(+)-Verussurine, a New Steroidal Alkaloid from the Roots and Rhizomes of *Veratrum nigrum* var. *ussuriense* and Structure Revision of (+)-Verabenzoamine[1]

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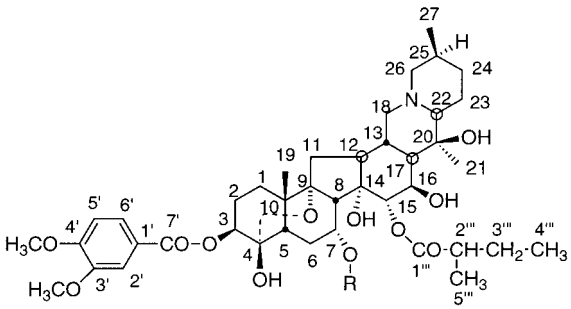
Two minor steroidal alkaloids, **1** and **2**, have been isolated from the roots and rhizomes of *Veratrum nigrum* var. *ussuriense*. Their structures have been determined by the use of spectral data as 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**1**) and 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**2**). By spectral data comparison with verabenzoamine, the structure of the latter compound has been revised from the previously reported 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**1**) to 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**2**). Accordingly, alkaloid **1** [7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine] must be new, and it was given the trivial name verussurine.

The Chinese crude drug "Li-lu", which is used for treating aphasia arising from apoplexy, dysentery, jaundice, headache, scabies, and chronic malaria,[2,3] is prepared from the dried roots and rhizomes of several *Veratrum* species (Liliaceae) such as *V. nigrum* L., *V. nigrum* L. var. *ussuriense* Loes., *V. maackii* Reg., *V. puberulum* Loes. f., *V. dahuricum* Loes. f., *V. schindleri* Loes. f., *V. grandiflorum* (Maxim.) Loes. f., and *V. mengtzeanum* Loes. f. Constituents of *Veratrum* species have been examined extensively, and more than 100 steroidal alkaloids have been isolated so far.[4−10] We have reported a new steroidal alkaloid (maackinine) and five known alkaloids (germanitrine, angeloylzygadenine, zygadenine, verazine, verazinine) from *V. maackii*[11] and a new alkaloid (verussurinine) and six known alkaloids (germidine, germerine, 15-*O*-(2-methylbutyroyl)germine, verazine, jervine, neogermbudine) from *V. nigrum* var. *ussuriense*.[1] In our continuing study, we have isolated two minor alkaloids **1** and **2** from *V. nigrum* var. *ussuriense*. Their structures were determined by spectral data interpretation as 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**1**) and 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**2**). In the present study the structure of verabenzoamine has been revised from the previously reported 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine[12] (**1**) to 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**2**). Accordingly, alkaloid **1** [7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine] must be new, and thus, we named it verussurine.

Alkaloid **2** was obtained as a colorless amorphous solid having $[\alpha]^{23}_{D}$ +8.7° ($CHCl_3$). Its EIMS showed a molecular ion at *m*/*z* 757 ($C_{41}H_{59}O_{12}N$ by HREIMS) and fragment ions at *m*/*z* 739, 575, 557, 473, 456, 182, 167, 112, and 98. The fragment ions at *m*/*z* 575 (M^+ − veratroic acid, $C_{32}H_{49}O_8N$) and 182 (veratroic acid, $C_9H_{10}O_4$) suggested the presence of a veratroyl group, while those at *m*/*z* 112 ($C_7H_{14}N$) and 98 ($C_6H_{12}N$) were characteristic of ceveratrum-type steroidal alkaloids.[13,14] In the IR spectrum, **2** showed absorptions at 3450 (OH), 2870, 2830, 2760 (*trans*-quinolizidine[15]), 1705 (ester CO), 1600, and 1510 (benzene ring) cm^{-1}. These data suggested that **2** is an ester-containing ceveratrum alkaloid.



