Studies on the Constituents of *Veratrum* Plants. I. Constituents of *Veratrum maackii* REG.; Isolation and Structure Determination of a New Alkaloid, Maackinine

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The constituents of the roots and rhizoma of *Veratrum maackii* REG. (Liliaceae), which are used as a Chinese crude drug "Li-lu," were examined and a new steroidal alkaloid named maackinine was isolated along with five known alkaloids, germanitrine, verazine, angeloylzygadenine, zygadenine, and verazinin. The structure of maackinine was determined by the use of two-dimensional nuclear magnetic resonance (2-D NMR) techniques (fl-decoupled ^{1}H–^{1}H chemical shift correlation spectroscopy (COSY), ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and long-range ^{1}H–^{13}C COSY spectra). Assignments of ^{1}H- and ^{13}C-NMR signals of these steroidal alkaloids were also performed.

Keywords *Veratrum maackii*; Liliaceae; maackinine; germanitrine; angeloylzygadenine; zygadenine; verazinine; steroidal alkaloid; fl-decoupled ^{1}H–^{1}H COSY; 2-D NMR

The Chinese crude drug "Li-lu (藜蘆)" is prepared from dried roots and rhizoma of the plants belonging to *Veratrum* genus (Liliaceae) and is useful against phasia from apoplexy, wind-type dysentery, jaundice, headache, scabies, chronic malaria, *etc.*[1] The constituents of *Veratrum* plants have been examined extensively and more than ninety steroidal alkaloids have been isolated so far.[2] Some of them show hypotensive activity,[1] *in vitro* cytotoxic activity against mouse leukemic P_{388} cells,[3] and transmitter-releasing activity.[4] In China, several species of *Veratrum* plants, such as *V. nigrum* L., *V. maackii* REG., *V. puberulum* LOES. f., and *V. schindleri* LOES. f., are used for preparation of "Li-lu",[1] but few studies have been done on the chemical constituents of Chinese *Veratrum* species.[5] *Veratrum maackii* REG., is grown mainly in Liaoning and Jilin Provinces in the northern part of China, and the total alkaloids of this plant have been reported to show a hypotensive effect.[6] Recently, constituents of this plant were examined by Zhao, *et al.* (the Chinese group of the present authors), who reported the isolation and identification of two steroidal alkaloids, verazine (M_2, **5**) and angeloylzygadenine (M_3, **3**).[7] Further examination of the extract led to the isolation of four additional steroidal alkaloids including a new one named maackinine (M_{1-1}, **1**). The structures of these alkaloids were determined by means of spectral methods involving two-dimensional nuclear magnetic resonance (2-D NMR) spectra. In this paper, we wish to report the isolation and the stucture determination of these four alkaloids.

The dried roots of *V. maackii* REG. were cut into small pieces and extracted with EtOH. The EtOH extract was treated as shown in Chart 1 to give three fractions A, B, and C. Fraction A was subjected to column chromatography over alkali-treated silica gel (see experimental section) and the eluates were separated repeatedly by preparative thin-layer chromatography (preparative TLC) and high performance liquid chromatography (HPLC) to give a new alkaloid M_{1-1}, named maackinine (**1**), and a known alkaloid, germanitrine (M_{1-2}, **2**).[8] The other fractions, B and C, were also separated by a combination of silica gel column chromatography and preparative TLC, and angeloylzygadenine (M_3, **3**)[7,9] was obtained from fraction B and zygadenine (M_4, **4**)[10] and verazinine (M_5, **6**)[11] were obtained from fraction C. Among these, **3** is a major alkaloid and its identity was confirmed by comparison with an authentic sample.[12]

Maackinine (**1**) is a minor alkaloid obtained as colorless prisms, mp 218—221 °C, $[\alpha]_D$ +3.85° ($CHCl_3$), and its molecular formula was determined to be $C_{39}H_{59}NO_{11}$ by mass spectral (MS) and high-resolution MS (HR-MS) measurements. It showed an ultraviolet (UV) absorption at λ 218 nm ($\log\varepsilon$ 4.18) and infrared (IR) absorptions at ν 3520 (OH), 2870, 2820, 2790, 2780 (*trans*-quinolizidine),[13] 1750, and 1715 cm^{-1} (ester CO). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table I), **1** showed signals due to two *tert*-methyls at δ 1.01 and 1.19 (19- and 21-CH_3, respectively), a *sec*-methyl at δ 1.08 (J=7 Hz, 27-H_3),[14] and an acetyl methyl at δ 2.13, and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** showed three signals due to carbonyl carbons at δ 169.4, 167.2, and 166.8 (Table II), suggesting that **1** may be a steroidal alkaloid having the cevane skeleton and having three ester groupings.

Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra with the aid of ^{1}H–^{1}H shift correlation spectroscopy (COSY), f1-decoupled ^{1}H–^{1}H COSY[15] (Fig. 1), and ^{1}H–^{13}C COSY allowed us to deduce the partial structures shown in Chart 3. Among three ester groupings in **1**, one is obviously an acetyl, while the other two are believed to be angeloyl groupings based on the ^{1}H- and ^{13}C-NMR data[16] (Tables I and II) and the observations of nuclear Overhauser effect (NOE) between the vinyl methyl protons at δ 1.87 (quintet, J=1.5 Hz, 5″-H_3) and 1.90 (quintet, J=1.5 Hz, 5′-H_3) and the olefinic protons at δ 5.97 (qq, J=7, 1.5 Hz, 3″-H) and 6.07 (qq, J=7, 1.5 Hz, 3′-H) respectively.

In the ^{1}H–^{1}H and f1-decoupled ^{1}H–^{1}H COSY (Fig. 4) spectra, the proton at δ 5.83 (7-H) showed correlation peaks with the protons at δ 2.93 (8-H), 2.30 and 2.04 (6-H_2), and the proton at δ 2.04 (6-H) with the signal at δ 2.28 (5-H). The other proton signals were analyzed by stepwise tracing of the correlation peaks observed in the COSY spectra. On the other hand, the protonated carbon signals could be assigned by ^{1}H–^{13}C COSY.

Next, we measured the long-range ^{1}H–^{13}C COSY of **1** in order to determine the connectivities of these partial structures and to determine the position of each ester group. As shown in Figs. 2 and 3, the carbon signal at δ 45.8 (s, C-10) showed long-range correlations with the proton signals at δ

