Full Papers

Two New Steroidal Alkaloids, 20-Isoveratramine and Verapatuline, from the Roots and Rhizomes of *Veratrum patulum*

Yasuhiro Tezuka,*[,†] Tohru Kikuchi,[†] Weijie Zhao,[‡] Jun Chen,[‡] and Yongtian Guo[‡]

Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan, and Research Institute for Medical and Pharmaceutical Science, Dalian, Chunyangjie 21, Dalian, People's Republic of China

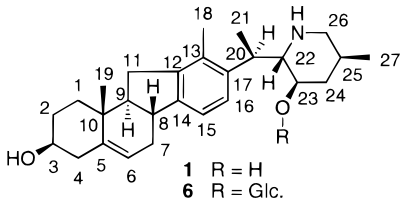
Received April 16, 1998

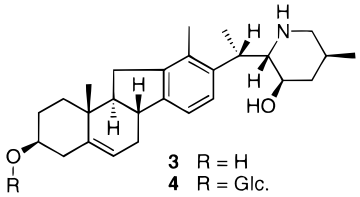
Roots and rhizomes of *Veratrum patulum* L. (Liliaceae), used as a source of the Chinese crude drug "Li-lu", have yielded two new steroidal alkaloids, 20-isoveratramine (**1**) and verapatuline (**2**), along with three known alkaloids, veratramine (**3**), veratrosine (**4**), and jervine (**5**). Structures of new alkaloids **1** and **2** were determined to be a C-20 epimer of **3** and *N*-(methoxycarbonyl)jervine, respectively, by the use of spectral data including 2D NMR.

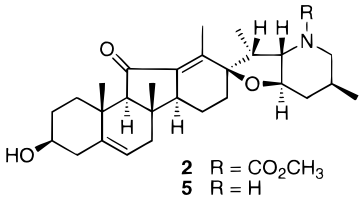
The Chinese crude drug "Li-lu" is prepared from dried roots and rhizomes of several *Veratrum* species (Liliaceae) such as *V. nigrum* L., *V. maackii* Reg., and *V. dahuricum* Loes. f. and is used to treat aphasia arising from apoplexy, wind-type dysentery, jaundice, headache, scabies, chronic malaria, and so forth.[1,2] Constituents of *Veratrum* plants have been examined extensively, and more than a hundred steroidal alkaloids have been isolated so far.[3−9] We previously examined the constituents of *V. maackii*[10] and *V. nigrum* L. var. *ussuriense* Nakai[11,12] and isolated three new alkaloids (maackinine, verussurinine, and verussurine) and 11 known ones [germanitrine, angeloylzygadenine, zygadenine, verazine, verazinine, germidine, germerine, 15-*O*-(2-methylbutyroyl)germine, neogermbudine, jervine, and verabenzoamine]. We have now examined the constituents of *V. patulum*, one of the sources of "Li-lu" recently reported to have anti-herpetic activity.[13] From the EtOH extract of dried roots and rhizomes of *V. patulum*, two new alkaloids, 20-isoveratramine (**1**) and verapatuline (**2**), were isolated along with three known ones, veratramine (**3**),[14,15] veratrosine (**4**),[15] and jervine (**5**).[10,14,16] In this paper, we wish to report the isolation and structure elucidation of the new alkaloids (**1** and **2**).

Results and Discussion

Isoveratramine (**1**) was obtained as colorless prisms, and its molecular formula was determined to be $C_{27}H_{39}NO_2$ (*m/z* 409) by EIMS and HREIMS. The EIMS was identical to that of veratramine (**3**),[17] and its ^{1}H and ^{13}C NMR spectra were also similar to those of **3**, except for slight differences in chemical shifts in the piperidine ring (Table 1). These observations led us to consider **1** to be a stereoisomer of **3**; that is, 20-iso-**3** or 22,23,25-triiso-**3**.







The ^{1}H NMR spectra of both **1** and **3** showed *trans*-diaxial coupling for H-22, H-23, and H-25, indicating that the piperidine rings have the chair form with axial H-22, H-23, and H-25. On the other hand, the *J* value for H-20 and H-22 (**1**, 7 Hz; **3**, 4 Hz) suggested that they should be gauche in **1** and anti in **3**. Moreover, the $[\alpha]_D$ values of **1** (+102.18°, *c* 0.09, MeOH) and **3** (−60.21°, *c* 0.29, MeOH) suggested that they would have almost enantiomeric conformations. The most stable conformers for **3**, 20-iso-**3**, and 21,23,25-triiso-**3**, by using molecular mechanics (MM2) and semiempirical quantum mechanical (PM3) methods, revealed that H-20 and H-22 of **3** are anti and 20-iso-**3** and 21,23,25-triiso-**3** are gauche and that 20-iso-**3**, but not 21,23,25-triiso-**3**, has almost enantiomeric conformation to **3**. From these observations, **1** is concluded to be 20-isoveratramine.