The ^1H and ^{13}C NMR spectra of **2** were similar to those of 15-*O*-(2-methylbutyroyl)germine, isolated previously from the same extract,[1] and analysis of the $^1H-^1H$ and $^1H-^{13}C$ COSY spectra indicated the presence of two *tert*-methyls, a *sec*-methyl, eight methylenes, 11 methines (five of which are oxygen- or nitrogen-substituted), three oxygen-substituted quaternary carbons, a ketal carbon, and a 2-methylbutyroyl functionality (Table 1 and Experimental Section). However, they were characterized by the presence of signals due to a veratroyl group, with low-field chemical shifts of H-3 [**2**, δ_H 5.17; 15-*O*-(2-methylbutyroyl)-germine, δ_H 3.85] and C-3 [**2**, δ_C 74.9; 15-*O*-(2-methylbutyroyl)germine, δ_C 72.7] and high-field chemical shifts of C-2 [**2**, δ_C 26.7; 15-*O*-(2-methylbutyroyl)germine, δ_C 27.9] and C-4 [**2**, δ_C 105.6; 15-*O*-(2-methylbutyroyl)germine, δ_C 107.0].

On the basis of the spectral data mentioned above, **2** was considered to be a germine-type alkaloid containing two ester (a 2-methylbutyroyl and a veratroyl) groups. Furthermore, the low-field shift of H-3 and C-3 and high-field shift of C-2 and C-4, in comparison with 15-*O*-(2-methylbutyroyl)germine, indicated the location of the veratroyl group to be at the C-3 position; i.e., **2** was 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine. The planar struc-

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

	1	**2**			**1**	**2**	
position	δ	δ	^1H l.r.-coupled[a]	position	δ	δ	^1H l.r.-coupled[a]
1	32.8 t	32.6 t	19	ester group at C-3			
2	26.5 t	26.7 t		1′	122.9 s	123.0 s	5′
3	74.9 d	74.9 d		2′	112.4 d	112.3 d	
4	105.1 s	105.6 s	2, 3	3′	148.7 s	148.6 s	2′, 5′, OCH_3
5	46.3 d	46.5 d	3, 19	4′	153.2 s	153.1 s	2′, 5′, 6′, OCH_3
6	27.8 t	28.8 t		5′	110.2 d	110.2 d	
7	67.9 d	66.8 d		6′	123.5 d	123.6 d	
8	48.0 d	48.0 d	11, 15	7′	165.8 s	165.8 s	3, 2′, 6′
9	92.6 s	93.0 s	5, 8, 11, 19	3′-OCH_3	56.1 q	56.0 q	
10	45.6 s	46.1 s	2, 6, 11, 19	4′-OCH_3	56.1 q	56.0 q	
11	33.0 t	33.1 t					
12	46.6 d	47.2 d		ester group at C-7			
13	33.2 d	33.4 d	11, 16	1″	169.3 s		
14	80.0 s	81.1 s	8, 12, 15, 16	2″	21.5 q		
15	69.5 d	69.9 d					
16	69.4 d	69.3 d		ester group at C-15			
17	45.9 d	45.4 d		1‴	175.3 s	175.7 s	15, 2‴, 3‴, 5‴
18	61.4 t	61.3 t		2‴	41.3 d	41.2 d	4‴
19	20.0 q	19.2 q		3‴	26.9 t	26.8 t	5‴
20	72.9 s	72.9 s	21	4‴	11.7 q	11.6 q	2‴
21	19.4 q	20.2 q		5‴	17.0 q	16.8 q	3‴
22	69.8 d	69.9 d					
23	18.4 t	18.3 t					
24	29.0 t	28.8 t					
25	27.4 d	27.3 d					
26	61.4 t	61.3 t					
27	17.1 q	17.1 q					

[a] Long-range coupled protons observed in the HMBC spectrum.

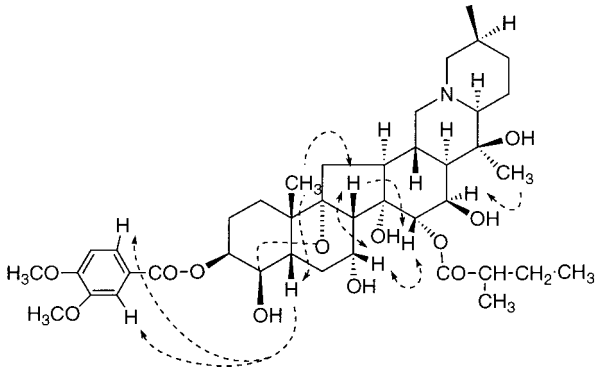

Figure 1. NOEs observed in the difference NOE spectra of **2**.

ture of **2** was confirmed by the long-range correlations observed in the HMBC spectrum, especially from those between C-7′ and H-3, H-2′, H-6′ and between C-1″ and H-15, H-2″, H_2-3″, H_3-5″ (Table 1), while the stereochemistry was established by a series of difference NOE experiments (Figure 1).