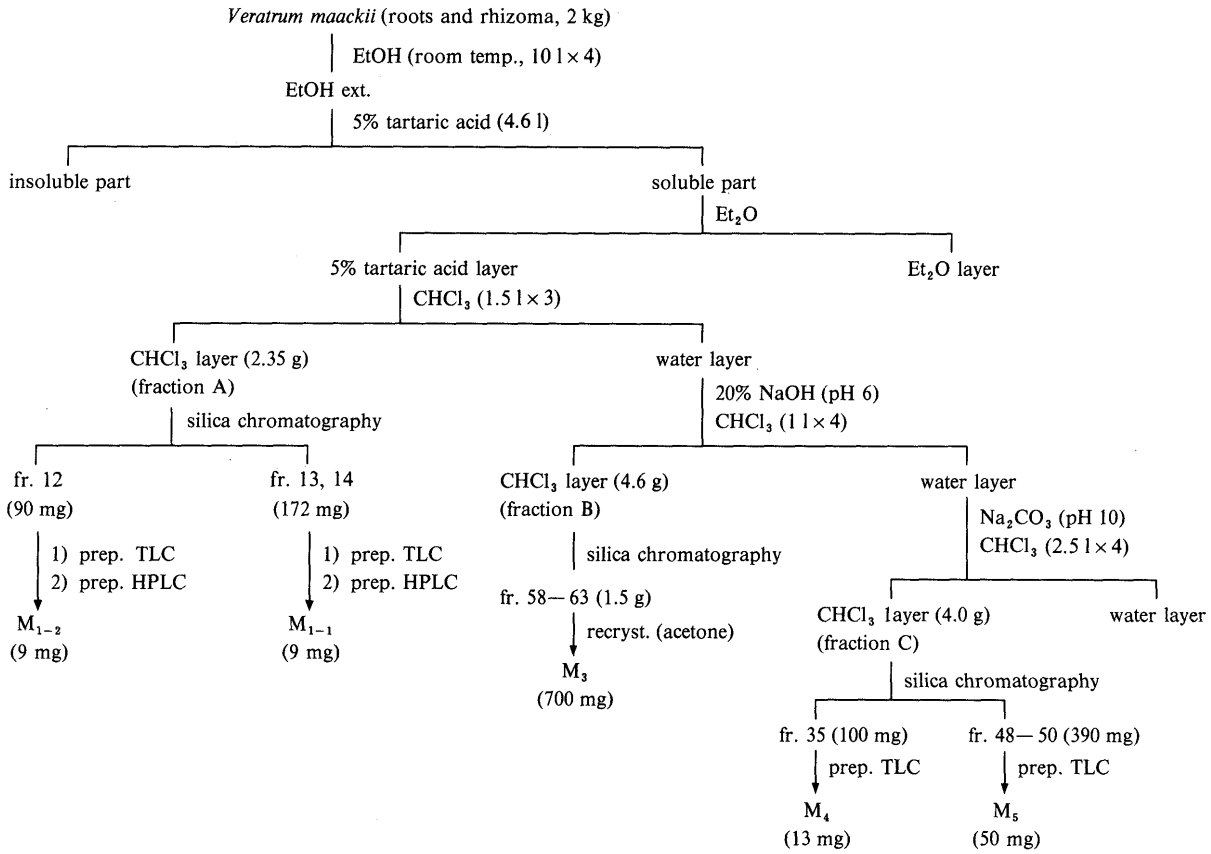

Chart 1

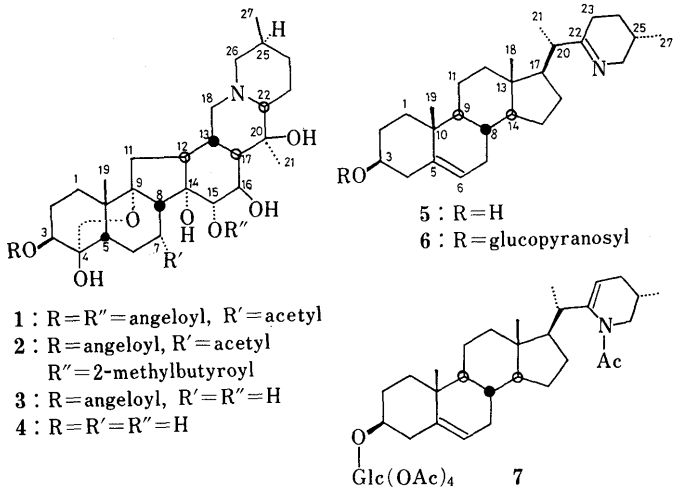

Chart 2

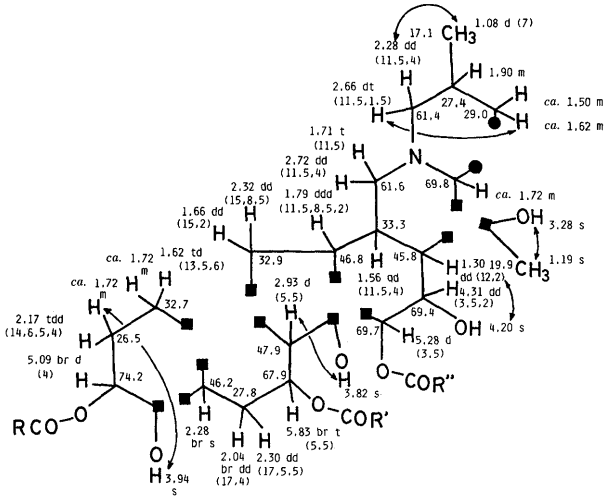

Chart 3. Partial Structures of Maackinine (**1**)
: Long-range coupling observed in ^1H–^1H COSY.

1.01 (19-H_3) and 2.04 (6-H), and the carbon signal at δ 92.5 (s, C-9) with the proton signals at δ 1.01 (19-H_3), 2.28 (5-H), and 2.32 (11-H). Therefore, the quaternary carbon C-10 should be connected with the carbons C-5 (δ 46.2), C-9 (δ 92.5), and C-19 (δ 19.4), and the quaternary carbon C-9 with the carbon C-11 (δ 32.9). Next, the carbon signal at δ 32.7 (t, C-1) showed long-range correlation with the methyl proton signal at δ 1.01 (19-H_3), so that it is reasonable to connect the carbons C-1 and C-10. On the other hand, the carbon signal at δ 80.0 (s, C-14) showed long-range correlations with the proton signals at δ 1.66 (H-11), 1.79 (H-12), 4.31 (H-16), 5.28 (H-15), and 3.82 (14-OH), while the proton signal at δ 3.82 (14-OH) showed long-range correlations with the carbon signals at δ 46.8 (t, C-12) and 47.9 (d, C-8). Thus, the quaternary carbon C-14 must be connected with the carbons C-8, C-12, and C-15. Further, the carbon signal at δ 72.9 (C-20) showed long-range correlations with the proton signals at δ 3.28 (20-OH) and 1.19 (21-H_3), while the proton signal at δ 1.19 (21-H_3) showed long-range correlations with the carbon signals at δ 45.8 (C-17) and 69.8 (C-22), indicating the connectivities of the carbons C-17, C-21, and C-22 with the quaternary carbon C-20.

