Two New Chemical Constituents of *Veratrum dahuricum* (TURCZ.) LOES. f.

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Two new chemical constituents, one new steroid, neoveratrenone (**1**), and one new glycerol ester, 1-[11-(ferulyloxy)undecanoyl)]glycerol (**2**), were isolated and characterized from the roots and rhizomes of *Veratrum dahuricum* (TURCZ.) LOES. f., together with five known compounds, *i.e.*, hexacosanoic acid 2,3-dihydroxypropyl ester (**3**), syringaresinol (**4**), prosapogenin A of dioscin (**5**), verapatulin (**6**), and oxyresveratrol (**7**). Their structures were established by extensive analysis of spectroscopic data as well as by comparison with literature reports. The compounds were evaluated for cytotoxic activity against three tumor cell lines of HepG-2, HeLa, and K562/S.

Introduction. – *Veratrum dahuricum* (TURCZ.) LOES. f., belonging to the Liliaceae family, is a famous traditional medicinal plant in China [1]. The plant has been reported to possess a variety of biological properties, such as hypotensive activity, emetic, antifungus, anticancer activities, *etc.* [2]. In recent years, the antitumor activity of steroidal alkaloids from the roots and rhizomes of *Veratrum* genus has been studied [3][4]. However, studies on non-alkaloid constituents of *Veratrum* plants have been comparatively rare. Previously, we reported works based on steroidal alkaloids of this genus [5–7]. Continuing our investigations of non-alkaloid constituents of *Veratrum* genus, we have isolated and identified two new compounds, neoveratrenone (**1**) and 1-[11-(ferulyloxy)undecanoyl)]glycerol (**2**), along with the five known components, hexacosanoic acid 2,3-dihydroxypropyl ester (**3**), syringaresinol (**4**), prosapogenin A of dioscin (**5**), and oxyresveratrol (**6**) (*Fig. 1*). Here, we report the isolation and structure elucidation of the two new compounds, as well as the cytotoxicities of the isolated compounds against the human tumor cell lines of HepG-2, HeLa, and K562/S cells.

Results and Discussion. – Compound **1** was obtained as a white amorphous powder. The molecular formula $C_{19}H_{26}O_3$, based on HR-ESI-MS (*m/z* 303.1957 ($[M+H]^+$)), was supported by the ^{13}C-NMR and DEPT spectral data. The ^{1}H-NMR spectrum (*Table*) of compound **1** exhibited two Me signals at δ(H) 0.97 (Me(18)), 1.61 (Me(19)), one olefinic H-atom signal at δ(H) 5.21(br. *s*, H–C(6)), signals of two O-substituted CH groups at δ(H) 3.33 (*m*, H–C(3)), 3.97 (*dd*, $J = 8.0$, H–C(7)), and two OH group H-atom signals at δ(H) 4.70 (*d*, $J = 4.8$, HO–C(3)) and 4.68 (*d*, $J = 6.8$, HO–C(7)). The ^{13}C-NMR and DEPT spectra displayed 19 C-atom signals, including those of four olefinic C-atoms at δ(C) 142.7, 128.2, 127.7, and 167.7, of one C=O group at δ(C) 198.3, and of two O-substituted C-atoms at δ(C) 70.3 and 72.9. The two Me groups, one

