Studies on the Constituents of *Veratrum* Plants. II.¹⁾ Constituents of *Veratrum nigrum* L. var. *ussuriense*. (1). Structure and ¹H- and ¹³C-Nuclear Magnetic Resonance Spectra of a New Alkaloid, Verussurinine, and Related Alkaloids

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Alkaloidal constituents of the roots and rhizoma of *Veratrum nigrum* L. var. *ussuriense* (Liliaceae), which are used as a source of the Chinese crude drug "Li-lu," were examined and a new alkaloid named verussurinine and six known alkaloids have been isolated. The structure of verussurinine was determined to be 16-*O*-(2-methylbutyryl)germine (1) by means of spectroscopic methods, and six other alkaloids were identified as germinine (2), germerine (3), 15-*O*-(2-methylbutyryl)germine (4), verazine (5), jervine (6), and neogermbudine (7). Complete assignments of the proton and carbon-13 nuclear magnetic resonance (¹H- and ¹³C-NMR) signals of these alkaloids are also presented.

Keywords *Veratrum nigrum* var. *ussuriense*; steroidal alkaloid; verussurinine; 15-*O*-(2-methylbutyryl)germine; germinine; germerine; ¹H-NMR; ¹³C-NMR; 2D NMR

The Chinese crude drug "Li-lu" (藜蘆) is prepared from dried roots and rhizoma of several plants of *Veratrum* species (Liliaceae) such as *V. nigrum* L., *V. maackii* REG., *V. dahuricum* LOES. f., and so on, and is used to treat aphasia arising from apoplexy, wind type dysentery, jaundice, headache, scabies, chronic malaria, etc.²⁾ Constituents of *Veratrum* plants have been examined extensively and more than ninety steroidal alkaloids have been isolated so far.³⁾ In the preceding paper,¹⁾ we have reported the isolation and structure elucidation of a new alkaloid named maackinine (8) together with five known steroidal alkaloids from *V. maackii* REG. We have also examined the constituents of *Veratrum nigrum* L. var. *ussuriense*,⁴⁾ and isolated a new alkaloid named verussurinine (1) along with six known alkaloids. In this paper, we wish to report the structure elucidation of this new compound and the proton and carbon-13 nuclear magnetic resonance (¹H- and ¹³C-NMR) signal assignments of related alkaloids.

Dried roots and rhizoma of *V. nigrum* L. var. *ussuriense* were cut into small pieces and extracted with ethanol. The ethanol extract was treated as shown in Chart 2 to give four fractions A, B, C, and D. Among these, fractions B and C were separated by a combination of column chromatography over alkali-treated silica gel and preparative thin-layer chromatography (TLC) to give verussurinine (U₁₁, 1) together with seven alkaloids, U₅, U₆ (unidentified),

U₇, U₈, U₉, U₁₀, and U₁₂, among which U₅, U₈, and U₁₂ were identified as verazine (5), jervine (6), and neogermbudine (7),⁴⁾ respectively.

Verussurinine (U₁₁, 1) was obtained as a colorless amorphous powder, [α]_D²³ –28.40° (pyridine), and its molecular formula was determined to be C₃₂H₅₁NO₉ by mass spectral (MS) and high-resolution MS (HR-MS) measurements. It showed infrared (IR) absorptions at ν 3230 (br, OH), 2880, 2820, 2760 (*trans*-quinolizidine),⁵⁾ and 1740 (ester CO) cm^{–1}. The ¹H-NMR spectrum of 1 showed signals due to two *tert*-methyls (δ 0.77 and 1.33, 19- and 21-H₃, respectively), a *sec*-methyl (δ 1.01, d, *J* = 7 Hz, 27-H₃), and a 2-methylbutyryl group (δ 0.88, 3H, t, *J* = 7 Hz, 4'-H₃; 1.30, 3H, d, *J* = 7 Hz, 5'-H₃; 1.44 and 1.81, each 1H, dqd, *J* = 14, 7, and 7 Hz, 3'-H₂; 2.42, 1H, sextet, *J* = 7 Hz, 2'-H) (Table I), and the ¹³C-NMR spectrum showed thirty-two signals including a carbonyl carbon signal (δ 175.3) and eleven oxygen- or nitrogen-substituted carbon signals (Table II).