Although correlation peaks indicative of the direct con-

TABLE I. ^{1}H-NMR Data of the Alkaloids from *V. maackii* in $CDCl_3$

^{1}H	1	2	3	4
1-Hα	1.72 m	1.71 m	1.67 m	1.64 m
1-Hβ	1.62 td (13.5, 6)	1.58 m	1.55 m	1.57 m
2-Hα	2.17 tdd (14, 6.5, 4)	2.18 m	2.19 tdd (14, 7, 5)	2.01 tdd (15, 7, 4.5)
2-Hβ	1.72 m	1.74 m	1.67 m	1.63 m
3-Hα	5.09 brd (4)	5.09 brd (4)	5.01 d (4.5)	3.75 brd (4.5)
5-Hβ	2.28 brs	2.28 m	2.04 t (3)	2.21 t (2.5)
6-Hα	2.30 dd (17, 5.5)	2.29 dd (17, 6)	1.93 dddd (14, 7, 3, 1)	1.82 m
6-Hβ	2.04 dd (17, 4)	2.04 dd (17, 4)	1.75 dddd (14, 11, 6, 3)	
7-Hα			1.99 dtd (13, 11, 7)	1.91 dtd (13, 11, 2)
7-Hβ	5.83 brt (5.5)	5.79 t (6)	1.61 dtd (13, 6, 1)	1.63 m
8-Hβ	2.93 d (5.5)	2.93 d (5.5)	2.51 dd (11, 6)	2.51 dd (11, 6)
11-Hα	1.66 dd (15, 2)	1.65 m	1.56 dd (15, 3)	1.55 dd (15, 2.5)
11-Hβ	2.32 dd (15, 8.5)	2.31 m	2.18 dd (15, 9)	2.13 dd (15, 8.5)
12-Hα	1.79 ddd (11.5, 8.5, 2)	1.77 ddd (16, 12, 4)	1.87 ddd (11, 9, 2.5)	1.83 ddd (10, 8.5, 2.5)
13-Hβ	1.56 qd (11.5, 4)	1.52 qd (12, 3.5)	1.54 qd (12, 4)	1.55 m
15-Hβ	5.28 d (3.5)	5.18 d (3.5)	3.74 d (3)	3.72 d (3)
16-Hα	4.31 dd (3.5, 2)	4.24 dd (3.5, 2)	4.42 dd (3, 2)	4.40 brs
17-Hα	1.30 dd (12, 2)	1.27 dd (12, 2)	1.43 dd (12, 2)	1.41 brd (12)
18-Hα	1.71 t (11.5)	1.74 t (11.5)	1.70 dd (12, 11)	1.70 t (10)
18-Hβ	2.72 dd (11.5, 4)	2.72 dd (11.5, 3.5)	2.67 dd (11, 4)	2.68 brd (10)
22-Hα	1.72 m	1.74 m	1.72 dd (11, 3)	1.71 m
23-H_2	1.49 m 1.55 m	1.50 m 1.62 m	1.52 m 1.62 m	1.54 m 1.62 m
24-H_2	1.50 m 1.62 m	1.50 m 1.61 m	1.49 m 1.58 m	1.50 m 1.58 m
25-Hα	1.90 m	1.90 m	1.89 m	1.90 m
26-Hα	2.28 dd (11.5, 4)	2.27 dd (15, 4)	2.27 dd (11.5, 3.5)	2.28 brd (10)
26-Hβ	2.66 dt (11.5, 1.5)	2.66 brd (11.5)	2.64 dd (11.5, 2)	2.65 brd (10)
19-H_3	1.01 s	0.99 s	1.01 s	0.98 s
21-H_3	1.19 s	1.18 s	1.23 s	1.23 s
27-H_3	1.08 d (7)	1.08 d (7)	1.08 d (7)	1.08 d (7)
4-OH	3.94 s	3.97 s	3.70 s	
14-OH	3.82 s	3.75 s	3.33 s	
15-OH			3.24 s	
16-OH	4.20 s	4.22 s	4.17 s	
20-OH	3.28 s	Not observed	4.67 s	
3-Angeloyl group				
3′-H	6.07 qq (7, 1.5)	6.08 qq (7, 1.5)	6.14 qq (7, 1.5)	
4′-H_3	1.99 dq (7, 1.5)	1.99 dq (7, 1.5)	2.00 dq (7, 1.5)	
5′-H_3	1.90 quintet (1.5)	1.90 quintet (1.5)	1.90 quintet (1.5)	
7-Acetyl group				
	2.13 s	2.09 s		
15-Ester group				
2″-H		2.35 sextet (7)		
3″-H	5.97 qq (7, 1.5)	1.44 dqd (14, 7, 6) 1.63 dqd (14, 7, 6)		
4″-H_3	1.93 dq (7, 1.5)	0.89 t (7)		
5″-H_3	1.87 quintet (1.5)	1.12 d (7)		

nectivities between the carbons C-4 and C-5 and between the carbons C-8 and C-9 could not be detected in this long-range ^{1}H–^{13}C COSYexperiment, it is reasonable to deduce that **1** is a germine-type alkaloid having three ester groupings at the C-3, C-7, and C-15 positions on the basis of the close similarity of its ^{13}C-NMR data to those of angeloylzygadenine (**3**) (Table II) and the appearance of *trans*-quinolizidine bands in the IR spectrum.

The position of each ester grouping was determined by NOE difference experiments, which are reproduced in Fig. 4. Irradiation of the acetyl methyl protons caused the increase of intensity of the signals of 4- and 14-OH and *vice versa*, indicating the position of the acetyl grouping to be C-7. Since both of the remaining ester groupings are angeloyl groupings, they must be present at C-3 and 15. On the other hand, irradiation of 21-H_3, 19-H_3, 8-H, 16-H, and 15-H caused increases in the intensity of 16-H and 16-OH, of 5-H and 8-H, of 19-H_3, 16-OH, 15-H, and 7-H, of 21-H_3, 17-H, 16-OH, and 15-H, and of 14-OH, 16-H, and 7-H, respectively.

These findings coupled with the coupling constants of each proton indicated the stereostructure of **1** to be as shown in Fig. 4. Accordingly, maackinine was determined to be 7-*O*-acetyl-3,15-*O*,*O*-diangeloylgermine (**1**).

Compound M_{1-2} (**2**) is also a minor alkaloid obtained as colorless pillars, mp 221—224 °C, $[\alpha]_D$ −57.4° (pyridine), and has the molecular formula $C_{39}H_{59}NO_{11}$ as confirmed by MS and HR-MS measurements. It showed a UV absorption at λ 218 nm (log ε 3.96) and characteristic IR absorptions at ν 3500 (OH), 2860, 2820, 2780, 2770 (*trans*-quinolizidine),[12] and 1735 cm^{-1} (ester CO). The ^{1}H-NMR spectrum of **2** showd signals due to three methyl groups at δ 0.99 (s), 1.08 (d, J=7 Hz), and 1.18 (s) and the spectral pattern was similar to that of maackinine (**1**) except for the

TABLE II. ^{13}C-NMR Data of the Alkaloids from *V. maackii* in $CDCl_3$

^{13}C	1	2	3	4	^{13}C	1	2	3	4
1	32.7 t	32.6 t	32.8 t	32.2 t	22	69.8 d	69.7 d	69.7 d	69.7 d
2	26.5 t	26.5 t	26.9 t	27.8 t	23	18.4 t	18.4 t	18.4 t	18.5 t
3	74.2 d	74.2 d	75.2 d	73.6 d	24	29.0 t	28.9 t	29.0 t	29.0 t
4	105.0 s	105.0 s	104.8 s	106.3 s	25	27.4 d	27.3 d	27.4 d	27.4 d
5	46.2 d	46.2 d	46.5 d	44.5 d	26	61.4 t	61.4 t	61.4 t	61.4 t
6	27.8 t	27.7 t	19.0 t	18.8 t	27	17.1 q	17.1 q	17.1 q	17.1 q
7	67.9 d	67.9 d	17.2 t	17.4 t	3-Angeloyl group				
8	47.9 d	47.9 d	44.0 d	43.8 d	1′	167.2 s	167.2 s	168.6 s	
9	92.5 s	92.5 s	96.2 s	96.2 s	2′	127.9 s	127.8 s	127.6 s	
10	45.8 s	45.8 s	45.8 s	46.1 s	3′	138.2 d	138.2 d	139.1 d	
11	32.9 t	32.9 t	33.2 t	33.2 t	4′	15.7 q	15.8 q	16.0 q	
12	46.8 d	46.6 d	46.3 d	46.2 d	5′	20.7 q	20.6 q	20.6 q	
13	33.3 d	33.2 d	34.2 d	34.1 d	7-Acetyl group				
14	80.0 s	79.9 s	80.8 s	81.2 s	$\underline{C}OCH_3$	169.4 s	169.3 s		
15	69.7 d	69.5 d	69.9 d	69.9 d	$CO\underline{C}H_3$	21.6 q	21.4 q		
16	69.4 d	69.4 d	70.3 d	70.4 d	15-Ester group				
17	45.8 d	45.5 d	44.3 d	44.3 d	1″	166.8 s	175.3 s		
18	61.6 t	61.4 t	61.7 t	61.6 t	2″	128.4 s	41.2 d		
19	19.4 q	19.3 q	19.0 q	19.1 q	3″	136.3 s	26.8 t		
20	72.9 s	72.9 s	73.3 s	73.3 s	4″	15.9 q	11.6 q		
21	19.9 q	19.9 q	19.9 q	19.9 q	5″	20.7 q	16.9 q		

