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A NEW STILBENE GLYCOSIDE FROM THE *n*-BUTANOL FRACTION OF *Veratrum dahuricum*

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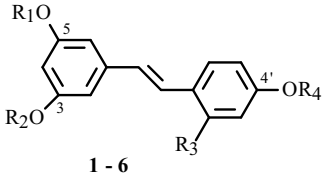
*A new stilbene glycoside, 5-methylresveratrol-3,4′-*O*-β-*D*-diglucopyranoside (**1**), was isolated from the* n*-butanol fraction of the rhizomes of* Veratrum dahuricum*, together with five known stilbenoids: resveratrol-3-*O*-β-*D*-glycoside (**2**), 4′-methylresveratrol-3-*O*-β-*D*-glycoside (**3**), oxyresveratrol-4′-*O*-β-*D*-glycoside (**4**), oxyresveratrol-3-*O*-β-*D*-glycoside (**5**), and oxyresveratrol-3,4′-*O*-β-*D*-diglycoside (**6**), and found for the first time in the investigated plant. The structures of six isolates were identified on the basis of 1D and 2D NMR data. Compounds **1–6** showed platelet aggregation inhibition, and compound **1** had an IC_{50} value of 383.6 μM against platelet aggregation induced by AA.*

Key words: *Veratrum dahuricum*, stilbene, 5-methylresveratrol-3,4′-*O*-*β*-D-diglucopyranoside, platelet aggregation.

The rhizome of *Veratrum dahuricum* (Liliaceae) is one of the sources of the traditional Chinese medicine *Li Lu* and is used for some diseases such as apoplexy, epilepsy, and acariasis [1, 2]. Previous phytochemical and pharmacological investigations of *Veratrum* plants focused mainly on alkaloids and reported about 100 steroidal alkaloids including 24 alkaloids from *Veratrum dahuricum* [3]. Only a few stilbenoids were reported from *Veratrum* plants, such as resveratrol, which has notable antithrombotic activity and which is intimately related to antiplatelet aggregation [4–8].

The present article describes the isolation, structure elucidation, and platelet aggregation inhibiting effect of six compounds: 5-methylresveratrol-3, 4′-*O*-*β*-D-diglucopyranoside (**1**), resveratrol-3-*O*-*β*-D-glycoside (**2**), 4′-methylresveratrol-3-*O*-*β*-D-glycoside (**3**), oxyresveratrol-4′-*O*-*β*-D-glycoside (**4**), oxyresveratrol-3-*O*-*β*-D-glycoside (**5**), and oxyresveratrol-3, 4′-*O*-*β*-D-diglycoside (**6**).



1 - 6

1: $R_1 = CH_3$, $R_2 = R_4 = Glc$, $R_3 = H$
2: $R_1 = R_3 = R_4 = H$, $R_2 = Glc$
3: $R_1 = R_3 = H$, $R_2 = Glc$, $R_4 = CH_3$
4: $R_1 = R_2 = H$, $R_3 = OH$, $R_4 = Glc$
5: $R_1 = R_4 = H$, $R_2 = Glc$, $R_3 = OH$
6: $R_1 = H$, $R_2 = R_4 = Glc$, $R_3 = OH$

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TABLE 1. ^{1}H NMR Data of Compound **1–6** (600 MHz, DMSO-d_6, δ, ppm, J/Hz)

C atom	**1**	**2**	**3**
2	6.88 (1H, br.s)	6.73 (1H, br.s)	6.75 (1H, br.s)
4	6.51 (1H, t, J = 1.8)	6.34 (1H, t, J = 1.8, 2.4)	6.36 (1H, m)
6	6.77 (1H, br.s)	6.57 (1H, br.s)	6.60 (1H, br.s)
2′	7.02 (1H, d, J = 9.0)	7.41 (1H, d, J = 8.4)	7.48 (1H, d, J = 8.4)
3′	7.51 (1H, d, J = 9.0)	6.84 (1H, d, J = 8.4)	6.92 (1H, d, J = 8.4)
5′	7.51 (1H, d, J = 9.0)	6.84 (1H, d, J = 8.4)	6.92 (1H, d, J = 8.4)
6′	7.02 (1H, d, J = 9.0)	7.41 (1H, d, J = 8.4)	7.48 (1H, d, J = 8.4)
α	7.03 (1H, d, J = 16.2)	6.86 (1H, d, J = 16.2)	6.87 (1H, d, J = 16.2)
β	7.19 (1H, d, J = 16.2)	7.02 (1H, d, J = 16.2)	7.09 (1H, d, J = 16.20
1″	4.88 (1H, br.s)	4.80 (1H, d, J = 7.2)	4.81 (1H, d, J = 7.2)
2″	3.32 (1H, dd, J = 9.6, 2.4)	3.33 (1H, dd, J = 9.6, 2.4)	3.38 (1H, m)
3″	3.20–3.34 (1H, m)	3.21–3.34 (1H, m)	3.20–3.34 (1H, m)
4″	3.20–3.34 (1H, m)	3.21–3.34 (1H, m)	3.20–3.34 (1H, m)
5″	3.17 (1H, td, J = 4.2, 3.6, 4.8)	3.18 (1H, td, J = 4.2, 3.6, 4.8)	3.16 (1H, m)
6″	3.51 (1H, dd, J = 12.0, 6.0)	3.50 (1H, dd, J = 11.4, 6.0)	3.48 (1H, m)
	3.74 (1H, d, J = 12.0)	3.73 (1H, d, J = 12.0)	3.70 (1H, m)
1‴	4.85 (1H, br.s)		
2‴	3.32 (1H, dd, J = 9.6, 2.4)		
3‴	3.20–3.34 (1H, m)		
4‴	3.20–3.34 (1H, m)		
5‴	3.18 (1H, td, J = 4.2, 3.6, 4.8)		
6‴	3.50 (1H, dd, J = 11.4, 6.0)		
	3.74 (1H, d, J = 12.0)		
$OC\underline{H}_3$	3.76 (3H, br.s)		3.73 (3H, br.s)