Detailed analyses of the ¹H- and ¹³C-NMR spectra by means of ¹H-¹H shift correlation spectroscopy (COSY) (Fig. 1) and ¹H-¹³C COSY indicated the presence in 1 of the partial structures shown in Chart 3.

These partial structures, coupled with the similarity of the ¹³C-NMR spectral pattern to that of maackinine (8)¹⁾ (Table II), suggested that 1 might be a monoester alkaloid

Chart 1

of the germine series. Furthermore, the ^1H -signal assignable to 16-H was shifted markedly down-field (δ 6.27) when compared with that of 8 (δ 4.31), indicating the location of the 2-methylbutyryl group to be at the C-16 position.

Based on these spectral data, verussurinine was concluded to be 16-*O*-(2-methylbutyryl)germine (1).

Compound U_7 (2) was obtained as colorless prisms, mp 231–233 °C, $[\alpha]_D^{23} + 6.85^\circ$ (CHCl_3), and its molecular formula was determined to be $\text{C}_{34}\text{H}_{53}\text{NO}_{10}$ by MS and HR-MS measurements. It showed IR absorptions at ν 3450, 2860, 2830, 2780, and 1735 cm^{-1} , suggesting the presence of hydroxyl, *trans*-quinolizidine,⁵⁾ and ester groups in the molecule. In the ^1H -NMR spectrum, 2 showed signals due to two *tert*-methyls (δ 0.97 and 1.20, 19- and 21- H_3 , respectively), a *sec*-methyl (δ 1.08, d, $J=7\text{ Hz}$, 27- H_3), an acetyl methyl (δ 2.08), and a 2-methylbutyryl group (δ 0.91, 3H, t, $J=7\text{ Hz}$, 4''- H_3 ; 1.14, 3H, d, $J=7\text{ Hz}$, 5''- H_3 ; 1.47 and 1.66, each 1H, dqd, $J=14, 7$, and 7 Hz , 3''- H_2 ; 2.40, 1H, sextet, $J=7\text{ Hz}$, 2''-H) (Table I), and in the ^{13}C -NMR spectrum, 2 showed signals due to two carbonyl carbons at δ 170.5 and 175.7 (Table II). Extensive analyses of these ^1H - and ^{13}C -NMR spectra with the aid of ^1H - ^1H and ^1H - ^{13}C COSY led to unambiguous assignments of all the ^1H - and ^{13}C -signals except for quaternary carbon signals.

These spectral data suggested that 2 is also an ester alkaloid of the germine type. This was supported by the

long-range ^1H - ^{13}C COSY spectrum (Fig. 2), in which connectivities of all the quaternary carbons (C_4 , C_9 , C_{10} , C_{14} , and C_{20}) with relevant protons were observed as shown by arrows in the formula in Fig. 2. The positions of the acetyl and the 2-methylbutyryl groups were also elucidated by long-range ^1H - ^{13}C COSY. As can be seen in Fig. 2a, the carbonyl carbons at δ 170.5 and 175.7 showed long-range correlations with the protons at δ 2.08 (acetyl methyl) and 4.92 (3-H) and with the protons at δ 1.14 (5''- H_3), 2.40 (2''-H), and 5.34 (15-H), respectively (Fig. 2a and Table II).