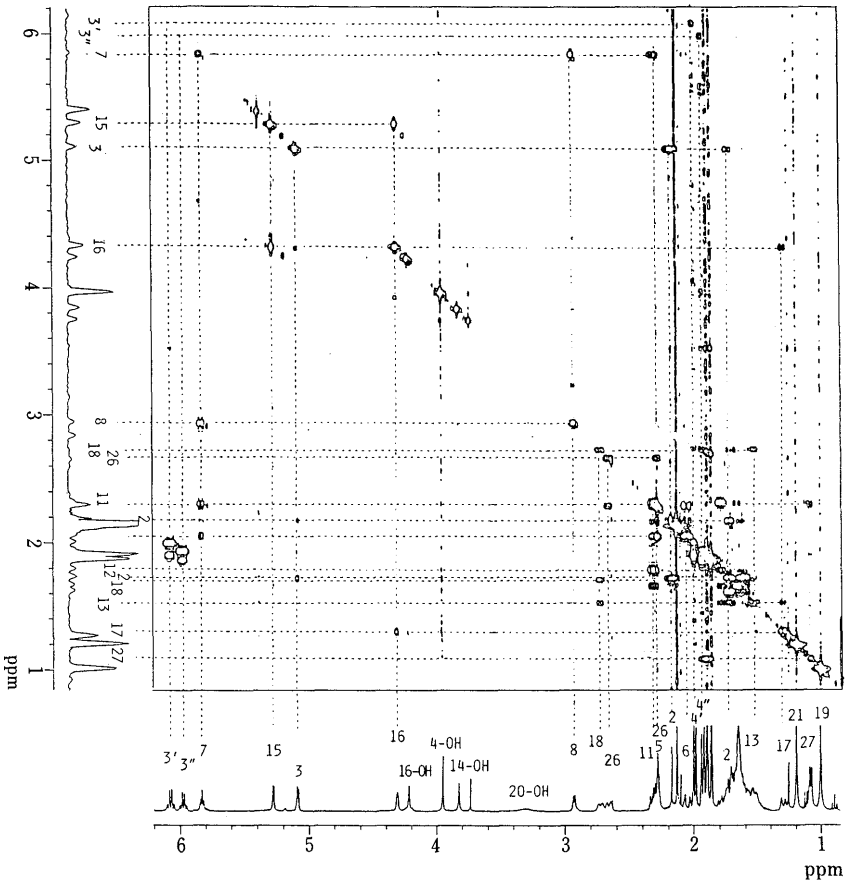

Fig. 1. F1-Decoupled ^1H–^1H Shift Correlated Spectrum of Maackinine (**1**) in $CDCl_3$ (Sample 9 mg, 1.5 h Run)

lack of signals due to an angeloyl grouping and the presence of signals due to a 2-methylbutyroyl grouping [δ 0.89 (3H, t, J=7 Hz, 4″-H_3), 1.12 (3H, d, J=7 Hz, 5″-H_3), 1.44, 1.63 (each 1H, dqd, J=14, 7, 6 Hz, 3″-H_2), and 2.35 (1H, sextet, J=7 Hz, 2″-H)] (Table I), suggesting that **2** may also be a steroidal alkaloid having the cevane skeleton.[13] The ^{13}C-NMR spectrum of **2** showed three signals due to carbonyl carbons at δ 167.2, 169.3, and 175.3, suggesting the presence of three ester groupings.

Extensive analysis of the ^1H- and ^{13}C-NMR spectra with the aid of ^1H–^1H COSY, f1-decoupled ^1H–^1H COSY, ^1H–^{13}C COSY, and long-range ^1H–^{13}C COSY allowed us to deduce that **2** is a triester (an acetyl, an angeloyl, and a 2-methylbutyroyl) of germine (Tables I and II). In the long-

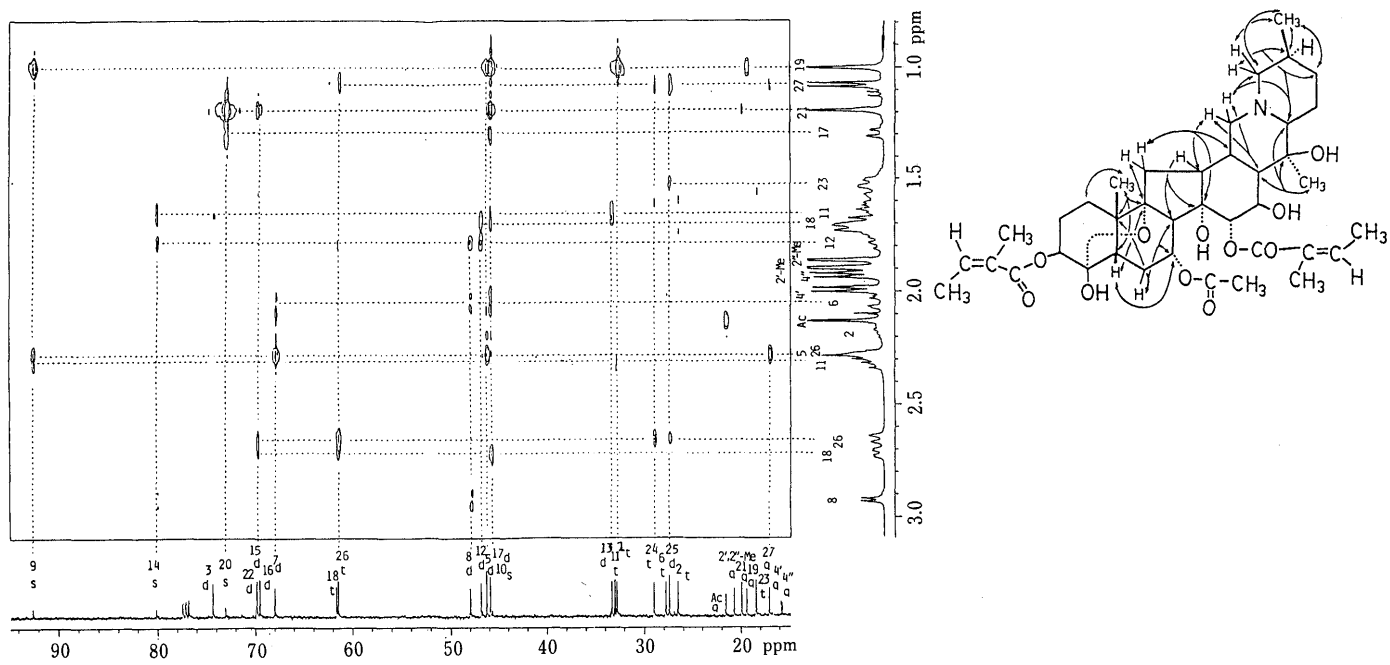

Fig. 2. Long-Range ^{1}H–^{13}C Shift Correlated Spectrum of Maackinine (**1**) in $CDCl_3$ in the Upfield Region (Sample 9 mg, $J_{CH}=10$ Hz, 16 h Run)