C atom	**4**	**5**	**6**
2	6.37 (1H, d, J = 1.8)	6.61 (1H, br.s)	6.64 (1H, br.s)
4	6.09 (1H, t, J = 2.4, 1.8)	6.32 (1H, br.s)	6.28 (1H, m)
6	6.37 (1H, d, J = 1.8)	6.55 (1H, br.s)	6.57 (1H, br.s)
2′			
3′	6.54 (1H, t, J = 2.4)	6.32 (1H, d, J = 2.4)	6.55 (1H, d, J = 2.4)
5′	6.51 (1H, dd, J = 8.4, 2.4)	6.25 (1H, dd, J = 9.0, 1.8)	6.52 (1H, dd, J = 6.0, 2.4)
6′	7.44 (1H, d, J = 8.4)	7.32 (1H, d, J = 8.4)	7.43 (1H, d, J = 8.4)
α	6.86 (1H, d, J = 16.2)	6.83 (1H, d, J = 16.2)	6.93 (1H, d, J = 16.2)
β	7.17 (1H, d, J = 16.2)	7.19 (1H, d, J = 16.2)	7.22 (1H, d, J = 16.2)
1″	4.80 (1H, br.s)	4.78 (1H, br.s)	4.79 (1H, d, J = 7.2)
2″	3.33 (1H, dd, J = 9.6, 2.4)	3.34 (1H, dd, J = 9.6, 2.4)	3.42 (1H, m)
3″	3.21–3.34 (1H, m)	3.21–3.34 (1H, m)	3.22–3.35 (1H, m)
4″	3.21–3.34 (1H, m)	3.21–3.34 (1H, m)	3.22–3.35 (1H, m)
5″	3.17 (1H, td, J = 4.2, 3.6, 4.8)	3.18 (1H, td, J = 4.2, 3.6, 4.8)	3.19 (1H, m)
6″	3.50 (1H, dd, J = 11.4, 6.0)	3.50 (1H, dd, J = 11.4, 6.0)	3.50 (1H, m)
	3.72 (1H, d, J = 12.0)	3.71 (1H, d, J = 12.0)	3.71 (1H, m)
1‴			4.79 (1H, d, J = 7.2)
2‴			3.42 (1H, m)
3‴			3.22–3.35 (1H, m)
4‴			3.22–3.35 (1H, m)
5‴			3.19 (1H, m)
6‴			3.50 (1H, m)
			3.71 (1H, m)

TABLE 2. ^{13}C NMR Data of Compounds **1–6** (150 MHz, DMSO-d_6, δ, ppm)

C atom	**1**	**2**	**3**	**4**	**5**	**6**
1	139.3	139.3	139.2	139.8	140.2	139.9
2	105.8	104.8	105.2	104.3	105.2	105.5
3	158.9	158.8	158.6	158.6	158.4	159.0
4	101.7	102.8	102.9	101.8	102.5	102.8
5	160.4	158.3	158.4	158.6	160.0	158.5
6	106.5	107.1	107.2	104.3	106.4	106.7
1′	128.6	128.0	129.1	118.1	115.3	118.0
2′	127.1	127.8	127.7	155.9	156.3	155.9
3′	116.5	115.5	114.2	104.0	102.7	104.0
4′	157.2	157.2	158.8	158.0	158.3	158.0
5′	116.5	115.5	114.2	107.6	107.3	107.6
6′	127.7	127.8	127.7	127.6	127.1	127.4
α	126.5	125.2	126.0	126.3	124.5	126.1
β	130.7	128.5	128.1	122.8	124.1	123.6
1″	100.7	100.7	100.8	100.8	100.5	100.8
2″	73.3	73.3	73.0	73.4	73.3	73.4
3″	77.2	77.1	76.9	77.1	77.0	76.7
4″	70.0	69.8	69.8	69.7	69.7	61.7
5″	76.8	76.7	76.2	76.7	76.7	77.0
6″	60.8	60.7	60.4	60.7	60.7	60.7
1‴	100.3					100.7
2‴	73.3					73.4
3‴	77.1					76.7
4‴	69.8					61.6
5‴	76.6					77.0
6‴	60.7					60.6
$O\underline{C}H_3$	55.2		55.1			