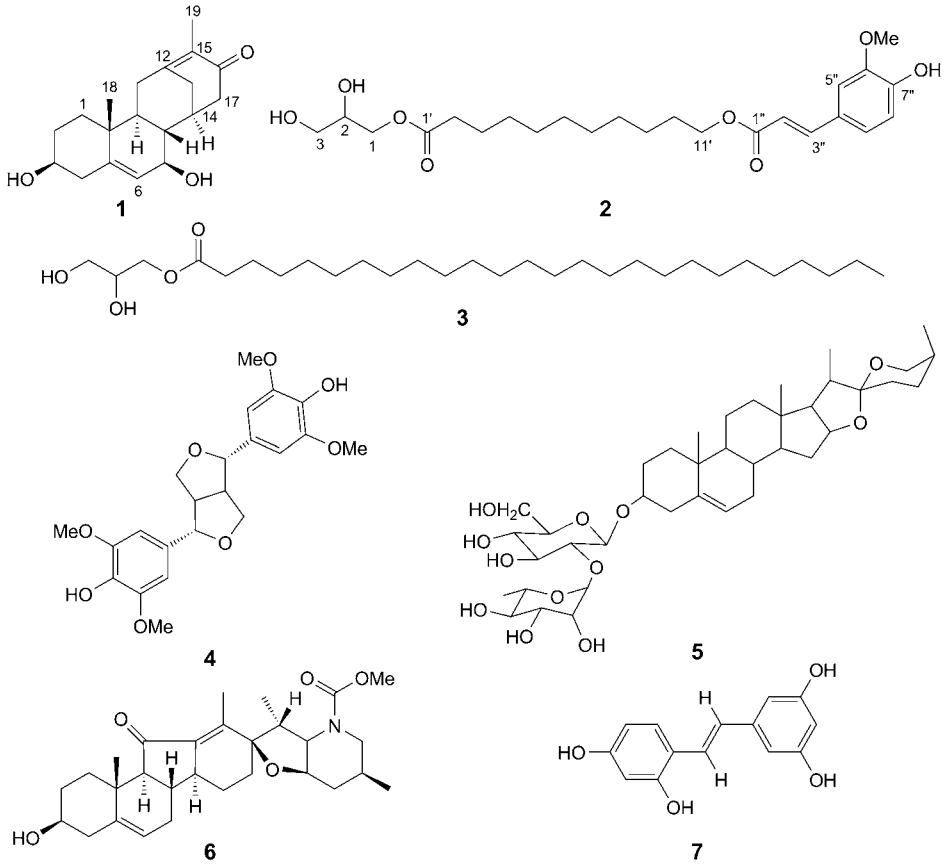

Fig. 1. *The structures of compounds* **1**–**7**

olefinic H-atom, and O-substituted CH groups were used as starting points to assign the signals of the other H-atoms and C-atoms by analysis of HMBC and HMQC correlations. In the HMBC spectrum (*Fig. 2*), δ(H) 0.97 (Me(18)) showed cross-peaks with δ(C) 38.0 (C(1)), 36.7 (C(10)), 49.9 (C(9)), and 142.7(C(5)), and δ(H) 1.61 (Me(19)) had correlations with δ(C) 127.7 (C(15)), 167.7 (C(12)), and 198.3 (C(16)), indicating that the two Me groups were at C(10) and C(15), respectively. The HMBC correlations between δ(H) 4.70 (*d*, $J = 4.8$, HO–C(3)) and δ(C) 70.3(C(3)), 41.6(C(4)), 31.4 (C(2)), and between δ(H) 4.68 (*d*, $J = 6.8$, HO–C(7)) and δ(C) 72.9 (C(7)), 128.2 (C(6)), 49.4 (C(8)) confirmed that the two OH groups were attached to C(3) and C(7), respectively. Regarding β-Me(18) group as reference point [8], the NOE cross-peaks observed between Me(18), and CH_2(4a), H–C(8), and CH_2(17a), between H–C(8) and HO–C(7), and between CH_2(4a) and HO–C(3), supported β-position of these H-atoms, whereas the NOE cross-peaks CH_2(4b)/H–C(3), H–C(7)/H–C(9) and H–C(14), H–C(9)/H–C(14) and CH_2(13b), and H–C(14)/CH_2(13b) confirmed their α-orientations (*Fig.2*). Based on these data, the structure of compound **1** was identified, and named neoveratrenone.

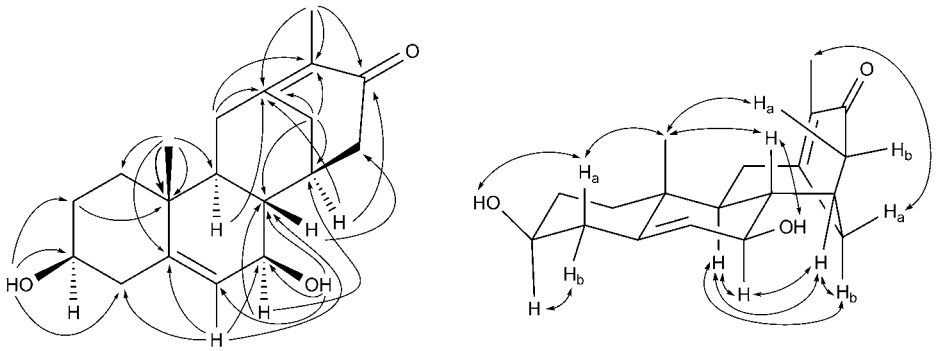

Fig. 2. *Key HMBC* (H → C) *and NOESY correlations* (H ↔ H) *of* **1**

Table. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.) *of* **1** (DMSO) *and* **2** ($CDCl_3$). δ in ppm, J in Hz.