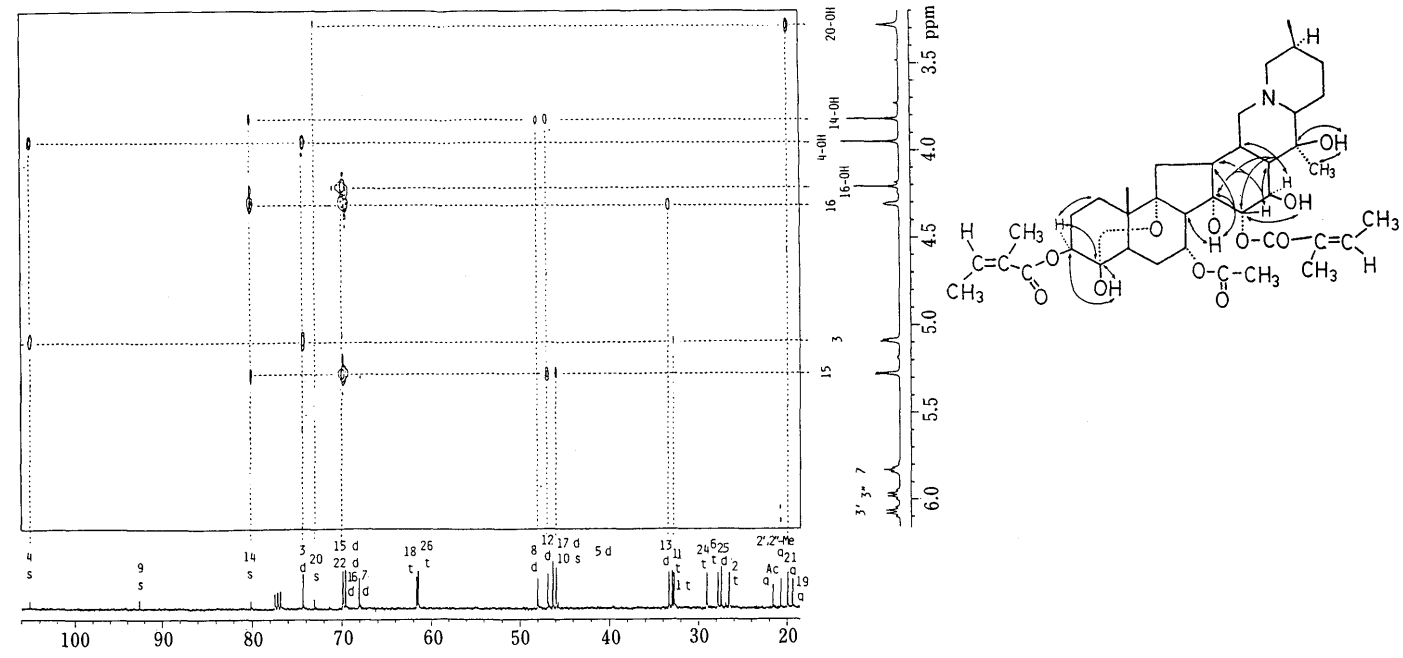

Fig. 3. Long-Range ^{1}H–^{13}C Shift Correlated Spectrum of Maackinine (**1**) in $CDCl_3$ in the Low-Field Region (Sample 9 mg, $J_{CH}=10$ Hz, 16 h Run)

range ^{1}H–^{13}C COSY spectrum, the carbon signals due to 1′-CO (δ 167.2), acetyl carbonyl (δ 169.3), and 1″-CO (δ 175.3) showed long-range correlations with the proton signals due to 3-H and 5′-H_3, with those due to acetyl methyl and 7-H, and with those due to 15-H and 5″-H, respectively (Fig. 5). Therefore, the angeloyl grouping must be attached to C-3, the acetyl grouping to C-7, and the 2-methylbutyroyl grouping to C-15.

Based on these spectral data, M_{1-2} (**2**) was determined to be germanitrine (7-*O*-acetyl-3-*O*-angeloyl-15-*O*-(2-methylbutyroyl)germine).[8]

Compound M_4 (**4**), $C_{27}H_{43}NO_7$, was obtained as colorless needles, mp 195—199 °C, $[\alpha]_D$ −47.6° ($CHCl_3$), and showed the M^+ peak at *m/z* 493 in the MS. In the IR spectrum, **4** showed absorptions of hydroxyl group(s) (ν 3440 cm^{-1}) and a *trans*-quinolizidine group (ν 2860, 2810, 2780, and 2760 cm^{-1}), but no carbonyl absorption. The ^{1}H-NMR spectrum of **4** showed three signals due to methyl protons at δ 0.98 (s, 19-H_3), 1.08 (d, $J=7$ Hz, 27-H_3), and 1.23 (s, 21-H_3) (Table I) and the ^{13}C-NMR spectrum was closely similar to that of **3** except for the carbon signals due to the angeloyl grouping. Therefore, **4** was also believed to be a steroidal alkaloid having the cevane skeleton but no ester grouping (Table II).

Detailed analysis of the f1-decoupled ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and long-range ^{1}H–^{13}C COSY spectra of **4**

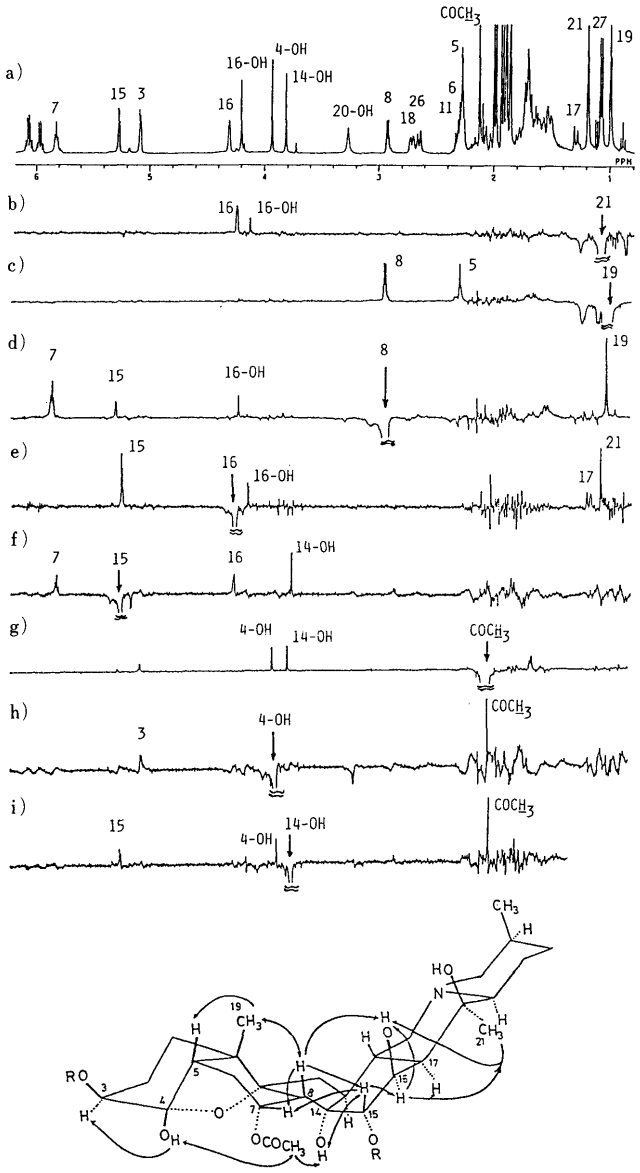

Fig. 4. NOE Difference Spectra of Maackinine (1) in $CDCl_3$
a) Normal spectrum, b—i) NOE difference spectra.