TABLE 3. The Effect of Compounds **1–6** on Platelet Aggregation Induced by Arachidonic Acid (AA), Adenosine Diphosphate (ADP), and Platelet Active Factor (PAF) (IC_{50}, μM)

Compounds	AA	ADP	PAF
1	383.6	558.1	500
2	75.6	272.3	697.8
3	108.2	556.4	500
4	175.3	480.8	500
5	256.2	500	500
6	305.7	500	500

Compound **1** was isolated as a white powder from the *n*-butanol fraction of the extract from rhizomes of *Veratrum dahuricum*. The molecular formula of **1** was determined to be $C_{27}H_{34}O_{13}$ by TOF-MS-ES analysis at *m/z* 565.1885 $[M-H]^-$. The ^1H NMR spectrum of **1** showed characteristic resonances for aromatic protons such as δ_H 7.51 (2H, d, J = 9.0 Hz, $H_{3',5'}$), 7.02 (2H, d, J = 9.0 Hz, $H_{2',6'}$), 6.88 (1H, br.s, H_2), and 6.77 (1H, br.s, H_6), 6.51 (1H, br.s, H_4). The presence of two *trans*-vinyl proton resonances at δ_H 7.19 (1H, d, J = 16.2 Hz, H_β) and 7.03 (1H, d, J = 16.2 Hz, H_α) in the ^1H NMR spectrum was observed, and the H_α–C_1, H_α–$C_{1'}$, and H_β–C_1, H_β–$C_{1'}$ were found in the HMBC spectrum. Moreover, the ^1H NMR spectrum of **1** was similar to that of **2**, except for the presence of three methyl protons at δ_H 3.76 (3H, br.s CH_3) and six sugar protons ranging from δ_H 4.88 to 3.74. Analysis of the ^{13}C NMR further showed that **1** had a methyl group (δ_C 55.2) and a sugar (δ_C 100.3, 77.1, 76.6, 73.3, 69.8, 60.7) compared to **2**. These implied that **1** might have the skeleton of stilbene. This inference was confirmed by the MS, MS^2, and MS^3 of **1**, which exhibited 589 $[M+Na]^+$, 565 $[M-H]^-$, 427 $[589\text{-Glc}]^+$, 265 $[427\text{-Glc}]^+$, 403 $[565\text{-Glc}]^-$, and 241 [403-Glc] quasi-ion peaks, revealing a stilbenoid containing two sugars. Furthermore, the two anomeric proton resonances

at δ_H 4.88 (1H, d, J = 7.8 Hz, $H_{1''}$) and 4.85 (1H, d, J = 7.8 Hz, $H_{1'''}$) in the ^{1}H NMR spectrum and two anomeric carbons $C_{1''}$ (δ_C 100.7) and $C_{1'''}$ (δ_C 100.3) from the ^{13}C NMR spectrum implied that the sugars were β-D-glucopyranosides, which was supported by the $^3J_{H1''-H2''}$, $^3J_{H1''-H2'''}$ coupling constant of 7.8 Hz and NOE connectivities detected between $H_{1''}$ and both $H_{3''}$ and $H_{5''}$, and $H_{1'''}$ and both $H_{3'''}$, and $H_{5'''}$. The correlation between the protons (δ_H) of the methoxyl group and the carbon C_5 (δ_C) in the HMBC spectrum indicated that the methoxyl group was attached at C_5. The positions of the two β-D-glucopyranosides were deduced to be connected to C_3 and $C_{4'}$, respectively, from the HMBC spectrum, in which the correlations $H_{1''}$–C_3 and $H_{1'''}$–$C_{4'}$ were found.

EXPERIMENTAL

The ^{1}H NMR spectra and ^{13}C NMR, NOESY, ^{1}H–^{13}C HMBC, and HMQC of these six stilbenoids were recorded in DMSO-d_6 on a Bruker AVANCE^{II} 600 NMR (600 MHz) spectrometer. Chemical shifts (δ) are given in ppm and coupling constants (J) in hertz. Mass spectra were obtained with a Micro mass spectrometer (Q-Tof micro) linked to an ESI source. TLC analysis was run on $HSGF_{254}$ silica gel plates (10–40 μm, Yantai, China). The stilbenoids were detected with a UV (254, 365 nm) lamp. Column chromatography was performed on silica gel (200–300 mesh, Yantai, China), polyamide (100–200 mesh, Taizhou, China) and Sephadex LH-20 (Pharmacia Co. Ltd.).