Position	**1**		Position	**2**	
	δ(H)	δ(C)		δ(H)	δ(C)
CH_2(1)	1.63–1.65 (*m*), 1.11 (*ddd*, *J* = 13.6, 3.2)	38.0	CH_2(1)	4.09–4.24 (*m*)	65.2
CH_2(2)	1.43–1.46 (*m*), 1.64–1.67 (*m*)	31.4	H–C(2)	3.93–3.95 (*m*)	70.3
H–C(3)	3.28–3.37 (*m*)	70.3	CH_2(3)	3.70 (*dd*, *J* = 11.2, 4.0), 3.60 (*dd*, *J* = 11.2, 5.6)	63.3
CH_2(4)	2.10 (br. *t*, *J* = 12.8), 2.26 (*dd*, *J* = 12.8, 2.4)	41.6	C(1′)		174.4
C(5)		142.7	CH_2(2′)	2.35 (*dd*, *J* = 7.6)	34.2
H–C(6)	5.21 (br. *s*)	128.2	CH_2(3′)	1.61–1.70 (*m*)	24.9
H–C(7)	3.97 (*dd*, *J* = 8.0)	72.9	CH_2(4′)	1.25–1.28 (*m*)	29.6
H–C(8)	1.38–1.42 (*m*)	49.4	CH_2(5′)	1.25–1.28 (*m*)	29.6
H–C(9)	1.59–1.61 (*m*)	49.9	CH_2(6′)	1.25–1.28 (*m*)	29.6
C(10)		36.7	CH_2(7′)	1.25–1.28 (*m*)	29.3
CH_2(11)	1.56–1.59 (*m*), 1.21 (br. *s*)	29.4	CH_2(8′)	1.25–1.28 (*m*)	29.1
C(12)		167.7	CH_2(9′)	1.25–1.28 (*m*)	28.8
CH_2(13)	2.46–2.49 (*m*), 2.30–2.32 (*m*)	30.3	CH_2(10′)	1.25–1.28 (*m*)	26.0
H–C(14)	2.34–2.38 (*m*)	48.8	CH_2(11′)	4.09–4.24 (*m*)	64.7
C(15)		127.7	C(1″)		167.5
C(16)		198.3	H–C(2″)	6.29 (*d*, *J* = 15.8)	115.7
CH_2(17)	2.27–2.30 (*m*), 2.29–2.32 (*m*)	37.7	H–C(3″)	7.61 (*d*, *J* = 15.8)	144.7
Me(18)	0.97 (*s*)	18.4	C(4″)		127.1
Me(19)	1.61 (*s*)	11.4	H–C(5″)	7.04 (*d*, *J* = 1.7)	109.3
			C(6″)		146.8
			C(7″)		147.9
			H–C(8″)	6.92 (*d*, *J* = 8.2)	114.7
			H–C(9″)	7.08 (*dd*, *J* = 8.2, 1.7)	123.1
			MeO	3.93 (*s*)	56.0

Compound **2** was obtained as a white amorphous powder and had the molecular formula $C_{24}H_{36}O_8$ as deduced from HR-ESI-MS (*m*/*z* 453.2487 ($[M+H]^+$)). In the ^{1}H-NMR spectrum of compound **2**, the presence of ferulic acid was confirmed by an *ABX pattern of signals at δ(H) 7.04 (d, J* = 1.7, H–C(5″)), 7.08 (*dd*, *J* = 8.2, 1.7,

H–C(9″)), and 6.92 (*d*, $J = 8.2$, H–C(8″)), and signals of one phenolic OH group at δ(H) 5.89(HO–C(7″)), of one MeO group at δ(H) 3.93 (MeO(6″)), and of a (*E*)-C=C bond at δ(H) 7.61 (*d*, $J = 15.8$, H–C(3″)) and 6.29 (*d*, $J = 15.8$, H–C(2″)), together with the C-atom singals δ(C) 147.9 (C(7″)), 146.8 (C(6″)), 109.3 (C (5″)), 127.1 (C(4″)), 123.1 (C(9″)), 114.7 (C(8″)), 144.7 (C(3″)), 115.7 (C(2″)), and 167.5 (C(1″)) in the ^{13}C-NMR spectrum of **2**. Meanwhile, the ^{1}H- and ^{13}C-NMR spectra revealed the presence of a 1-(ω-hydroxyfattyacyl)-glycerol in **2** [9]. The HR-ESI mass spectrum with a strong peak corresponding to $[M+H]^+$ at *m*/*z* 453.2486 indicated that the esterifying acid was 11-hydroxylundecanoic acid. The assignment and connectivity were determined using HMQC and HMBC spectra. In the HMBC spectrum (*Fig. 3*), CH_2 (11′) (δ(H) 4.20) showed a cross-peak with C(1″) (δ(C) 167.5), indicating that the feruloyloxy group was located at C(11′). Based on these data, the structure of compound **2** was established as 1-[11-(ferulyloxy)undecanoyl)]glycerol.

