suspended in H_2O and then partitioned successively with petroleum ether, $CHCl_3$, AcOEt, and BuOH. The $CHCl_3$ extract (151 g) was separated by CC (silica gel, $CHCl_3$/MeOH gradient): *Fractions 1.1–1.14. Fr. 1.4* (3.7 g) was purified by repeated CC (*MCI* gel, MeOH/H_2O 10:1; *Sephadex LH-20*, MeOH; silica gel $CHCl_3$/acetone 20:1 → 6:1): **1** (9 mg). By a similar procedure, the AcOEt extract (172 g) was fractionated: *Fr. 2.1–2.12. Fr. 2.3* was further subjected to various CCs: **2** (32 mg).

23-Methoxycyclopamine ((= rel-*(2′*R*,3*S*,3′*R*,3′a*S*,6′*S*,6a*S*,6b*S*,7′a*R*,11a*S*,11b*R*)-1,2,3,3′a,4,4′,5′,6,6′,6a,6b,7,7′,7′a,8,11,11a,11b-Octadecahydro-7′a-methoxy-3′,6′,10,11b-tetramethylspiro[9*H*-benzo[a]fluorene-9,2′(3′*H*)-furo[3,2-b]pyridin]-3-ol*; **1**): Yellow amorphous powder. $[\alpha]_D^{25} = 44.0$ ($c = 0.05$, $CHCl_3$). IR ($CHCl_3$): 3360, 2940, 2180, 1624, 1060. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 442 (M^+). HR-ESI-MS: 442.3324 (M^+, $C_{28}H_{50}O_3^+$; calc. 442.3321).

*15-*O*-(2-Methylbutanoyl)-3-*O*-veratroylprotoverine* (= rel-*(3β,4α,6α,7α,15α,16β)-4,9-Epoxycevane-3,4,6,7,14,15,16,20-octol 3-(3,4-Dimethoxybenzoate) 15-(2-Methylbutanoate)*; **2**): White amorphous powder. $[\alpha]_D^{25} = +12.5$ ($c = 0.02$, CH_3Cl). IR ($CHCl_3$): 3480, 2860, 2760, 1610, 1160, 820. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 774 (M^+). HR-ESI-MS: 774.4067 (M^+, $C_{41}H_{59}NO_{13}^+$; calc. 774.4065).

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