Jeveratrum-type alkaloids usually have the (20*R*)-

* To whom correspondence should be addressed. Tel.: +81-764-34-2281 (ex. 2826). Fax: +81-764-34-5059. E-mail: tezuka@ms.toyama-mpu.ac.jp.
† Research Institute for Wakan-Yaku.
‡ Research Institute for Medical and Pharmaceutical Science.

S0163-3864(98)00150-5 CCC: $15.00
Published on Web 07/23/1998

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data for Alkaloids 1 and 3 in C_5D_5N

	1[a]		**3**[b]	
C	δ_H	δ_C	δ_H	δ_C
1	1.25 td (13.5, 3.5) 1.76 dt (13.5, 3)	38.4 t	1.31 td (13.5, 4) 1.80 br d (13.5)	38.5 t
2	1.88 m 2.11 m	32.1 t	1.88 dddd (13.5, 12, 11, 3.5) 2.12 br d (12)	32.1 t
3	3.82 tt (11, 4.5)	71.2 d	3.84 tt (11, 4)	71.3 d
4	2.59 dd (13, 11) 2.69 ddd (13, 4.5, 1.5)	42.9 t	2.60 br dd (13, 11) 2.71 br dd (13, 4)	42.9 t
5		143.2 s		142.7 s
6	5.44 br d (4)	121.4 d	5.40 br d (4)	121.5 d
7	1.91 m 2.41 ddd (14, 11, 4)	30.5 t	2.02 br dd (15, 12) 2.56 m	30.8 t
8	2.45 ddd (12, 11, 5)	41.2 d	2.95 td (12, 5)	41.5 d
9	1.64 td (12, 7.5)	57.2 d	1.80 td (12, 7)	57.5 d
10		37.2 s		37.2 s
11	2.11 dd (14, 12) 2.60 dd (14, 7.5)	30.4 t	2.50 dd (14.5, 12) 2.78 dd (14.5, 7)	30.7 t
12		133.1 s[c]		133.1 s[c]
13		143.7 s[c]		143.65 s[c]
14		144.3 s[c]		143.69 s[c]
15	6.87 d (7.5)	120.9 d	7.10 d (7.5)	119.9 d
16	7.68 d (7.5)	125.9 d	7.66 d (7.5)	126.7 d
17		139.6 s		141.2 s
18	2.66 s	16.4 q	2.57 s	16.1 q
19	1.07 s	19.3 q	1.11 s	19.3 q
20	4.34 quintet (7)	36.6 d	4.06 qd (7, 4)	35.7 d
21	1.86 d (7)	21.7 q	1.64 d (7)	21.1 q
22	3.47 dd (10, 7)	67.0 d	2.84 dd (9, 4)	68.3 d
23	4.51 ddd (11, 10, 4)	68.0 d	3.56 ddd (11, 9, 4.5)	70.7 d
24	1.55 td (12, 11) 2.39 br dd (12, 4)	43.5 t	1.35 q (11) 2.25 br d (11)	45.2 t
25	2.53 m	27.4 d	1.48 m	32.5 d
26	2.59 t (11) 3.42 br d (11)	51.1 t	2.20 t (11.5) 2.99 br d (11.5)	54.6 t
27	0.75 d (6)	18.4 q	0.74 d (6.5)	19.0 q

[a] Assigned by the COSY and HETCOR spectra and by comparison with the data for **3**. [b] Assigned by the COSY and HETCOR spectra and by comparison with the data in $CDCl_3$, where long-range $^1H-^{13}C$ COSY spectrum was also measured. [c] May be interchanged in each column.

configuration, but four (20*S*)-alkaloids have been reported.[15,18−20] In addition, a (20*S*) steroidal alkaloid glycoside, 20-isoveratramin-23-*O*-*β*-D-glucopyranoside (**6**), has been isolated from the roots of *V. patulum* in Korea.[15] The aglycon of **6** (i.e., 20-isoveratramine), however, has not been found before, and thus **1** is a novel veratrum alkaloid.

