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A New Glycoside of Resveratrol Dimer from Stem Bark of *Vitis vinifera*

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Vitis vinifera (common grapevine) is a perennial and deciduous woody vine of the genus *Vitis*, native to the Mediterranean region, central Europe, and southwestern Asia and commonly cultivated on every Continent on Earth nowadays. Lots of health benefits of grapevine such as chemopreventive, antimicrobial, antioxidant and anti-inflammatory activities[1-4] has been reported and resveratrol (3,5,4'-trihydroxystilbene), a reputed constituent of grapevine is regarded as an active principle of above mentioned biological activities. Grapevine is known as an important source of resveratrol and many naturally occurring hydroxystilbene components.[5]

In the course of examining the inhibitory effect of various kinds of plant extracts on the proliferation of tumor cells, we have found that a methanol extract from stem bark of grapevine exhibited a potent inhibition on the proliferation of cultured human tumor cells such as A549 (non small cell), SK-OV-3 (ovary) and SK-MEL-2 (melanoma) in a dose dependent manner, *in vitro*.

Thus, a bioassay-guided fractionation of the extract had performed and finally led to the isolation of a new glycoside of resveratrol dimer, (-)-ε-viniferin glycoside (**1**) and nine related resveratrol oligomers, i.e., ε-viniferin (**2**),[6] ampelopsin A (**3**),[7] isoampelopsin F (**4**),[8] caraphenol B (**5**),[8] malibatol A (**6**),[9] viniferaether B (**7**),[10] vitisin A (**8**),[6] cis-vitisin A (**9**),[11] vitisin B (**10**)[6] as active ingredients responsible for the antitumoral property. Structures of the isolated active components (**1**-**10**) were established by chemical and spectroscopic means. In the present paper, we describe the identification of a new glycoside of resveratrol dimer (**1**), together with the cytotoxic effect of ten resveratrol oligomers (**1**-**10**) isolated from the stem bark extract of grapevine (Fig. 1).

Compound **1**, an amorphous yellow powder, $[\alpha]_D^{20}$: −15.0

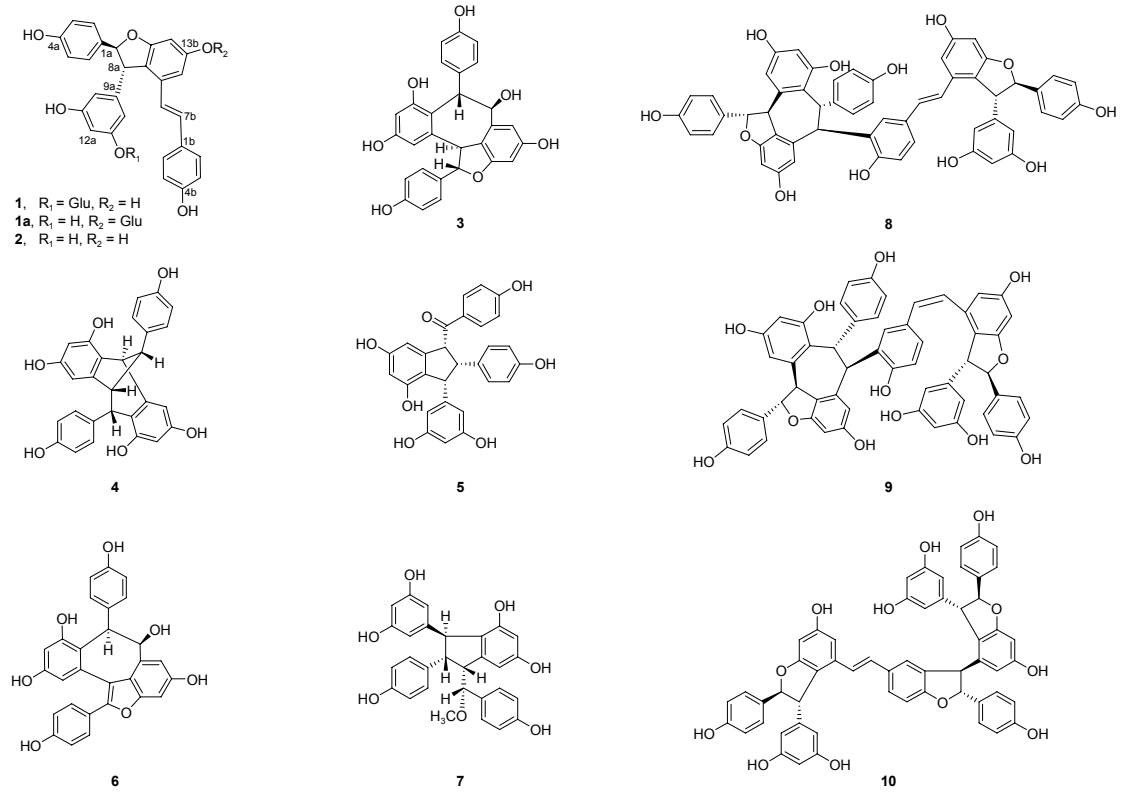

Figure 1. Structures of isolated resveratrol and its derivatives isolated from stem bark extracts of *Vitis vinifera*.

