STEROIDAL ALKALOIDS FROM *Veratrum dahuricum*

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Veratrum dahuricum (*Liliaceae*), growing in the northeast of China, is one of the 14 species of the genus *Veratrum* [1, 2]. The rhizomes of this plant are used in traditional Chinese medicine for some diseases, such as apoplexy, epilepsy, and acariasis [2].

In previous phytochemical studies on the genus *Veratrum*, about 100 steroidal alkaloids have been reported, including 11 steroidal alkaloids from *V. dahuricum*, namely: jervine, pseudojervine, veratramine, veratrosine, verazine, verdine, germine, rubijervine, isorubijervine, 3-veratroylgermine, and veramine [3, 4].

Herein we present the results from the investigation of steroidal alkaloids of *V. dahuricum* collected in Jilin Province, P. R. China. The air-dried and powdered rhizomes of the plant (25 kg) were refluxed with 75% ethanol for 2×3 h. The alcoholic extract was concentrated to an aqueous residue, and then partitioned successively with petroleum ether, chloroform, ethyl acetate, and *n*-butanol to give four portions. The chloroform portion (125 g) was subjected to silica gel column chromatography (200–300 mesh, 1.5 kg), eluting with the gradient $CHCl_3$:CH_3OH (10:0–20:1–10:1–5:1–2:1–0:1), and gave six fractions: I (16 g), II (11 g), III (13 g), IV (13 g), V (12 g), VI (19 g). Fraction I (16 g) was subject to silica gel column chromatography to afford four subfractions, and the fourth subfraction was purified over silica gel column chromatography, eluting with petroleum ether: acetyl acetate (4:1–2:1–1:1) containing 0.5% $(CH_3CH_2)_2NH$ to yield compounds **1** (6 mg) and **2** (9 mg).

Fraction II (11 g) was dissolved in 2% H_2SO_4 and then filtered. The filtrate was extracted with acetyl acetate after basification with ammonia to give crude alkaloids, which were purified over silica gel column chromatography, eluting with petroleum ether: acetyl acetate (3:1–2:1–1:1) containing 0.5% $(CH_3CH_2)_2NH$ to yield compounds: **3** (7 mg), **4** (5 mg), **5** (107 mg), and **6** (84 mg). Analogously, compounds **7** (41 mg), **8** (105 mg), and **9** (62 mg) from fraction III (13 g), compound **10** (59 mg) from fraction V (12 g), and compound **11** (17 mg) from fraction VI (19 g) were obtained.

The *n*-butanol portion (130 g) was submitted to silica gel column chromatography (200–300 mesh, 1.5 kg), eluting with the gradient $CHCl_3$:CH_3OH (20:1–10:1–5:1–2:1–0:1), and gave five fractions:A (21 g), B (13 g), C (11 g), D (17g), E (20 g). The fraction F (13 g) was dissolved in 2% H_2SO_4, and filtered. The filtrate was made basic with ammonia and partitioned with EtOAc. The crude alkaloids obtained were purified repeatedly over silica gel and subjected to Sephadex LH-20 column chromatography, yielding compounds: **12** (5 mg) and **13** (26 mg).

On the basis of the analysis of UV, PMR (600 MHz), ^{13}C NMR (150 MHz), NOESY, ^{1}H–^{13}C HMBC, HSQC, and mass spectra, these compounds were determined as: maackinine (**1**) [5], 3-acetyl-15-methylbutylgermine (**2**) [6], veramarine (**3**) [4], cyclopamine (**4**) [7], veratramine **5** [3], jervine (**6**) [3], 3,15-diangeloylgermine (**7**) [8], 3-angeloylzygadenine **8** [5], 3-veratroylzygadenine **9** [4], 15-angeloylgermine (**10**) [9], zygadenine (**11**) [5], germine (**12**) [10], and veratrosine (**13**) [11]. Compounds **1**–**4**, **7**, **8**, **10**, **11** were isolated from *V. dahuricum* for the first time.

The potential antitumor activities of compounds **4**–**10** were evaluated by the MTT assay [12] with A549, LOVO, QGY-7703, and 6T-CEM cell lines (Table 1). Compounds **4**–**10** were moderately inhibitory to the four cell lines, and on the whole, the jerveratrum-type alkaloids **4**–**6** exhibited stronger antitumor activity than cevine alkaloids **7**–**10**, while compounds **6**, **10** showed superior inhibitory effect on the 6T-CEM cell line with IC_{50} at 6.83 and 8.74 μg/mL, respectively.

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TABLE 1.The Inhibition of Compounds **4-10** Against the Proliferation of A549, LOVO, QGY-7703, and 6T-CEM Cell Lines, μg/mL

Compounds	A549		LOVO		QGY-7703		6T-CEM	
	IC_{50}	IC%	IC_{50}	IC%	IC_{50}	IC%	IC_{50}	IC%
4	38.36	50.27	40.16	73.95	88.36	59.46	~100	49.43
5	13.47	100	23.23	77.83	35.35	100	15.21	100
6	53.10	100	25.69	57.02	54.98	81.20	6.83	100
7	>100	10	103.33	53.23	>100	23.13	>100	10.63
8	>100	16.32	>100	34.93	>100	32.06	>100	13.03
9	>100	23.25	55.45	67.53	>100	52.02	>100	30.79
10	>100	30.20	>100	32.24	91.04	56.58	8.74	94.81

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