Verapatuline (**2**) was obtained as a colorless amorphous solid, and its IR spectrum showed absorptions of hydroxyl (ν 3400 cm^{-1}) and conjugated carbonyl (ν 1700 and 1620 cm^{-1}) groups. The molecular formula of **2** was determined by HREIMS to be $C_{29}H_{41}NO_5$ (*m*/*z* 483), $C_2H_2O_2$ (58 amu) more than jervine (**5**). EIMS of **2** was similar to that of **5**, with 58 amu higher fragment ions at *m*/*z* 183 and 165.[17] The ^{1}H and ^{13}C NMR spectra of **2** were also similar to those of **5** (Table 2), but they were characterized by the presence of additional signals ascribable to an *N*-(methoxycarbonyl) group (δ_H 3.72, s; δ_C 52.7, q; 158.5, s). These data suggested that **2** should be a derivative of **5** with a methoxycarbonyl group on the nitrogen atom. This conclusion was confirmed by the $^1H-^1H$, $^1H-^{13}C$, and long-range $^1H-^{13}C$ COSY spectra. The former two spectra clarified the partial structures, while the last one revealed the connectivities among the partial structures (Table 2). The methyl protons at δ_H 1.02 (H_3-19) showed long-range correlations with the carbons at δ_C 36.8 (t, C-1), 37.0 (s, C-10), 62.6 (d, C-9), and 142.3 (s, C-5). Thus, C-1, C-5, C-9, and C-19 were connected with the quaternary carbon C-10. On the other hand, the quaternary carbon C-10 was also correlated with the protons H-4 and H-6, indicating that C-4 and C-6 should be connected to C-5. Similarly, the quaternary carbons at δ_C 206.8 (C-11), 137.1 (C-12), 146.2 (C-13), and 85.0 (C-17) were assigned based on the long-range correlations with H-9, with H-15 and H_3-18, with H-16 and H_3-18, and with H-16, H_3-18, and H_3-21, respectively. From these evidences, verapatuline has been determined to be *N*-(methoxycarbonyl)jervine (**2**).

Approximately 20 alkaloids with *N*-(methoxycarbonyl) groups have been reported. These include indole alkaloids (e.g., ajmalicidine, fruticosine, kopsine, and pleiocarpine) from Apocynaceaeous plants[21] and three aporphinoid alkaloids (romucosine, cathafiline, and cathaformine) from Annonaceaeous[22] and Lauraceaeous[23] plants. However, verapatuline (**2**) is the first veratrum alkaloid possessing an *N*-(methoxycarbonyl) group.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-4 polarimeter at 23 °C, and IR spectra were recorded on a JASCO IRA-2 spectrometer. NMR spectra were recorded with a JEOL JNM-GX400 spectrometer with TMS as internal standard. EIMS and HREIMS were obtained on a JEOL GC-mate mass spectrometer at the ionization potential of 70 eV. Column chromatography was performed over alkali-treated Si gel,[11] and preparative TLC was carried out with precoated Merck Kieselgel GF_{254} plates. For drying organic solvents, anhydrous $MgSO_4$ was used.

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data for Alkaloids 2 and 5 in $CDCl_3$

	2[a]			**5**[b]	
C	δ_H	δ_C	^{1}H l.−r. coupled[c]	δ_H	δ_C
1	1.19 td (13.5, 3.5) 2.57 dt (13.5, 3.5)	36.8 t	19	1.19 td (14, 4) 2.58 dt (14, 3.5)	36.8 t
2	1.56 tdd (13.5, 11, 3.5) 1.85 m	31.1 t	4	1.55 m 1.86 br d (13)	31.3 t
3	3.52 tt (11, 4.5)	71.6 d	1	3.52 tt (11, 4.5)	71.7 d
4	2.18 br dd (12.5, 11) 2.35 ddd (12.5, 4.5, 2)	41.4 t	2, 6	2.17 m 2.36 m	41.5 t
5		142.3 s	1, 4, 7, 19		142.4 s
6	5.38 br d (5)	120.9 d	4, 7	5.38 br d (5)	121.0 d
7	1.92 m 2.35 m	30.7 t	9	1.89 m 2.36 m	30.8 t
8	1.60 m	37.9 d	6, 9	1.60 m	38.0 d
9	1.67 d (13)	62.6 d	1, 7, 19	1.66 d (12)	62.6 d
10		37.0 s	2, 4, 6, 9, 19		37.1 s
11		206.8 s	9		206.8 s
12		137.1 s	15, 18		137.2 s
13		146.2 s	16, 18		145.9 s
14	1.97 m	44.7 d	16	1.95 m	44.9 d
15	1.35 br dt (13, 10) 1.95 m	24.3 t		1.37 br t (12.5) 1.94 m	24.4 t
16	1.60 m 1.92 m	31.6 t		1.52 m 1.92 m	31.0 t
17		85.0 s	16, 18, 21		85.6 s
18	2.24 d (1.5)	12.2 q		2.17 s	12.2 q
19	1.02 s	18.4 q	1, 9	1.01 s	18.5 q
20	2.95 quintet (7)	42.4 d	21	2.52 dq (9, 7)	40.4 d
21	0.95 d (7)	10.6 q		0.96 d (7)	10.8 q
22	3.21 dd (11, 7)	63.5 d		2.72 t (9)	66.6 d
23	3.61 td (11, 4.5)	73.6 d		3.30 ddd (11.5, 9, 4)	76.5 d
24	1.11 ddd (12, 11, 9) 2.21 ddd (12, 6, 4.5)	35.3 t	26, 27	1.21 q (11.5) 2.19 dt (11.5, 4)	39.0 t
25	1.87 m	28.5 d	27	1.61 m	31.6 d
26	2.84 dd (13, 8.5) 3.68 dd (13, 4.5)	51.5 t		2.33 t (12) 3.08 dd (12, 4)	54.7 t
27	1.03 d (6.5)	20.1 q	26	0.95 d (6.5)	18.9 q
CO_2CH_3		158.5 s	CO_2CH_3		
CO_2CH_3	3.72 s	52.7 q			