From the foregoing evidence, compound U_7 (2) was determined to be germidine [3-*O*-acetyl-15-*O*-(2-methylbutyryl)germine].⁶⁾

Compound U_9 (3), $\text{C}_{37}\text{H}_{59}\text{NO}_{11}$, mp 207–209 °C, $[\alpha]_D^{23} + 5.16^\circ$ (CHCl_3), and compound U_{10} (4), $\text{C}_{32}\text{H}_{51}\text{NO}_9$, mp 221–223 °C, $[\alpha]_D^{23} - 24.10^\circ$ (CHCl_3), were also believed to be germine-type alkaloids. Extensive analyses of their ^1H - and ^{13}C -NMR spectra by means of ^1H - ^1H COSY, ^1H - ^{13}C COSY, and long-range ^1H - ^{13}C COSY led to exact assignments of all the ^1H - and ^{13}C -signals as shown in Tables I and II. Finally, compounds U_9 and U_{10} were determined to be germerine (3) [3-*O*-(2-hydroxy-2-methylbutyryl)-15-*O*-(2-methylbutyryl)germine]⁶⁾ and 15-*O*-(2-methylbutyryl)germine (4),⁷⁾ respectively, on the basis of the above-mentioned spectral data.

Verussurinine (1) has the interesting structural feature of

Fig. 1. ^1H - ^1H COSY Spectrum of Verussurinine (1) in $\text{C}_5\text{D}_5\text{N}$

a) Whole region, b) high-field region.

Chart 3. Partial Structures of Verussurinine (1) Deduced from the ^1H - and ^{13}C -NMR Data

~ : Long-range coupling observed in ^1H - ^1H COSY.

an ester group at the $\text{C}-16\beta$ position, which is considered to be sterically hindered. To our knowledge, this is only the second example of a natural germine-type alkaloid carrying a 16- O -acyl group.⁸⁾ Structure elucidation of U_6 and purification of fractions A and D are now under investigation and will be reported in forthcoming papers.

Experimental

Melting points were determined on a Kofler-type apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-4 polarimeter at 23°C , and IR spectra were recorded on a JASCO IRA-2 spectrometer. MS and HR-MS were taken on a JEOL D-300 mass spectrometer in the electron impact mode at the ionization potential of 70 eV, and NMR spectra were recorded with a JEOL JNM-GX400 spectrometer with tetramethylsilane as an internal standard. Column chromatography was done over alkali-treated silica gel,¹⁾ and preparative TLC was carried out with precoated Merck Kieselgel GF₂₅₄ plates. For drying organic solvents, anhydrous MgSO_4 was used.

Isolation of Alkaloids Dried roots and rhizoma (7 kg) of *Veratrum nigrum* L. var. *ussuriense*, collected at Qianshan in Liaoning Province, China in 1985, were cut into small pieces and extracted with EtOH ($20\text{ l} \times 4$) at room temperature. The EtOH solutions were combined and concentrated in *vacuo*. The residue was dissolved in 5% aqueous tartaric acid solution (7.3 l) and insoluble material was removed by filtration. The filtrate was defatted with ether ($1\text{ l} \times 4$) and then extracted with CHCl_3 ($2\text{ l} \times 3$). The CHCl_3 layer was dried and concentrated to give fraction A (4.5 g). The 5% tartaric acid layer was adjusted to pH 6 by careful addition of 20% aqueous Na_2CO_3 solution and extracted with CHCl_3 ($2\text{ l} \times 3$). The CHCl_3 layer was dried and concentrated to give fraction B (10 g). The residual aqueous solution was then adjusted to pH 10 with 20% aqueous Na_2CO_3 solution and extracted with CHCl_3 ($2\text{ l} \times 4$). Drying and concentration of the CHCl_3 layer gave fraction C (11.6 g). Then, the aqueous solution was further basified (pH > 10) by addition of 20% aqueous Na_2CO_3 solution and extracted with CHCl_3 ($1\text{ l} \times 4$). The CHCl_3 layer was dried and concentrated to give fraction D (5.6 g).

Treatment of Fraction B Fraction B (10 g) was chromatographed over alkali-treated silica gel (520 g) with $\text{MeOH}-\text{CHCl}_3$ (2:98, 4:96, and then 6:94). The eluates were monitored by TLC and separated into fifty-one fractions.

Fractions 5–7 (295 mg) were combined and recrystallized from acetone to give U_5 (120 mg), mp $178\text{--}180^\circ\text{C}$, which was identified as verazine (5) by comparison with an authentic sample.⁴⁾

Fractions 22–25 (35 mg) and fraction 26 (290 mg) were recrystallized from acetone to give U_6 (10 mg) and U_7 (155 mg), respectively.