Plant Material. The plant were harvested in Yanbian, Jilin province, PR China, in September of 2005, and authenticated as *Veratrum dahuricum* by Prof. Yong-Zhen Liu of the School of Pharmacy, Yanbian University. The voucher specimen (collection No. 211) was deposited at the Herbarium of the School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.

Extraction and Isolation. The air-dried and powdered rhizomes of *Veratrum dahuricum* (25 kg) were refluxed with 75% ethanol for 2×3 h. The alcoholic extract was concentrated *in vacuo* to an aqueous residue, which was successively partitioned with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The partial *n*-butanol extract (152 g) was subjected to polyamide (100–200 mesh) column chromatography, eluting with the gradient H_2O–EtOH (90:10, 80:20, 70:30, 50:50, 5:95) to yield five fractions F_1–F_5. The F_1 fraction was submitted to silica gel column chromatography (200–300 mesh), eluting with the gradient $CHCl_3$–CH_3OH (20:1–10:1–5:1–2:1–0:1), and gave five subfractions: F_{1A} (106 mg), F_{1B} (130 mg), F_{1C} (110 mg), F_{1D} (170 mg), and F_{1E} (85 mg). The subfraction F_{1C} (110 mg) was purified repeatedly over Sephadex LH-20 and ODS column chromatography to yield compounds **1** (6 mg) and **2** (36 mg), and the subfraction F_{1B} (130 mg) gave compound **3** (11 mg). The F_2 fraction was submitted to silica gel (200–300 mesh), Sephadex LH-20 and ODS column chromatography, and preparative HPLC, yielding compounds **4** (9 mg) and **5** (7 mg); analogously, the F_3 fraction yielded **6** (78 mg).

Compound 1. White powder, mp 155–157°C (MeOH), $[\alpha]_D^{20}$ –40.3° (*c* 0.212; MeOH); ESMS: *m/z* 589 $[M+Na]^+$, 605 $[M+K]^+$, 565 $[M-H]^-$, MS^2: 427 $[589\text{-Glc}]^+$, 403 $[565\text{-Glc}]^-$, MS^3: 265 $[427\text{-Glc}]^+$, 241 $[403\text{-Glc}]^-$; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 2).

Compound 2. White powder; ESMS: *m/z* 413 $[M+Na]^+$, 389 $[M-H]^-$, 425 $[M+Cl]^-$, MS^2: 229 $[413\text{-Na+H-Glc}]^+$, 227 $[M\text{-H-Glc}]^-$; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 2). The spectral data were comparable to published values for **2** [9].

Compound 3. White powder; ESMS: *m/z* 405 $[M+H]^+$, 427 $[M+Na]^+$, 403 $[M-H]^-$, MS^2: 243 $[427\text{-Na+H-Glc}]^-$; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 2). The spectral data were comparable to published values for **3** [10].

Compound 4. White powder; ESMS: *m/z* 429 $[M+Na]^+$, 441 $[M+Cl]^-$, 405 $[M-H]^-$, MS^2: 245 $[429\text{-Na+H-Glc}]^+$, 243 $[M\text{-H-Glc}]^-$; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 2). The spectral data were comparable to published values for **4** [11].

Compound 5. White powder; ESMS: *m/z* 429 $[M+Na]^+$, 441 $[M+Cl]^-$, 405 $[M-H]^-$, MS^2: 245 $[429\text{-Na+H-Glc}]^+$, 243 $[M\text{-H-Glc}]^-$; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 2). The spectral data were comparable to published values for **5** [12, 13].

Compound 6. White powder; ESMS: *m/z* 591 $[M+Na]^+$, 567 $[M-H]^-$, 603 $[M+Cl]^-$, MS^2: 429 $[M\text{-Na+H-Glc}]^+$; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 2). The spectral data were comparable to published values for **6** [13].

Anti-Platelet Aggregation Assay. The anti-platelet aggregation activity of compounds **1–6** was assayed according to the method reported in the literature [14] with some modifications. As shown in Table 3, compounds **2–5** containing a sugar

showed a higher inhibition of platelet aggregation than compounds **1** and **6**, and all the compounds were more sensitive to AA-induced platelet aggregation than to other two blood media. Compound **2** was the most active against platelet aggregation, the IC_{50} values of 75.6, 272.3, and 697.8 μM, while the new compound **1** showed only moderate inhibition of platelet aggregation induced by AA and ADP, 383.6 and 558.1 μM.

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