Alkaloid **1** was also obtained as colorless amorphous solid, $[\alpha]^{23}_D$ +8.0° ($CHCl_3$), and its EIMS pattern was similar to that of **2**, except for a shift of 42 (C_2H_2O) of the molecular ion (*m/z* 799, $C_{43}H_{61}O_{13}N$) and some key fragment ions. The IR, ^1H NMR, and ^{13}C NMR spectral data were also similar to those of **2** (Table 1 and Experimental Section), but the ^1H and ^{13}C NMR spectra were characterized by the presence of signals due to an acetyl group [δ_H 2.10 (3H, s, H_3-2″); δ_C 21.5 (q, C-2″), 169.3 (s, C-1″)], with the absence of a signal of a hydroxyl proton, and a low-field shift of H-7 [**1**, δ_H 5.82 (1H, t, J = 5.5 Hz); **2**, δ_H 4.62 (1H, t, J = 3.5 Hz)] and C-7 [**1**, δ_C 67.9 (d); **2**, δ_C 66.8 (d)], and a high-field shift of C-6 [**1**, δ_C 27.8 (t); **2**, δ_C 28.8 (t)].

From these spectral data and the analysis of the $^1H-^1H$ and $^1H-^{13}C$ COSY spectra, it could be suggested that **1** is a germine-type alkaloid having three ester (a 2-methylbutyroyl, a veratroyl, and an acetyl) groups. The positions of the ester groups were determined to be C-15, C-3, and C-7, respectively, on the basis of similarity of the spectra mentioned above, the low-field shift of H-7 and C-7, and the high-field shift of C-6, which was also supported by comparison with the ^1H and ^{13}C NMR spectra of germanitrine [7-*O*-acetyl-3-*O*-angeloyl-15-*O*-(2-methylbutyroyl)germine].[1] Thus, **1** was determined to be 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine.

Verabenzoamine, an alkaloid isolated from rhizomes of *Veratrum album*, has been reported to have the structure formula **1** [7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine].[12] However, the reported NMR data for verabenzoamine were compatible with those of **2** but not with those of **1**, especially those for H-7 and C-7 (verabenzoamine, δ_H 4.62, δ_C 66.93; **1**, δ_H 5.82, δ_C 67.9; **2**, δ_H 4.62, δ_C 66.8). Moreover, the δ values of H-7 and C-7 of verabenzoamine were compatible with those of germine-type alkaloids without an ester group at C-7 [H-7, δ_H 4.59−5.03; C-7, δ_C 66.6−66.8][1,16] but not with those of germine esters with an ester group at C-7 [H-7, δ_H 5.79−5.83; C-7, δ_C 67.9].[11] On the other hand, the FABMS data of verabenzoamine were reported to show no molecular ion and a [$M^+ - CH_3CO$] ion at *m/z* 756, but with the EI ionization method which is more destructive than FAB, alkaloids **1** and **2** showed weak, but clear, molecular ions at *m/z* 799 ($C_{43}H_{61}O_{13}N$) and at *m/z* 757 ($C_{41}H_{59}O_{12}N$), respectively. These data would suggest that the structure of verabenzoamine should be revised from the previously reported 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**1**) to 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**2**). Accordingly, structure **1** must be new and has been named verussurine.

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 tetramethylsilane as an internal standard. EIMS and HREIMS were taken on a JEOL GC-Mate mass

spectrometer at an ionization potential of 70 eV. Column chromatography was conducted over alkali-treated silica gel,[11] and preparative TLC was carried out with precoated Merck Kieselgel GF_{254} plates. For drying organic solvents, anhydrous $MgSO_4$ was used.

Plant Material. Roots and rhizomes of *V. nigrum* var. *ussuriense* were collected at Qianshan in Liaoning Province, People's Republic of China, in 1985 and identified by Dr. Guo Yun-Zhen at Shenyang Pharmaceutical University. A voucher specimen is deposited at the Research Institute for Medical and Pharmaceutical Science, Dalian.