Fraction 29 (35 mg) was also recrystallized from acetone to afford U_8 (20 mg), mp $236.5\text{--}238^\circ\text{C}$, which was identified as jervine (6).⁴⁾

Combined fractions 30–33 (540 mg) were recrystallized from acetone to give U_9 (350 mg).

Treatment of Fraction C Fraction C (11.6 g) was also chromatographed over alkali-treated silica gel (670 g) with $\text{MeOH}-\text{CHCl}_3$ (2:98, 4:96, and then 8:92), and separated into fifty fractions on the basis of TLC

Fig. 2. Long-Range ^1H - ^{13}C COSY Spectrum of **2** in CDCl_3 (Sample ca. 80 mg, $J_{\text{CH}} = 10 \text{ Hz}$, 12 h Run)

a) Whole region, b) high-field region. Dotted circles indicate the correlation peaks, which are significant but weak at this threshold level.

monitoring.

Fractions 17–19 (1.32 g) were combined and recrystallized from acetone to give **U**₁₀ (350 mg).

Fraction 20 (880 mg) was further purified by preparative TLC with $\text{MeOH}-\text{CHCl}_3$ (5:95) to give **U**₁₁ (180 mg), while fraction 28 (880 mg) was recrystallized from acetone to give **U**₁₂ (410 mg), mp 147–149 °C,

which was identified as neogermbudine (**7**).⁴⁾

Verussurinine (**U**₁₁, **1**) Colorless amorphous powder, $[\alpha]_{\text{D}} -28.40^\circ$ ($c=0.48$, pyridine). IR ν_{max} (KBr) cm^{-1} : 3230, 2880, 2820, 2760, 1740. ^1H - and ^{13}C -NMR: Tables I and II. MS m/z (%): 593 (M^+ , 100), 575 ($\text{M}^+ - \text{H}_2\text{O}$, 25), 550 (15), 492 (32), 474 (15), 331 (8), 154 (17), 112 (100), 98 (61). HR-MS: Found 593.3561, Calcd for $\text{C}_{32}\text{H}_{51}\text{NO}_9$ (M^+) 593.3563.

TABLE I. ^1H Chemical Shifts (in ppm) and Coupling Constants (in Hz, in Parentheses) for the Alkaloids from *V. nigrum* L. var. *ussuriense*