[a] Assigned by the COSY, HETCOR, and long-range ^{1}H−^{13}C COSY spectra. [b] Assigned by the COSY and HETCOR spectra. [c] Long-range coupled protons observed in the long-range ^{1}H−^{3}C COSY.

The most stable conformations were deduced by the MM2 and PM3 programs in CS Chem3D/MOPAC Pro software (ChembridgeSoft Corporation, MA).

Plant Material. Roots and rhizomes of *Veratrum patulum* L. were collected at Qianshan in Liaoning Province, People's Republic of China in 1986, and identified by Dr. Guo Y.-Z. at Shenyang Pharmaceutical University. A voucher specimen is deposited in the Research Institute for Medical and Pharmaceutical Science, Dalian.

Isolation of Alkaloids. Dried roots and rhizomes (2.2 kg) of *V. patulum* were cut into small pieces and extracted with EtOH (4 × 7 L) at room temperature. The EtOH solutions were combined and concentrated in vacuo. The residue was dissolved in 5% aqueous tartaric acid solution (2.5 L), and insoluble material was removed by filtration. The tartaric acid solution was defatted with ether (4 × 3 L), basified with 20% Na_2CO_3 to pH 10, and extracted with $CHCl_3$ (4 × 500 mL). Drying and concentration of the $CHCl_3$ layer gave a total alkaloid (7 g).

The total alkaloid (6.1 g) was chromatographed over alkali-treated Si gel (400 g) with MeOH−$CHCl_3$ (2:98, 6:94, 10:90, and then 15:85). The eluates were monitored by TLC and separated into 26 fractions. Fraction 3 [MeOH−$CHCl_3$ (2:98) eluate, 80 mg] was recrystallized from $CHCl_3$ to give isoveratramine (**1**, 5 mg). Fraction 6 [MeOH−$CHCl_3$ (2:98) eluate, 230 mg] was separated by preparative TLC with MeOH−$CHCl_3$ (2.5:97.5) to give verapatuline (**2**, 160 mg). Fraction 8 [MeOH−$CHCl_3$ (2:98) eluate, 1.4 g] was recrystallized from Me_2CO to afford a mixture of alkaloids (230 mg). The mixture was subjected to preparative TLC with Me_2CO, and the less polar zone gave jervine (**5**, 135 mg) as colorless needles, mp 243−244 °C, $[\alpha]_D$ −78.92° (EtOH, *c* 0.24), while the more polar zone yielded veratramine (**3**, 75 mg) as colorless needles, mp 208−210 °C, $[\alpha]_D$ −60.21° (MeOH, *c* 0.29). Fraction 23 [MeOH−$CHCl_3$ (15:85) eluate, 190 mg] was recrystallized from Me_2CO to give veratrosine (**4**, 57 mg) as colorless needles, mp 242−244 °C, $[\alpha]_D$ −40.76° (EtOH−$CHCl_3$ 1:1, *c* 0.25).

Isoveratramine (1): colorless prisms; mp 267−270°; $[\alpha]^{23}_D$ +102.18° (*c* 0.09, MeOH); UV (EtOH) λ_{max} (log ϵ) 207 (4.32) nm; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz), Table 1; EIMS *m/z* 409 (M^+, 3), 408 (6), 391 (6), 376 (6), 362 (5), 295 (11), 115 (100); HREIMS *m/z* 409.2998 (calcd for $C_{27}H_{39}NO_2$, 409.2981).