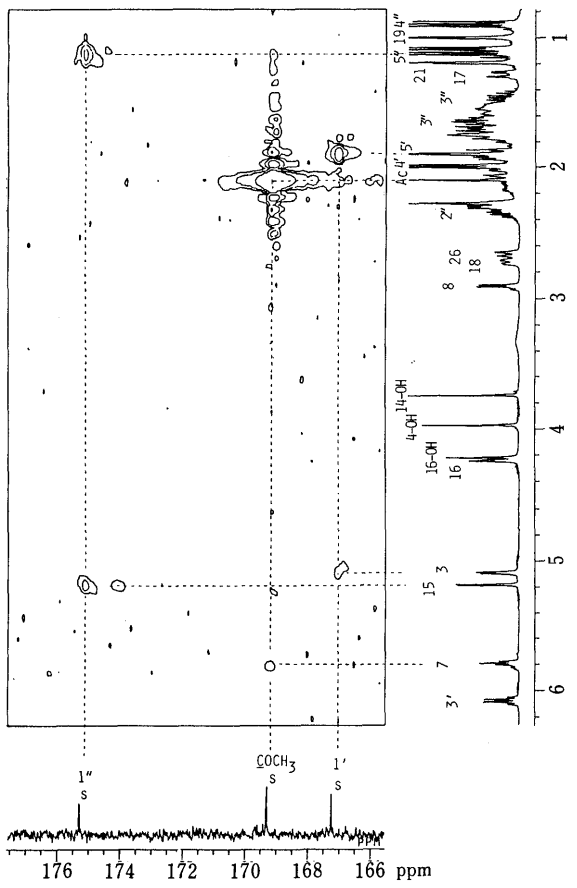

Fig. 5. Long-Range ^1H–^{13}C Shift Correlated Spectrum of Germanitrine (**2**) in $CDCl_3$ (Sample 9 mg, J_{CH} = 10 Hz, 16 h Run)

led to the conclusion that **4** may be zygadenine,[10] and this was confirmed by comparison of its spectral data with the published values.

Compound M_5 (**6**), colorless amorphous powder, $[\alpha]_D$ −67.1° (MeOH), showed the quasi-molecular ion peak at *m/z* 560 ($C_{27}H_{43}NO+H^+$) in the fast atom bombardment MS (FAB-MS). In the IR spectrum, **6** showed a strong absoption of hydroxyl group(s) at *v* 3400 cm^{-1}, but no absorption of a *trans*-quinolizidine group or a carbonyl group.

The ^1H-NMR spectrum of **6** showed the signals due to two *tert*-methyls (δ 0.67 and 0.93, 18- and 19-H_3), two *sec*-methyls (δ 0.85 and 1.14, 27- and 21-H_3), and several protons geminal to oxygen functions (Table III). On the other hand, the ^{13}C-NMR spectrum of **6** exhibited a signal at δ 173.4 which was attributable to an imino carbon. Therefore, **6** was thought to be an alkaloid having a verazine-like skeleton.

On acetylation, **6** gave a pentaacetate (**7**) [δ_H 1.97, 1.98, 1.99, 2.06, and 2.13; δ_C 20.4 (2×C), 20.60 (2×C), 23.6 (br), 169.6, 169.8, 170.0 (br), 170.3, and 170.5], which showd IR absorptions at *v* 1750 and 1630 cm^{-1}, suggesting that one of the acetyl groupings was forming an amide. It should be noted that in the ^{13}C-NMR spectrum measured at room temperature, some signals of this pentaacetate were broadened or almost vanished, perhaps due to the restricted rotation of the N-acetyl grouping, but these carbon signals were clearly obseved when measured at elevated temperature (50—80 °C) (Fig. 6).

Then we measured the ^1H–^1H, ^1H–^{13}C, and long-range ^1H–^{13}C COSY spectra of **6** and its pentaacetate (**7**) (at 80 °C) and detailed analysis of these spectra led to the complete proton and carbon signal assignments as shown in Table III. On the basis of these spectral data, **6** was determined to be verazinine (3-*O*-β-glucopyranosylverazine).[11]

Experimental

Melting points were determined on a Kofler-type apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-4 polarimeter at 22 °C. UV spectra were recorded on a Shimadzu 202 UV spectrometer in MeOH solutions and IR spectra with a JASCO IRA-2 spectrometer or a Nicolet 5DX FT-IR spectrometer in $CHCl_3$ solutions unless otherwise noted. MS were taken on a JEOL D-300 mass spectrometer with a direct inlet system. ^1H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-GX400 spectrometer in $CDCl_3$ or C_5D_5N solutions with tetramethylsilane as an internal standard. Chemical shifts are recorded in δ values and coupling constants are in Hz. Multiplicities of ^{13}C-NMR spectra were determined by means of the distortionless enhancement by polarization transfer (DEPT) method. ^1H–^1H, ^1H–^{13}C, and long-range ^1H–^{13}C COSY spectra were measured by the use of JEOL

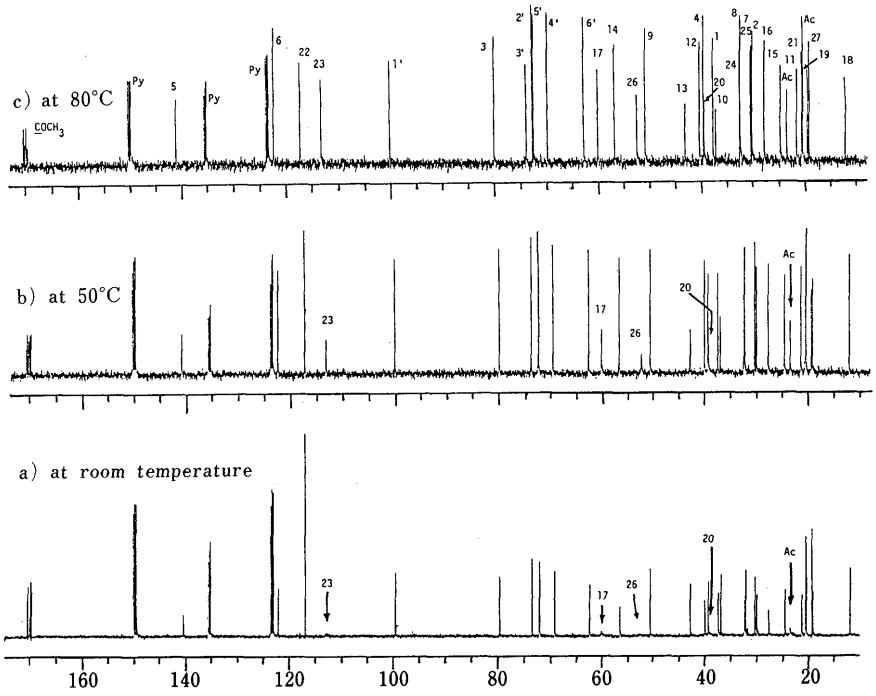

Fig. 6. ^{13}C-NMR Spectra of Verazinine Pentaacetate (7) in C_5D_5N

standard pulse sequences (^{1}H–^{1}H COSY: VCOSYN, 45° mixing pulse; ^{1}H–^{13}C COSY: VBDCHSHF, $J=140$ Hz; long-range ^{1}H–^{13}C COSY: VCHSHF, $J=10$ Hz) and f1-decoupled ^{1}H–^{1}H COSY spectra were measured by the use of the pulse sequence reported by Bax and Freeman.[15] All collected data were treated with JEOL standard software. NOE difference spectra were measured by the use of the JEOL standard sequence (DIFNOE2) with irradiation for 5 s. Column chromatography was done over alkali-treated silica gel (*vide infra*). Preparative TLC was carried out with pre-coated Merck Kieselgel GF_{254} plates and plates were examined under UV light. Extraction of substances from silica gel was done with MeOH–CH_2Cl_2 (5:95 or 10:90) or MeOH–$CHCl_3$ (10:90 or 30:70) and the eluates were concentrated *in vacuo*. TLC analysis was done on pre-coated Merck Kieselgel GF_{254} plates with MeOH–$CHCl_3$ (5:95, 10:90, or 15:85) as developing solvents and spots were detected by spraying Dragendorff reagent. HPLC separations were carried out on a Shimadzu LC-5 HPLC system [conditions: column, TSK gel ODS-120A column, 21.5×300 mm (Tosoh Co., Ltd.); mobile phase, 0.1 M ammonium acetate–MeOH (3:7); flow rate, 5 ml/min; detector setting, 254 nm]. Eluates were concentrated *in vacuo* and the residues were alkalized by addition of dilute Na_2CO_3 solution and extracted with CH_2Cl_2 to give the recovered alkaloids.