^1H	1 ^{a)}	2 ^{b)}	3 ^{b)}	4 ^{b)}
1-H	1.90 m 1.63 dd (13, 6.5)	1.66 m 1.56 m	1.70 m 1.61 m	1.70 m 1.57 m
2-H	2.39 m 1.90 m	2.11 m 1.68 m	2.15 m 1.65 m	2.02 m 1.59 m
3-H	4.47 br d (4)	4.92 br d (4)	5.03 br d (4)	3.85 br d (4.5)
5-H	2.87 t (3)	2.32 t (3)	2.22 t (3)	2.37 t (3)
6-H	2.44 dd (15, 3) 2.22 ddd (15, 5, 3)	2.14 br s	2.17 m 2.08 dd (17, 5)	2.13 m 2.07 m
7-H	4.67 br t (5)	4.59 br t (5)	4.60 br t (5)	4.59 br t (5)
8-H	2.67 d (5)	2.58 d (5)	2.60 d (5)	2.56 d (5)
11-H	2.59 dd (15, 9) 1.84 br d (15)	2.22 dd (15, 8.5) 1.59 m	2.22 dd (15, 9) 1.59 m	2.18 dd (15, 8.5) 1.58 m
12-H	2.16 m	1.79 ddd (11.5, 8.5, 2)	1.79 ddd (11.5, 8.5, 2)	1.77 ddd (11.5, 8.5, 2)
13-H	1.92 m	1.54 m	1.52 m	1.53 m
15-H	4.34 d (3.5)	5.34 d (3.5)	5.34 d (3)	5.34 d (3.5)
16-H	6.27 br d (3.5)	4.29 dd (3.5, 2)	4.29 dd (3, 2)	4.28 dd (3.5, 2)
17-H	1.92 m	1.29 dd (12, 2)	1.28 dd (12, 2)	1.28 dd (12, 2)
18-H	3.04 dd (11, 3) 1.70 t (11)	2.72 dd (11.5, 4) 1.74 t (11.5)	2.72 dd (11.5, 4) 1.74 t (11.5)	2.73 dd (11.5, 4) 1.73 t (11.5)
22-H	1.55 dd (11, 3)	1.75 m	1.77 m	1.74 m
23-H	1.82 m 1.43 m	1.54 m	ca. 1.5	1.54 m
24-H	1.43 m	1.60 m 1.51 m	1.58 m 1.51 m	1.61 m 1.51 m
25-H	1.76 br s	1.90 br s	1.91 br s	1.91 br s
26-H	2.74 br d (11) 2.18 dd (11, 3)	2.65 br d (11.5) 2.29 dd (11.5, 3.5)	2.66 br d (11.5) 2.29 dd (11.5, 3.5)	2.67 br d (11.5) 2.28 dd (11.5, 3.5)
19-H ₃	0.77 s	0.97 s	0.98 s	0.94 s
21-H ₃	1.33 s	1.20 s	1.20 s	1.20 s
27-H ₃	1.01 d (7)	1.08 d (7)	1.08 d (7)	1.08 d (7)
4-OH	6.71 br s	6.21 br s	6.12 br s	6.34 br s
7-OH	Not observed	4.97 br s	4.90 br s	5.07 br s
14-OH	Not observed	4.53 s	4.43 s	4.63 s
15-OH	5.97 br s			
16-OH		4.31 s	4.29 s	4.32 s
20-OH	3.92 s	3.36 br s	3.30 br s	Not observed
2'-H(H ₃)	2.42 sextet (7)	2.08 s		2.40 sextet (7)
3'-H	1.81 dqd (14, 7, 7) 1.44 dqd (14, 7, 7)		1.77 m 1.67 m	1.67 dqd (14, 7, 7) 1.47 dqd (14, 7, 7)
4'-H ₃	0.88 t (7)		0.90 t (7)	0.91 t (7)
5'-H ₃	1.30 d (7)		1.40 s	1.14 d (7)
2''-H		2.40 sextet (7)	2.40 sextet (7)	
3''-H		1.66 dqd (14, 7, 7) 1.47 dqd (14, 7, 7)	1.67 m 1.47 dqd (14, 7, 7)	
4''-H ₃		0.91 t (7)	0.91 t (7)	
5''-H ₃		1.14 d (7)	1.15 d (7)	