Isolation of Alkaloids. Dried roots and rhizomes (7 kg) of *V. nigrum* var. *ussuriense* were cut into small pieces and extracted with EtOH (20 L × 4) at room temperature. The EtOH solutions were combined and concentrated in vacuo, and the residue was dissolved in 5% aqueous tartaric acid solution (7.3 L). The tartaric acid soluble part was defatted with ether (1 L × 4) and then extracted with $CHCl_3$ (2 L × 3) to give fraction A (4.5 g).[1] This was chromatographed over alkali-treated silica gel (670 g) with $CHCl_3$ and then 2% MeOH−$CHCl_3$ to give 32 fractions. Fractions 10 (470 mg) and 11 (975 mg) were combined and rechromatographed over silica gel with 0.5% MeOH−$CHCl_3$ to give nine fractions. Among them, fraction 2 (290 mg) and fraction 4 (245 mg) were separately subjected to preparative TLC with 10% hexane−AcOEt to give alkaloids **1** (verussurine, 3.5 mg) and **2** (verabenzoamine, 23 mg), respectively.

Verussurine (1): colorless amorphous solid; $[\alpha]^{23}_D$ +8.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3500, 2850, 2830, 2760, 1735, 1705, 1600, 1515 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 0.89 (3H, t, J = 7 Hz, H-4‴), 1.03 (3H, s, H_3-19), 1.08 (3H, d, J = 7 Hz, H_3-27), 1.12 (3H, d, J = 7 Hz, H-5‴), 1.19 (3H, s, H_3-21), 1.29 (1H, br d, J = 12 Hz, H-17), 1.44 and 1.64 (each 1H, dqd, J = 14, 7, 6 Hz, H_2-3‴), 1.78 (1H, m, H-12), 1.91 (1H, m, H-25), 2.10 (3H, s, H_3-2‴), 2.30 (1H, m, H-26), 2.36 (1H, qt, J = 7, 6 Hz, H-2‴), 2.40 (1H, br s, H-5), 2.67 (1H, br d, J = 12 Hz, H-26), 2.93 (1H, d, J = 5.5 Hz, 8-H), 3.74 (1H, s, HO-14), 3.92 (3H, s, CH_3O-3′), 3.93 (3H, s, CH_3O-4′), 4.02 (1H, s, HO-4), 4.20 (1H, br s, HO-16), 4.25 (1H, br s, H-16), 5.20 (1H, d, J = 3 Hz, H-15), 5.24 (1H, br d, J = 4 Hz, H-3), 5.82 (1H, t, J = 5.5 Hz, H-7), 6.87 (1H, d, J = 8.5 Hz, H-5′), 7.54 (1H, d, J = 2 Hz, H-2′), 7.65 (1H, dd, J = 8.5, 2 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m/z* 799 (M^+, 3.6), 781 (M^+ − H_2O, 1.2), 617 (M^+ − veratroic acid, 21), 215 (30), 182 (veratroic acid, 100), 167 (*m/z* 182 − CH_3, 40), 112 (*N*-methyl-3-methylquinolizidinium cation, 100); HREIMS *m/z* 799.4150 (calcd for $C_{43}H_{61}O_{13}N$, 799.4142), 781.4027 (calcd for $C_{43}H_{59}O_{12}N$, 781.4037), 617.3603 (calcd for $C_{34}H_{51}O_9N$, 617.3563), 182.0570 (calcd for $C_9H_{10}O_4$, 182.0579), 112.1146 (calcd for $C_7H_{14}N$, 112.1126).