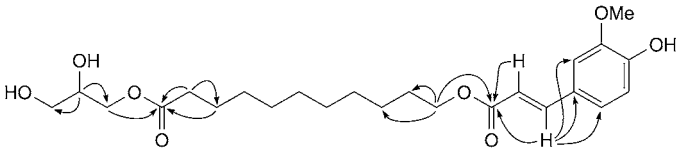

Fig. 3. *Key HMBCs* (H → C) *of* **2**

Compounds **3**–**7** were identified as hexacosanaic acid 2,3-dihydroxypropyl ester (**3**) [10], syringaresinol (**4**) [11], prosapogenin A of dioscin (**5**) [12], verapatulin (**6**) [13], and oxyresveratrol (**7**) [14] respectively, by various spectral analysis and comparison with literature values. Compounds **3**–**5** were obtained from this genus for the first time, and compound **6** was isolated from the title plant for the first time.

Cytotoxic Activities. Compounds **1**–**7** were tested for their cytotoxic activities *in vitro* against human tumor cell lines of HepG-2, HeLa, and K562/S cells using MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method. Compound **5** exhibited cytotoxic activity with IC_{50} values of 29.59, 25.07, and 24.31 μmol/l , while other compounds exhibited no activity with IC_{50} values > 100 μmol/l.

Financial support from the *Natural Science Foundation of China* (21102035) and the *Natural Science Item of Education Department of Henan Province* (2011B360001) is gratefully acknowledged.

Experimental Part

General. TLC: precoated SiO_2 *GF-254* (*Qingdao Haiyang Chemical Group Co.*) and SiO_2 *60 RP-18* $F_{254}S$ plates (*Merck*). Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Group Co.*). Optical rotations: *Perkin-Elmer 241MC* polarimeter. UV Spectra: *Shimadzu UV-2201* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker IFS-55* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AV-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *MDS Sciex ESI-Q-TOF* mass spectrometer, in *m*/*z*.

Plant Material. The plant material of *Veratrum dahuricum* (TURCZ.) LOES. f. was collected in Liaoning Province of China in july 2008 and identified by Assoc. Prof. *Wang-Jun Yuan*, Henan University. A voucher specimen (2008007) was deposited with the Institute of Pharmacy, Pharmaceutical College, Henan University, China.

Extraction and Isolation. The dried roots and rhizomes of *Veratrum dahuricum* (TURCZ.) LOES. f. (10 kg) were extracted with 95% EtOH (3 × 50 l, 3 h each) under reflux. The extract was concentrated

under reduced pressure to yield a residue (387 g), which was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:1 → 1:1) to yield five combined fractions. *Fr. 1* (2 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 30:1) to give compound **3** (50 mg). *Fr. 2* (1g) was repeatedly separated by CC (SiO_2; petroleum ether (PE)/Me_2CO 2:1) to yield compound **4** (20 mg). *Fr. 3* (2.7g) was further subjected to CC (SiO_2; $CHCl_3$/AcOEt 1:1) to yield combined *Frs. C1 – C3*. *Fr. C1* was further purified by CC (SiO_2; $CHCl_3$/MeOH 50:1) to afford compound **2** (34 mg), *Fr. C2* was subjected to CC (SiO_2; PE/AcOEt 1:1) to give compound **1** (20 mg), and *Fr. C3* was further separated by CC (SiO_2; PE/Me_2CO 3:1) to yield compound **6** (73 mg). *Fr. 4* (0.5 g) was separated by CC (SiO_2, PE/Me_2CO 1:1) to yield compound **7** (95 mg). *Fr. 5* (1.4 g) was submitted to CC (SiO_2; PE/Me_2CO 1:7) to give compound **5** (72 mg).

Neoveratrenone (=*(3S,6R,6aS,7S,12aS,12bR)-1,3,4,6,6a,7,8,12,12a,12b-Decahydro-3,6-dihydroxy-10,12b-dimethyl-7,11-methanocycloocta[a]naphthalen-9(2H)-one*; **1**). White powder. $[\alpha]_D^{20} = +11.0$ (c = 0.025, MeOH/$CHCl_3$ 1:2). UV (MeOH): 221 (2.82), 282 (3.03). IR (KBr): 3410, 1701, 1635. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 303.1957 ($[M+H]^+$, $C_{19}H_{27}O_3^+$; calc. 303.1960).

1-[11-(Ferulyloxy)undecanoyl]glycerol (=*2,3-Dihydroxypropyl 11-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy}undecanoate*; **2**). White powder. UV (MeOH): 224 (3.17), 325 (3.45). IR (KBr): 3435, 1718, 1605. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 453.2486 ($[M+H]^+$, $C_{24}H_{37}O_8^+$; calc. 453. 2488).

Cytotoxicity Assays. The cytotoxicity assays towards three tumor cell lines including HepG-2 (human hepatoma cell line), HeLa (human cervical-cancer cell line), and K562/S (human erythroleukemia cell line) were carried out by MTT method as described in [6][7].

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Received July 20, 2012