a, b) Measured in $\text{C}_5\text{D}_5\text{N}$ and CDCl_3 , respectively.**U₆** Colorless amorphous powder, $[\alpha]_{\text{D}} -62.8^\circ$ ($c=1.0$, CHCl_3).**Germinine (U₇, 2)** Colorless prisms, mp $231-233^\circ\text{C}$, $[\alpha]_{\text{D}} +6.85^\circ$ ($c=0.62$, CHCl_3). [Lit., ^{6a)} mp $230-231^\circ\text{C}$, $[\alpha]_{\text{D}} +13^\circ$ ($c=1.67$, CHCl_3)]. IR ν_{max} (CHCl_3) cm^{-1} : 3450, 2860, 2830, 2780, 1735. ^1H - and ^{13}C -NMR: Tables I and II. MS m/z (%): 635 (M^+ , 17), 617 ($\text{M}^+ - \text{H}_2\text{O}$, 4), 575 ($\text{M}^+ - \text{CH}_3\text{CO}_2\text{H}$, 6), 557 ($\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3\text{CO}_2\text{H}$, 3), 535 (4), 472 (3), 112 (100), 98 (10). HR-MS: Found 635.3667, Calcd for $\text{C}_{34}\text{H}_{53}\text{NO}_{10}$ (M^+) 635.3668.**Germerine (U₉, 3)** Colorless prisms, mp $207-209^\circ\text{C}$, $[\alpha]_{\text{D}} +5.16^\circ$ ($c=0.63$, CHCl_3). [Lit., ^{6a)} mp $200-203^\circ\text{C}$, $[\alpha]_{\text{D}} +15.7^\circ$ ($c=1.02$, CHCl_3)]. IR ν_{max} (CHCl_3) cm^{-1} : 3450, 2860, 2820, 2770, 1770. ^1H - and ^{13}C -NMR: Tables I and II. MS m/z (%): 693 (M^+ , 4), 635 (3), 575 ($\text{M}^+ - \text{C}_4\text{H}_8(\text{OH})\text{CO}_2\text{H}$, 20), 557 ($\text{M}^+ - \text{C}_4\text{H}_8(\text{OH})\text{CO}_2\text{H} - \text{H}_2\text{O}$, 5), 473 (3), 456 (12), 112 (100), 98 (11), 73 (31). HR-MS: Found 693.4056, Calcd for $\text{C}_{37}\text{H}_{59}\text{NO}_{11}$ (M^+) 693.4087.**15-O-(2-Methylbutyryl)germine (U₁₀, 4)** Colorless prisms, mp $221-223^\circ\text{C}$, $[\alpha]_{\text{D}} -24.10^\circ$ ($c=0.77$, CHCl_3). [Lit., ⁷⁾ mp $224-226^\circ\text{C}$, $[\alpha]_{\text{D}} -21.48^\circ$ (pyridine)]. IR ν_{max} (CHCl_3) cm^{-1} : 3450, 2860, 2820, 2790, 1730. ^1H - and ^{13}C -NMR: Tables I and II. MS m/z (%): 593 (M^+ , 100), 575 ($\text{M}^+ - \text{H}_2\text{O}$, 49), 535 (4), 491 (14), 474 (12), 331 (10), 154 (8), 112 (100), 98 (31). HR-MS: Found 593.3561, Calcd for $\text{C}_{32}\text{H}_{51}\text{NO}_9$ (M^+) 593.3563.**Acknowledgements** This study was supported in part by a Grant-in-Aid for Scientific Research to T. K. (No. 01571145) from the Ministry of Education, Science and Culture of Japan. 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**

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TABLE II. ^{13}C Chemical Shifts (in ppm) for the Alkaloids from *V. nigrum* L. var. *ussuriense*