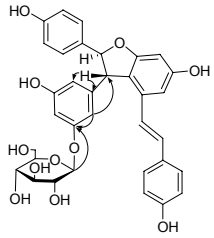

Figure 2. Important HMBC correlations for **1**.

Table 1. ^{1}H- and ^{13}C-NMR spectral data for compounds **1**[a] and **1a**

	^{1}H (**1**)	^{1}H (**1a**)[b]	^{13}C (**1**)	^{13}C (**1a**)
1a			133.8	133.7
2a, **6a**	7.22 (d, 8.4)	7.20 (d, 8.5)	128.0	128.0
3a, **5a**	6.85 (d, 8.4)	6.83 (d, 8.5)	116.2	116.2
4a			158.2	158.3
7a	5.49 (d, 5.4)	5.45 (d, 5.4)	94.0	94.0
8a	4.56 (d, 5.4)	4.51 (d, 5.4)	57.0	57.0
9a			147.4	147.1
10a	6.43, br s	6.23(s)	109.3	107.0
11a			159.6	159.9
12a	6.52, br s	6.23(s)	103.0	102.2
13a			160.4	159.9
14a	6.46, br s	6.23(s)	108.6	107.0
1b			129.8	129.9
2b, **6b**	7.19 (d, 8.7)	7.18 (d, 8.7)	128.8	128.8
3b, **5b**	6.77 (d, 8.7)	6.73 (d, 8.7)	116.4	116.3
4b			158.2	158.3
7b	6.93 (d, 16.2)	7.01 (d, 15.8)	130.3	130.7
8b	6.70 (d, 16.2)	6.71 (d, 15.8)	123.2	123.2
9b			136.4	136.4
10b			119.6	122.3
11b			162.5	162.1
12b	6.36 (d, 1.8)	6.53 (d, 2.0)	96.9	98.0
13b			159.7	160.4
14b	6.76 (d, 1.8)	7.01 (d, 2.0)	104.3	105.5
1'	4.74 (d, 7.6)	4.99 (d, 7.3)	102.1	102.5
2'	3.47, m	3.47, m	74.6	74.8
3'	3.51, m	3.45, m	71.1	71.5
4'	3.47, m	3.57, m	77.5	78.0
5'	3.56, m	3.53, m	77.8	78.4
6'	3.73, m	3.95, m	62.4	62.8

1 and **1a** were measured in CD_3COCD_3. 300 MHz (^{1}H) and 75 MHz (^{13}C). [a]TMS was used as the internal standard; chemical shifts are shown in the δ scale with *J* values in parenthesis. br s: broad singlet; d: doublet; m: multiple.

(*C* 0.1, CH_3OH). The HRESIMS exhibited a molecular ion peak at *m/z* 639.1838 $[M+Na]^+$, consistent with a molecular formula of $C_{34}H_{32}O_{11}$. The ^{1}H and ^{13}C signals of **1** in the aromatic region were almost superimposable with those of ε-viniferin (**2**),[6] a well known resveratrol dimer found in grapevines. The molecular formula and additional six carbon signals (five oxygenated methines and one methylene) of **1** compared with those of **2** strongly implied that **1** was a monoglycosidic congener of **2**. All

Table 2. Inhibition of tumor cell proliferation by resveratrol and its derivatives isolated from stem bark extracts of *Vitis vinifera*

Compound	ED_{50} (μM)[a]		
	A549	SK-OV-3	SK-MEL-2
2	18.64 ± 2.24	13.91 ± 1.17	13.75 ± 3.14
3	26.41 ± 2.17	14.80 ± 0.30	> 30.0
8	3.49 ± 0.14	1.71 ± 0.31	33.28 ± 3.14
9	0.93 ± 0.21	0.17 ± 0.06	13.04 ± 1.34
10	18.63 ± 2.31	4.11 ± 0.12	4.85 ± 0.94
Etoposide	2.61 ± 0.13	2.90 ± 0.37	2.73 ± 0.29

[a]ED_{50} value of compound against each cancer cell line, which was defined as a concentration (μM) that caused 50 % inhibition of cell proliferation *in vitro*. Data are expressed as mean ± SEM of three separate experiments.

proton signals and carbon signals of **1** were completely assigned by the aid of two-dimensional NMR experiments, COSY, DEPT, HMQC and HMBC. The enzymatic hydrolysis of **1** with hesperidinase gave a D-glucose and **2**, respectively. D-glucose determined by TLC over silica gel (CH_2Cl_2-MeOH-H_2O = 8:5:1) by comparison with authentic samples. The linkage pattern of D-glucose was established as β-orientation by the coupling constant (*J* = 7.6 Hz) of anomeric proton (H-1') and the attached point of glycosidic linkage was confirmed by HMBC spectral data (Fig. 2), i.e., the anomeric proton (H-1') showed a correlations with C-11a, which suggested that a D-glucose was linked at the hydroxyl group of C-11a of **2** *via* β-glycosidic linkage as depicted in Fig. 1. A monoglycosidic compound 2, paucifloroside A (1a) has been recently isolated from the stem bark of Vatica pauciflora.[12] However, **1**a had a different glycosidic linkage point (C-13b) as depicted in Fig. 1. All proton signals and carbon signals of **1** were observed to be slightly different with those of **1**a, which were summarized in Table 1. And absolute stereochemistry of C-7a and C-8a determined using CD spectroscopic evidence.[14] In circular dichroism (CD) experiments, compound **1** showed a positive cotton effect near 236 nm. To the best of our knowledge, **1** has never been isolated before neither from grapevines nor from any other natural resources.

