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Steroidal alkaloids from *Veratrum schindleri* and *Veratrum maackii*

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1. Subject and source

Veratrum schindleri Loes. f. and *Veratrum maackii* Regel (Liliaceae), two important species of the genus *Veratrum*, have been locally used to prepare the traditional Chinese medicine ''Li-lu'' in some parts of China. The whole plants of *V. schindleri* and *V. maackii* were collected from Mount Lu (Shanxi, China) and Jilin province, China, respectively, and were authenticated by Prof. Hanchen Zheng, Department of Pharmacognosy, Second Military Medical University. The voucher specimens are kept in the Herbarium of Second Military Medical University, Shanghai, PR China (No. V.S.20050925 and No. V.M. 200500937, respectively).

2. Previous work

Several species of the genus *Veratrum* have been extensively studied and more than 100 steroidal alkaloids have been isolated from them, together with stilbenoids, dipeptide and flavonoid compounds (Atta-ur-Rahman et al., 1993; China Herbal, 1999). There are two reports on the chemical composition of *V. schindleri* and six steroidal alkaloids (angeloylzygadenine, isorubijervine, jervine, veratroylzygadenine and veratrosine) have been described (Zhao et al., 2003; Zhou et al., 2006). The compounds isolated from *V. maackii* were seven steroidal alkaloids: maackinine, zygadenine, germanitrine, angeloylzygadenine, verazine, *epi*-verazine and verazinine (Zhao et al., 1986, 1989; Han and Riiegger, 1992) and two stilbenoids: resveratrol and 2,3′,4,5′-tetrahydroxystilbene (Zhao et al., 1998).

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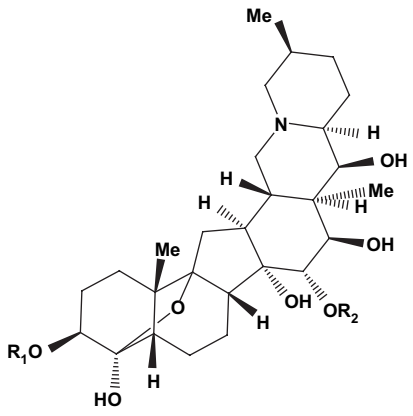
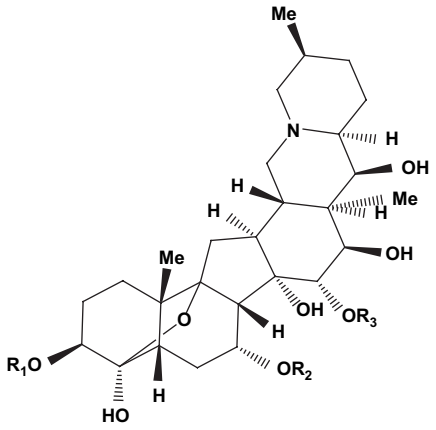
3. Present study

Herein, we report the isolation of 16 steroidal alkaloids: germanitrine (**1**), verussurine (**2**), verazine (**3**), verabenzoamine (**4**), veralosidine (**5**), 3,15-diangeloylgermine (**6**), veralomine (**7**), germitrine (**8**), 2-methylbutyrylzygadenine (**9**), neogermitrine (**10**), verazinine (**11**), germidine (**12**), 3-angeloygermine (**13**), protoveratridine (**14**), zygacine (**15**) and zygadenine (**16**) from *V. schindleri*. And eight steroidal alkaloids: verussurine (**2**), veralosidine (**5**), veratroylzygadenine (**17**), 2-methylbutyrylzygadenine (**9**), neogermitrine (**10**), germidine (**12**), 3-angeloygermine (**13**) and protoveratridine (**14**) from *V. maackii*.

The air-dried and powdered whole plants of *V. schindleri* and *V. maackii* were extracted with ethanol (75% v/v) three times for 2 h each time. After removal of the solvent under reduced pressure, the residue was partitioned sequentially with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH (five times), respectively.

The $CHCl_3$ extract (104 g) of *V. schindleri* was subjected to silica gel chromatography using mixtures of $CHCl_3$–MeOH (30:1; 10:1; 5:1; 1:1; 0:1) to yield five fractions (Fr. A1–Fr. A5). Further purification of fraction A4 ($CHCl_3$–MeOH 10:1) by repeated silica gel, Sephadex LH-20 chromatography and recrystallization yielded compounds **1** (15 mg) (Klohs et al., 1953), **2** (7 mg) (Tezuka et al., 1998), **3** (22 mg) (Adam et al., 1967), **4** (12 mg) (Tezuka et al., 1998), **5** (10 mg) (Shakirov and Yunusov, 1975) and **6** (11 mg) (Yang et al., 1987a,b). The EtOAc extract (148 g) of *V. schindleri* was subjected to silica gel chromatography using mixtures of $CHCl_3$–MeOH (20:1; 10:1; 5:1; 1:1; 0:1) to afford six fractions (Fr. B1–Fr. B6). Fraction B5 ($CHCl_3$–MeOH 5:1) was submitted to repeated silica gel and Sephadex LH-20 chromatography leading to compounds **7** (14 mg) (Shakirov et al., 1976), **8** (20 mg) (Kupchan, 1959), **9** (7 mg) (Yagi, 1962), **10** (16 mg) (Kupchan, 1959), **11**