^{13}C	1 ^{a)} δ	8 ^{b)} δ	2 ^{b)} δ	^1H L.r. coupled		3 ^{b)} δ	^1H L.r. coupled		4 ^{b)} δ
				$^3J_{\text{CH}}^{\text{c)}$	$^2J_{\text{CH}}^{\text{c)}$		$^3J_{\text{CH}}^{\text{c)}$	$^2J_{\text{CH}}^{\text{c)}$	
1	33.0 t	32.7 t	32.4 t	19		32.4 t	3, 19		32.1 t
2	30.2 t	26.5 t	26.6 t			26.5 t			27.9 t
3	73.2 d	74.2 d	74.6 d	2, 4-OH		75.5 d			72.7 d
4	107.4 s	105.0 s	105.4 s	2, 6	3, 4-OH	105.2 s	6		107.0 s
5	45.5 d	46.2 d	46.0 d	19		46.3 d			44.5 d
6	30.3 t	27.8 t	28.6 t			28.6 t			28.6 t
7	67.8 d	67.9 d	66.7 d	5	6	66.6 d	5	6	66.8 d
8	48.0 d	47.9 d	47.9 d	6, 12		47.9 d	6, 14-OH		47.8 d
9	93.0 s	92.5 s	92.9 s	5, 7, 12, 19	11	92.9 s	5, 7, 19	11	92.6 s
10	47.0 s	45.8 s	45.9 s	2, 6	5, 19	45.9 s	6	1, 19	46.2 s
11	34.3 t	32.9 t	33.1 t			33.1 t			33.1 t
12	47.6 d	46.8 d	47.3 d	15		47.2 d	15		47.2 d
13	34.7 d	33.3 d	33.7 d	11, 16		33.6 d	11, 16		33.6 d
14	82.5 s	80.0 s	81.2 s	8, 11, 16	12, 15, 14-OH	81.1 s	11, 16	8, 12, 15, 14-OH	81.1 s
15	70.2 d	69.7 d	69.71 d	14-OH, 16-OH		69.8 d	14-OH	16	69.9 d
16	70.7 d	69.4 d	69.3 d			69.3 d			69.3 d
17	44.7 d	45.8 d	45.5 d	15, 18, 21		45.4 d	15, 18, 21		45.4 d
18	63.1 t	61.6 t	61.4 t			61.4 t			61.3 t
19	19.2 q	19.4 q	19.1 q			19.1 q			19.2 q
20	73.2 s	72.9 s	73.0 s		21	72.9 s		21	72.9 s
21	21.3 q	19.9 q	19.9 q			19.9 q			20.0 q
22	71.2 d	69.8 d	69.65 d	21, 26		69.6 d	18, 21, 26		69.7 d
23	19.0 t	18.4 t	18.4 t			18.3 t			18.3 t
24	29.6 t	29.0 t	28.9 t	26, 27		28.9 t	26		28.9 t
25	28.0 d	27.4 d	27.3 d		27	27.3 d		27	27.3 d
26	61.9 t	61.4 t	61.3 t			61.3 t			61.2 t
27	17.4 q	17.1 q	17.1 q	26		17.0 q	26		17.1 q
1'	175.3 s	167.2 s	170.5 s	3	2'	176.4 s	3, 5'		175.7 s
2'	42.1 d	127.9 s	21.5 q			74.8 s	4'	5'	41.1 d
3'	26.9 t	138.2 d				33.2 t	5'	4'	26.7 t
4'	12.0 q	15.7 q				7.7 q			11.5 q
5'	16.7 q	20.7 q				25.6 q			16.8 q
1''		169.4 s	175.7 s	15, 5''	2''	175.6 s	15, 5''		
2''		21.6 q	41.2 d	4''	5''	41.1 d	4''	5''	
3''			26.8 t	5''	4''	26.7 t	5''	4''	
4''			11.6 q			11.5 q			
5''			16.8 q			16.8 q			
1'''		166.8 s							
2'''		128.4 s							
3'''		136.3 d							
4'''		15.9 q							
5'''		20.7 q							

a, b) Measured in $\text{C}_5\text{D}_5\text{N}$ and CDCl_3 , respectively. c) $^2J_{\text{CH}}$ and $^3J_{\text{CH}}$ indicate the protons coupled with the carbon through two and three bonds, respectively, observed in the long-range ^1H - ^{13}C COSY.

- 6) a) J. Fried, H. L. White, and O. Wintersteiner, *J. Am. Chem. Soc.*, **72**, 4621 (1950); b) S. M. Kupchan, *ibid.*, **81**, 1921 (1959).
- 7) I. Nakhatov, R. Shakirov, and S. Yu. Yunusov, *Khim. Prir. Soedin.*, **1983**, 118 [*Chem. Nat. Prod.*, **1983**, 120].
- 8) Germinaline [16-*O*-acetyl-3,15-*O*,*O*-bis(2-methylbutyryl)germine] has been isolated from *V. lobelianum*; see K. Samikov, R. Shakirov, and S. Yu. Yunusov, *Khim. Prir. Soedin.*, **1971**, 790. It was reported

that synthetic 3,16-*O*,*O*-diacetylgermine is unstable and degraded rapidly in aqueous solution above pH 7 at room temperature, but is stable in nonaqueous systems (CH_2Cl_2 , CHCl_3 , acetonitrile, acetone, and pyridine). See E. M. Cohen and R. Aczel, *J. Pharmaceut. Sci.*, **60**, 193 (1971); E. M. Cohen, R. Aczel, M. Torchiana, R. Tull, and E. J. J. Grabowski, *J. Med. Chem.*, **17**, 769 (1974).