Alkali-Treated Silica Gel A portion of silica gel (Qing Dao, China) was taken, three times the amount of 0.5% aqueous NaOH solution was added, and the mixture was shaken thoroughly (pH value was adjusted to 7.8). The mixture was concentrated on a steam bath almost to dryness, then the residual silica gel was dried at 110 °C for 30 min.

Isolation of Alkaloids from *Veratrum maackii* Reg. Dried roots and rhizoma (2 kg) of *V. maackii*, collected at Zhuanghe Xian in Liaoning Province, China in 1985, were cut into small pieces and extracted with EtOH (10 l×4) at room temperature. The EtOH solutions were combined and concentrated *in vacuo*. The residue was dissolved in 5% aqueous tartaric acid solution (4.6 l) and insoluble material was removed by filtration. The filtrate was defatted with ether and then extracted with $CHCl_3$ (1.5 l×3). The $CHCl_3$ layer was dried and concentrated to give fraction A (2.35 g). The 5% tartaric acid layer was adjusted to pH 6 by careful addition of 20% aqueous NaOH solution and extracted with $CHCl_3$ (1 l×4). The $CHCl_3$ layer was dried and concentrated to give fraction B (4.6 g). The residual aqueous solution was then adjusted to pH 10 with 2% aqueous Na_2CO_3 solution and extracted with $CHCl_3$ (3 l×3). The $CHCl_3$ layer was dried and concentrated to give fraction C (4.0 g).

Treatment of Fraction A Fraction A (2.35 g) was chromatographed over silica gel (200 g) with hexane–$CHCl_3$ (10:90). The eluates were monitered by TLC and separated into fifty fractions.

Fraction 12 (90 mg) was subjected to preparative TLC with MeOH–$CHCl_3$ (5:95) to give crude M_{1-2} (30 mg), which was further purified by HPLC followed by recrystallization from acetone–ether to give a pure sample of M_{1-2} (9 mg)

Fractions 13 (115 mg) and 14 (57 mg) were combined and subjected to preparative TLC with MeOH–$CHCl_3$ (5:95) to give crude M_{1-1} (40 mg), which was further purified by HPLC followed by recrystallization from acetone–ether to give a pure sample of M_{1-1} (9 mg).

Treatment of Fraction B Fraction B (4.6 g) was chromatographed over silica gel (640 g) with MeOH–$CHCl_3$ (4:96) and separated into one hundred fractions. Fractions 58—63 (1.5 g) were combined and crystallized from acetone to give M_3 (700 mg), which was identified as angeloylzygadenine (**3**).[9]

Treatment of Fraction C Fraction C (4.0 g) was chromatographed over silica gel (750 g) with MeOH–$CHCl_3$ (7:93) and separated into eighty-four fractions.

Fraction 35 was subjected to preparative TLC with MeOH–$CHCl_3$ (10:90) to give M_4 (13 mg).

Fractions 48 (80 mg), 49 (80 mg), and 50 (230 mg) contained a very polar compound. These were combined and a part (100 mg) was subjected repeatedly to preparative TLC with MeOH–$CHCl_3$ (10:90) to give M_5 (16 mg).

Maackinine (M_{1-1}, 1) Colorless prisms, mp 218—221 °C, $[\alpha]_D$ +3.85° ($c=0.65$, $CHCl_3$). UV λ_{max} nm (log ε): 218 (4.18). IR ν_{max} cm^{-1}: 3520, 2870, 2820, 2790, 2780, 1750, 1715, 1235, 1165. ^{1}H- and ^{13}C-NMR: see Tables I and II. EI-MS (ionization voltage, 20 eV) *m/z* (%): 716 (M^+-1, 13), 601 (49), 569 (37), 463 (35), 353 (9), 297 (17), 112 (100). HR-MS: Found 717.4087, Calcd for $C_{39}H_{59}NO_{11}$ (M^+) 717.4073.

Germanitrine (M_{1-2}, 2) Colorless pillars, mp 221—224 °C, $[\alpha]_D$ −57.4° ($c=0.63$, pyridine), 0.0±2° ($c=0.64$, $CHCl_3$). [Lit.,[7] mp 228—229 °C, $[\alpha]_D$ −61±2° ($c=1.0$, pyridine), 0.0±2° ($c=1.15$, $CHCl_3$)]. UV ν_{max} nm (log ε): 218 (3.96). IR ν_{max} cm^{-1}: 3500, 2860, 2820, 2780, 2770, 1735, 1230, 1160. ^{1}H- and ^{13}C-NMR: see Tables I and II. EI-MS *m/z* (%): 717 (M^+, 1), 658 (1), 410 (4), 256 (39), 149 (27), 100 (100). HR-MS: Found 717.4086, Calcd for $C_{39}H_{59}NO_{11}$ (M^+) 717.4087.

Zygadenine (M_4, 4) Colorless needles (EtOH), mp 195—199 °C, $[\alpha]_D$ −47.6° ($c=0.37$, $CHCl_3$). [Lit.,[10] mp 218—222 °C, $[\alpha]_D$ −48.5° ($CHCl_3$)]. IR ν_{max} cm^{-1}: 3440, 2860, 2810, 2780, 2760, 1050. ^{1}H- and ^{13}C-NMR: see Tables I and II. EI-MS *m/z* (%): 493 (M^+, 40), 476 (11), 429 (15), 386 (11), 368 (10), 355 (17), 256 (29), 149 (31), 112 (100). HR-MS: Found 493.3033, Calcd for $C_{27}H_{43}NO_7$ (M^+) 493.3038.

Verazinine (M_5, 6) Colorless amorphous powder, $[\alpha]_D$ −67.1° ($c=0.44$, MeOH). [Lit.,[11] mp 259—261 °C (acetone), $[\alpha]_D$ −112.6° ($c=0.49$, $CHCl_3$)]. IR ν_{max} (KBr) cm^{-1}:3400, 1075, 1025, 1070—1020 (br). ^{1}H- and ^{13}C-NMR: see Table III. FAB-MS *m/z*: 560 ($M+H^+$) ($C_{23}H_{43}NO+H$),