	R_1	R_2
17 veratroylzygadenine	Ve	H
9 2-methylbutyrylzygadenine	MB	H
15 zygacine	Ac	H
16 zygadenine	H	H

	R_1	R_2	R_3
1 germanitrine	An	Ac	MB
2 verussurine	Vc	Ac	MB
4 verabenzoamine	Vc	H	MB
6 3,15-diangeloylgermine	An	An	H
8 germitrine	HMB	Ac	MB
10 neogermitrine	Ac	Ac	MB
12 germidine	Ac	H	MB
13 3-angeloygermine	An	H	H
14 protoveratridine	MB	H	H

[a]Ac=acetate, MB=2-methylbutyrate, HMB=2-hydroxy-methylbutyrate, An=angelate, Ve=veratroate

Fig. 1. Chemical structures of cevanine type steroidal alkaloids isolated from *V. schindleri* and *V. maackii*.

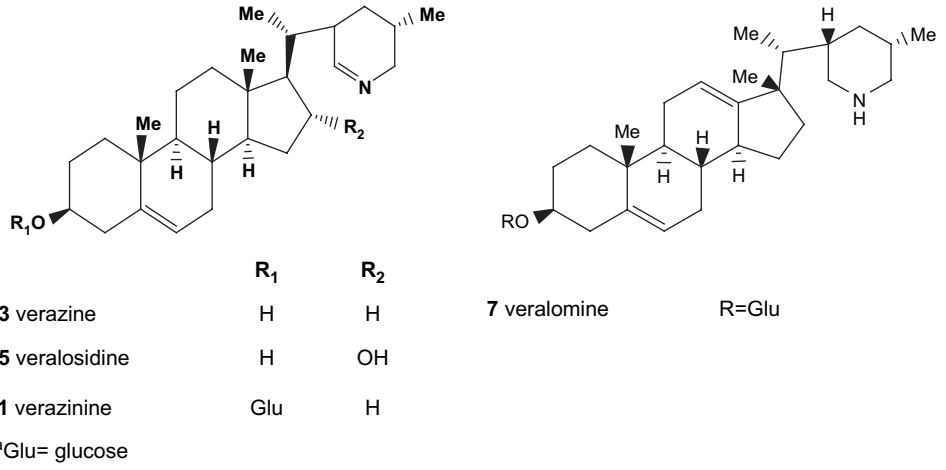


Fig. 2. Chemical structures of other type steroidal alkaloids isolated from *V. schindleri* and *V. maackii*.

(16 mg) (Taskhanova et al., 1985), **12** (13 mg) (Zhao et al., 1991), **13** (24 mg) (Yang et al., 1987a,b), **14** (13 mg) (China Pharmaceutical University, 1998), **15** (5 mg) (Gilbertson, 1973) and **16** (14 mg) (Gilbertson, 1973) (Figs. 1 and 2).

The $CHCl_3$ extract (104 g) of *V. maackii* was subjected to silica gel chromatography using mixtures of $CHCl_3$–MeOH (30:1; 10:1; 5:1; 1:1; 0:1) to yield four fractions (Fr. A1–Fr. A4). Fraction A3 ($CHCl_3$–MeOH 5:1) was then purified by successive column chromatography on silica gel monitored by TLC and led to the isolation of compounds **2** (11 mg), **5** (7 mg), **17** (12 mg), **9** (6 mg), **10** (11 mg), **12** (5 mg), **13** (8 mg) and **14** (11 mg).

The structures were identified based on chemical reactions, spectral analysis (^{1}H NMR, ^{13}C NMR, 2D NMR, MS, UV and IR) and by comparison of their spectral data with those reported previously in the literatures.

4. Chemotaxonomic significance

The genus *Veratrum* contains about 40 species and is an important genus in the Liliaceae family due to its significant bioactivities (Li et al., 2006). The main class of compounds isolated from this genus is the steroidal alkaloids, which can be divided into five types on the basis of the carbon framework: cevanine, veratramine, jervine, solanidine and verazine types (Li et al., 2006). Here, chemical constituents of *V. schindleri* and *V. maackii* were studied and 16 steroidal alkaloids from *V. schindleri* and eight from *V. maackii* were obtained. To the best of our knowledge, the occurrence of these compounds was reported for the first time in *V. schindleri* and *V. maackii*, respectively.

Both *V. maackii* and *V. schindleri* contain high amounts of cevanine type alkaloids, including verussurine (**2**), 2-methylbutyrylzygadenine (**9**), neogermitrine (**10**), germidine (**12**), 3-angeloygermine (**13**) and protoveratridine (**14**). Moreover, the content of this type of steroidal alkaloids is relatively high in comparison with the other types of alkaloids. Previous studies have shown cevanine type alkaloids which are structurally characterized by 4,9-olide cevanines or ester-alkaloids and usually with the substituted groups at 3-, 6-, 7- and 15-position have been reported in other species of *Veratrum* (Atta-ur-Rahman et al., 1993; Li et al., 2006). Also the related genera *Schoenocaulon* and *Zygadenus* are sources of veratrum alkaloids (Grancai and Grancaiova, 1994). This suggests that the presence of 4,9-olide cevanine alkaloids could be considered as a chemosystematic marker for the genus *Veratrum*.

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