All isolated resveratrol oligomers (**1**-**10**) were evaluated for the inhibitory activity on the proliferation of cultured human tumor cell lines such as A549 (non small cell lung), SK-OV-3 (ovary) and SK-MEL-2 (melanoma) by SRB method, *in vitro*. The concentration of compounds required for 50% proliferation of tested tumor cells (ED_{50}) were summarized in Table 2. However, **1** and **4**-**8** did not exhibit a significant inhibition on the proliferation of tested tumor cells below 30 μM, respectively. Moreover, Anti-proliferative activity of resveratrol dimers (**1**-**7**) were much less potent than those of resveratrol tetramers (**8**-**10**) on each tested tumor cells.

Experimental Section

General procedures. The High resolution electrospray ionization (HRESI) and electron impact (EI) mass spectra were obtained using a Q-Tof micro LC-MS/MS instrument (Waters, USA) and CP3800-1200L (Varian, USA) mass spectrometer, respectively. ^{1}H-NMR (nuclear magnetic resonance), and ^{13}C-

NMR spectra were recorded on a Bruker (Rheinstetten, Germany) AM 300, AMX 500 and AMX 800 NMR spectrometer using TMS as an internal standard. Column chromatography was performed using a silica gel (Kieselgel 60, 70 - 230 mesh, Merck, Darmstadt, Germany), and Lichroprep RP-18 (40 - 63 mm, Merck). Thin layer chromatography (TLC) analysis was performed on Kieselgel 60 F254 plates (silica gel, 0.25 mm, Merck)and spots were detected under a UV lamp Spectroline Medel ENF-240 C/F (Spectronics Corporation, Westbury, NY) followed by 10% H_2SO_4 reagent. Solvents and reagents were obtained from commercial sources and used without further purification. Hesperidinase was a purchased from Tokyo Tanabe Pharmaceutical Co., Ltd. (Tokyo, Japan).

Plant material. The stem bark of *Vitis vinifera* were harvested in October 2008 from the vineyard, Yuseong, Korea. A voucher specimen (KR0331) was deposited at the herbarium of the Korea Research Institute of Chemical Technology.

Extraction and isolation. The stem bark of *Vitis vinifera* (10 kg) were extracted twice with 50 L of ethanol (EtOH) by maceration at room temperature for 7 days. After filtration with cotton ball, the filtrate was combined and evaporated to dryness to give 708.8 g of dark syrupy extract. The ethanol extract was suspended in 20 L of water and partitioned sequentially with equal volume of CH_2Cl_2, EtOAc and *n*-BuOH. The EtOAc soluble fraction (463.8 g) was subjected to silica gel (5.0 kg) column (15 × 60 cm) chromatography, eluted with MeOH in CH_2Cl_2 in a step-gradient manner from 1% to 50% to give six fractions (F1: 32.9 g, F2: 39.8 g, F3: 28.1 g, F4: 195.8 g, F5: 39.2 g and F6: 110.2 g). The fraction F2 was further purified by ODS (500 g) column (2.0 × 40 cm) chromatography, which produced 230 mg of **2**. Fraction F3 was further purified by silica gel column chromatography in a similar manner to that described above and eluted with MeOH in CH_2Cl_2 in a step-gradient manner, from 1% to 50% to produce six fractions (F31-F38). F34 was also purified by a similar manner with RP-18 column chromatography eluted with MeOH in H_20 (1% to 100%) in a stepwise gradient, which finally gave 15 mg of compound **3** and 48 mg of **7**, respectively. Fifteen mg of **4** was obtained by crystallization of F343 in MeOH. After removal of **4**, the remained mother liquor of F343 was further purified by RP-18 column chromatography eluted with MeOH in H_2O (1% to 100%) in a stepwise gradient, which finally gave 9 mg of **6** and 3 mg of **5**. Repeated RP-18 chromatography of F4 and F5 with step-gradient elution of MeOH in H_2O finally resulted in the purification of 9.1g of **10** and 90 mg of **1** from F4, and 8.1 g of **8** and 210mg **9** from F5.

(-)-ε-Viniferin glycoside (1): Amorphous yellow powder; $[\alpha]_D^{20}$: −15.0 (*C* 0.1, CH_3OH). ^1H and ^{13}C-NMR spectra data: Table 1; HRESIMS: *m/z* 639.1838 $[M+Na]^+$, calcd for $C_{34}H_{32}O_{11}Na$, 639.1842.

***In vitro* cytotoxicity assays.** All of the experimental procedures followed the NCI's protocols with some minor changes based on the sulforhodamine B (SRB) method as described previously.[15]

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