Verapatuline (2): colorless amorphous solid; $[\alpha]^{23}_D$ −49.93° (*c* 0.31, EtOH); UV (EtOH) λ_{max} (log ϵ) 252 (4.25) nm; IR (KBr) ν_{max} 3400, 1700, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), Table 2; EIMS *m/z* 483 (M^+, 5), 183 (100), 168 (70); HREIMS *m/z* 483.3026 (calcd for $C_{29}H_{41}NO_5$, 483.2984).

Acknowledgment. One of the authors (W. Z.) is grateful to Dr. M. Sasa, former President of Toyama Medical and Pharmaceutical University, and to the Fujisawa Foundation for a fellowship.

References and Notes

(1) *Dictionary of Chinese Crude Drugs*; Chiang Su New Medical College and Shanghai Scientific Technological Publishers: Shanghai, 1977; pp 2692−2695.
(2) Namba, T. In *The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures*; Hoikusha: Osaka, 1993; Vol. I, pp 183−184.
(3) Harrison, D. M. *Nat. Prod. Rep.* **1984**, *1*, 219−224.
(4) Harrison, D. M. *Nat. Prod. Rep.* **1986**, *3*, 443−449.
(5) Atta-ur-Rahman; Choudhary, M. I. *Nat. Prod. Rep.* **1995**, *12*, 361−379.
(6) Atta-ur-Rahman; Choudhary, M. I. *Nat. Prod. Rep.* **1997**, *14*, 191−203.
(7) Prelog, V.; Jeger, O. In *The Alkaloids: Chemistry and Physiology*; Manske, R. H. F., Ed.; Academic: New York, 1953; Vol. 3, Chapter 21, pp 247−312.
(8) Tomko, J.; Voticky, Z. In *The Alkaloids: Chemistry and Physiology*; Manske, R. H. F., Ed.; Academic: New York, 1973; Vol. 14, Chapter 1, pp 1−82.
(9) Greenhill, J. V.; Grayshan, P. In *The Alkaloids: Chemistry and Pharmacology*; Brossi, A., Cordell, G. A., Ed.; Academic: San Diego, 1992; Vol. 41, Chapter 4, pp 177−237.
(10) Zhao, W.; Tezuka, Y.; Kikuchi, T.; Chen, J.; Guo, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2920−2928.
(11) Part 4 in the series Studies on the Constituents of Veratrum Plants. For part 3, see: Tezuka, Y.; Kikuchi, T.; Zhao, W.; Chen, J.; Guo, Y. *J. Nat. Prod.*, in press.
(12) Zhao, W.; Tezuka, Y.; Kikuchi, T.; Chen, J.; Guo, Y. *Chem. Pharm. Bull.* **1991**, *39*, 549−554.
(13) Woo, E.-R.; Kim, H. J.; Kwak, J. H.; Lim, Y. K.; Park, S. K.; Kim, H. S.; Lee, C.-K.; Park, H. *Arch. Pharm. Res.* **1997**, *20*, 58−67.
(14) Sprague, P. W.; Doddrell, D.; Roberts, J. D. *Tetrahedron* **1971**, *27*, 4857−4862.
(15) Irsch, E.-M.; Pachaly, P.; Breitmaier, E.; Sin, K.-S. *Liebigs Ann. Chem.* **1993**, 281−285.
(16) Atta-ur-Rahman; Ali, R. A.; Parveen, T.; Choudhary, M. I.; Sener, B.; Turkoz, S. *Phytochemistry* **1991**, *30*, 368−370.
(17) Budzikiewicz, H. *Tetrahedron* **1964**, *20*, 2267−2278.
(18) Kaneko, K.; Kawamura, N.; Mitsuhashi, H.; Ohsaki, K. *Chem. Pharm. Bull.* **1979**, *27*, 2534−2536.
(19) Han, X.; Rüegger, H. *Planta Med.* **1992**, *58*, 449−453.
(20) Ripperger, H. *Phytochemistry* **1996**, *41*, 1629−1631.
(21) In *Dictionary of Alkaloids*; Southon, I. W., Buckingham, J., Ed.; Chapman and Hall: London, 1989; Vol. 1, pp 22, 446, 617, 618, 860.
(22) Chen, Y.-Y.; Chang, F.-R.; Wu, Y.-C. *J. Nat. Prod.* **1996**, *59*, 904−906.
(23) Wu, Y.-C.; Chao, Y.-C.; Chang, F.-R.; Chen, Y.-Y. *Phytochemistry* **1997**, *46*, 181−184.

NP980150B