Verabenzoamine (2): colorless amorphous solid; $[\alpha]^{23}_D$ +8.7° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3450, 2870, 2830, 2760, 1705 (br), 1600, 1510 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 0.91 (3H, t, J = 7 Hz, H-4‴), 1.02 (3H, s, H_3-19), 1.10 (3H, br d, J = 6.5 Hz, H_3-27), 1.15 (3H, d, J = 7 Hz, H-5‴), 1.22 (3H, s, H_3-21), 1.33 (1H, br d, J = 12 Hz, H-17), 1.47 and 1.68 (each 1H, dqd, J = 14, 7, 7 Hz, H_2-3‴), 1.82 (1H, m, H-12), 1.94 (1H, m, H-25), 2.36 (1H, m, H-26), 2.41 (1H, sextet, J = 7 Hz, H-2‴), 2.43 (1H, m, H-5), 2.63 (1H, d, J = 4.5 Hz, 8-H), 2.75 (1H, m, H-26), 3.93 (3H, s, CH_3O-3′), 3.94 (3H, s, CH_3O-4′), 4.31 (1H, dd, J = 3.5, 2 Hz, H-16), 4.34 (1H, br s, HO-16), 4.58 (1H, br s, HO-14), 4.62 (1H, t, J = 3.5 Hz, H-7), 4.93 (1H, br s, HO-7), 5.17 (1H, d, J = 4 Hz, H-3), 5.37 (1H, d, J = 3.5 Hz, H-15), 6.19 (1H, br s, HO-4), 6.88 (1H, d, J = 8.5 Hz, H-5′), 7.55 (1H, d, J = 2 Hz, H-2′), 7.67 (1H, dd, J = 8.5, 2 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m/z* 757 (M^+, 3.5), 739 (M^+ − H_2O, 4.1), 575 (M^+ − veratroic acid, 76), 557 (M^+ − H_2O − veratroic acid, 62), 473 (14), 456 (23), 182 ([veratroic acid]$^+$, 100), 167 ([*m/z* 182 − CH_3]$^+$, 26), 112 (*N*-methyl-3-methylquinolizidinium cation, 100), 98 (3-methylquinolizidinium cation, 22); HREIMS *m/z* 757.3990 (calcd for $C_{41}H_{59}O_{12}N$, 757.4037), 739.3966 (calcd for $C_{41}H_{57}O_{11}N$, 739.3931), 575.3419 (calcd for $C_{32}H_{49}O_8N$, 575.3458), 557.3308 (calcd for $C_{32}H_{47}O_7N$, 557.3352), 182.0549 (calcd for $C_9H_{10}O_4$, 182.0579), 112.1140 (calcd for $C_7H_{14}N$, 112.1126), 98.0955 (calcd for $C_6H_{12}N$, 98.0970).

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References and Notes

(1) Studies on the Constituents of *Veratrum* Plants. 3. For part 2, see: Zhao, W.; Tezuka, Y.; Kikuchi, T.; Chen, J.; Guo, Y. *Chem. Pharm. Bull.* **1991**, *39*, 549−554.
(2) Chiang Su New Medical College. In *Dictionary of Chinese Crude Drugs*; Shanghai Scientific Technology Publisher: Shanghai, 1977; pp 2692−2695.
(3) Namba, T. In *The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures*; Hoikusha: Osaka, 1993; Vol. I, pp 183−184.
(4) Harrison, D. M. *Nat. Prod. Rep.* **1984**, *1*, 219−224.
(5) Harrison, D. M. *Nat. Prod. Rep.* **1986**, *3*, 443−449.
(6) Atta-ur-Rahman; Choudhary, M. I. *Nat. Prod. Rep.* **1995**, *12*, 361−379.
(7) Atta-ur-Rahman; Choudhary, M. I. *Nat. Prod. Rep.* **1997**, *14*, 191−203.
(8) Prelog, V.; Jeger, O. In *The Alkaloids: Chemistry and Physiology*; Manske, R. H. F., Ed.; Academic Press: New York, 1953; Vol. 3, Chapter 21, pp 247−312.
(9) Tomko, J.; Voticky, Z. In *The Alkaloids: Chemistry and Physiology*; Manske, R. H. F., Ed.; Academic Press: New York, 1973; Vol. 14, Chapter 1, pp 1−82.
(10) Greenhill, J. V.; Grayshan, P. In *The Alkaloids: Chemistry and Pharmacology*; Brossi, A., Cordell, G. A., Ed.; Academic Press: San Diego, 1992; Vol. 41, Chapter 4, pp 177−237.
(11) Zhao, W.; Tezuka, Y.; Kikuchi, T.; Chen, J.; Guo, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2920−2928.
(12) Atta-ur-Rahman; Ali, R. A.; Choudhary, M. I.; Sener, B.; Turkoz, S. *J. Nat. Prod.* **1992**, *55*, 565−570.
(13) Budzikiewicz, H. *Tetrahedron* **1964**, *20*, 2267−2278.
(14) Atta-ur-Rahman; Choudhary, M. I. In *The Alkaloids: Chemistry and Biology*; Cordell, G. A., Ed.; Academic Press: San Diego, 1998; Vol. 50, Chapter 2, pp 61−108.
(15) Bohlmann, F. *Ber.* **1958**, *91*, 2157−2167.
(16) Han, X.; Grossmann, P.; Rüegger, H. *Magn. Reson. Chem.* **1991**, *29*, 100−112.

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