TABLE III. ^{1}H- and ^{13}C-NMR Data of Alkaloids from *V. mackii* in C_5D_5N

^{1}H	6	7	^{13}C	6	7 (r.t.)	7 (80 °C)
1-H_2	0.99 m 1.73 m	1.05 m 1.79 dt (13.5, 3.5)	1	37.5 t	37.4 t	37.8 t
			2	30.2 t	29.9 t	30.2 t
2-Hα	1.75 dtd (13, 11, 4)	1.68 m	3	78.1 d	79.7 d	80.1 d
2-Hβ	2.12 m	2.02 m	4	39.3 t	39.3 t	39.6 t
3-Hα	3.96 tt (11, 4.5)	3.71 tt (11, 4.5)	5	140.9 s	140.5 s	141.1 s
4-Hα	2.73 ddd (13, 4.5, 2)	2.49 ddd (13, 4.5, 2)	6	121.9 d	122.2 d	122.3 d
4-Hβ	2.47 ddq (13, 11, 2.5)	2.36 ddq (13, 11, 3)	7	32.2 t	32.1 t	32.5 t
6-H	5.35 dt (5, 2.5)	5.40 dt (5, 2)	8	32.1 d	32.2 d	32.6 d
7-Hα	1.56 m	1.56 m	9	50.4 d	50.4 d	51.0 d
7-Hβ	1.89 dtd (17, 5, 2.5)	1.96 m	10	36.9 s	36.9 s	37.8 s
8-Hβ	1.40 m	1.44 m	11	21.3 t	21.3 t	21.7 t
9-Hα	0.91 td (12, 5)	0.97 m	12	40.0 t	39.9 t	40.4 t
11-Hα	1.43 m	1.40 m	13	42.4 s	42.8 s	43.2 s
11-Hβ		1.48 m	14	56.7 d	56.5 d	57.0 d
12-Hα	1.18 td (12.5, 4.5)	1.22 td (13, 4.5)	15	24.6 t	24.6 t	24.8 t
12-Hβ	1.94 dt (12.5, 3)	2.03 m	16	27.7 t	27.7 t	28.0 t
14-Hα	0.97 m	0.99 m	17	54.0 d	a)	60.2 d
15-H_2	1.02 m 1.54 m	1.08 m 1.58 m	18	12.2 q	11.9 q	12.2 q
			19	19.4 q	19.4 q	19.6 q
16-H_2	1.37 m 1.59 m	1.48 m 1.74 m	20	46.9 d	a)	39.5 d
			21	18.2 q	20.63 q	20.8 q
17-Hα	1.63 m	1.40 m	22	173.4 s	a)	117.3 s
20-H	2.38 dq (9.5, 7)	2.81 dq (10, 7)	23	27.5 t	a)	113.2 d
23-H_2	2.01 br t (9) 2.14 m	5.18 t (3.5)	24	28.4 t	32.3 t	32.6 t
			25	28.0 d	30.3 d	30.5 d
24-H	1.13 m	1.62 ddd (19, 9.5, 3.5)	26	57.1 t	a)	52.6 t
24-H	1.65 m	2.21 ddd (19, 7, 3.5)	27	19.4 q	19.1 q	19.3 q
25-H	1.50 m	1.80 m	Glucopyranose			
26-H	3.09 dddd (17, 10, 2.5, 1.5)	2.73 dd (11, 9)	1′	102.6 d	99.7 d	100.1 d
26-H	3.83 ddt (17, 4.5, 2)	3.87 dd (11, 3)	2′	75.3 d	72.2 d	72.8 d
18-H_3	0.67 s	0.69 s	3′	78.6 d	73.5 d	74.1 d
19-H_3	0.93 s	0.96 s	4′	71.7 d	69.2 d	70.0 d
21-H_3	1.14 d (7)	1.33 d (7)	5′	78.5 d	72.1 d	72.6 d
27-H_3	0.85 d (6.5)	0.87 d (7)	6′	62.9 t	62.4 t	63.0 t
Glucopyranose			$\underline{C}OCH_3$		169.6 s	169.5 s
1′-H	5.06 d (8)	4.98 d (8)			169.8 s	169.8 s
2′-H	4.06 t (8)	5.30 dd (9, 8)			170.0 s	170.0 s
3′-H	4.30 m	5.59 t (9)			170.3 s	170.3 s
4′-H	4.28 m	5.36 t (9)			170.5 s	170.4 s
5′-H	3.99 ddd (8, 5.5, 2.5)	4.02 ddd (9, 5, 3)	$CO\underline{C}H_3$		20.4 q	20.5 q
6′-H_2	4.42 dd (12, 5.5) 4.57 dd (12, 2.5)	4.35 dd (12, 3) 4.47 dd (12, 5)			(×2)	(×2)
					20.60 q	20.6 q
$OCOCH_3$		1.97 s			(×2)	
		1.98 s				20.7 q
		1.99 s	$NCO\underline{C}H_3$		23.6 q	23.6 q
		2.06 s				
$NCOCH_3$		2.13 s				

a) Signals were not observed.

380 ($M+H^+-$glucose).

Acetylation of Verazinine (M_5, 6) Compound 6 was acetylated with pyridine–acetic anhydride and the reaction mixture was treated in the usual manner to give the pentaacetate (7), colorless needles (Et_2O), mp 199—200 °C, $[\alpha]_D+66°$ ($c=0.53$, $CHCl_3$). IR ν_{max} cm^{-1}: 1750, 1630, 1220, 1040, ^{1}H- and ^{13}C-NMR: see Table III.

Acknowledgments This study was supported in part by a Grant-in-Aid for Scientific Research to T. K. (No. 61470147) from the Ministry of Education, Science and Culture of Japan, which is gratefully acknowledged. 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 the fellowship.

References and Notes

1) Chiang Su New Medical College ed., "Dictionary of Chinese Crude Drugs," Shanghai Scientific Technologic Publisher, Shanghai, 1977, p. 2692.
2) D. M. Harrison, *Nat. Prod. Rep.*, **3**, 446 (1986); *idem, ibid.*, **1**, 221 (1984); J. Tomko and Z. Voticky, "The Alkaloids," Vol. XIV, ed. by R. H. F. Manske, Academic Press, Inc., New York, 1973, p. 5; and references cited therein.
3) J. Fuska, A. Fuskova, A. Vassova, and Z. Voticky, *Neoplasma*, **28**, 709 (1981).
4) M. C. W. Michin, *J. Neuroscience Methods*, **2**, 111 (1980).
5) G. Liang and N. Sun, *Zhongguo Yaoxue Xuebao*, **1984**, 131; *idem, ibid.*, **1984**, 190; *idem, ibid.*, **1984**, 431; Z. Jia, W. Li, Y. Li, L. Yang, and Z. Zhu, *Lanzhou Daxue Xuebao, Ziran Kexueban* (*Huaxue Jikan*), **19**, 203 (1983) [*Chem. Abstr.*, **100**, 99912*s* (1983)].
6) G. Zhao and G. Ding, *Zhongguo Shenglixue Xuebao*, **24**, 261 (1961).
7) W. Zhao, J. Chen, and Y. Guo, *Zhongyao Tongbao*, **11**, 294 (1986).
8) M. W. Klohs, M. D. Draper, F. Keller, S. Koster, W. Malesh, and F. J. Petracek, *J. Am. Chem. Soc.*, **75**, 4925 (1953).
9) M. Suzuki, Y. Murase, R. Hayashi, and N. Sanpei, *Yakugaku Zasshi*, **79**, 619 (1959).
10) S. M. Kupchan and C. V. Deliwala, *J. Am. Chem. Soc.*, **75**, 1025 (1953); S. M. Kupchan, *ibid.*, **81**, 1925 (1959).
11) E. M. Taskhanova, R. Shakirov, and S. Y. Yunusov, *Khim. Prir.*

Soedin., **3**, 368 (1985) [*Chemistry of Natural Compounds*, **3**, 343 (1985)]. They measured the optical rotation of this sample in $CHCl_3$ solution, but our sample, which had the same structure as that given by Taskhanova *et al.*, could not be dissolved in $CHCl_3$.

12) We examined in detail the ^1H- and ^{13}C-NMR spectra of angeloylzygadenine (**3**), which was used as a reference compound for the spectral analyses of other alkaloids. The results are given in Tables I and II.

13) F. Bohlmann, *Chem. Ber.*, **91**, 2157 (1958).

14) S. Ito, J. B. Stothers, and S. M. Kupchan, *Tetrahedron*, **20**, 913 (1964).

15) A. Bax and R. Freeman, *J. Mag. Reson.*, **44**, 542 (1981).

16) I. Yosioka, K. Hino, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.*, **20**, 1499 (1972); A. K. Chakravarty, B. Das, and S. C. Pakrashi, *Phytochemistry*, **26**, 2345 (1987).